

Postmortem Study to Evaluate Age Related Angiopathy in Memory Associated Brain Regions

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ABSTRACT

Objective: The present study reveals amyloid deposition in the brain parenchyma.

Design: It is cross sectional descriptive study.

Place and Duration: The study was conducted in the Department of Anatomy, University of Health Sciences, Lahore. The duration of the study was two years.

Methods: Sixty brain specimens (age 19-72 years) of human cadavers were collected using purposive non- probability sampling from the mortuary of the King Edward Medical University, Lahore. Human cerebrums obtained from autopsy table; were divided into two groups according to their age. Group I comprised of deceased less than sixty years of age, group II contains above sixty years of age. The study was confined to those areas of brain which are concerned with memory i.e. cerebral cortex and hippocampus. The microscopic slides were prepared from the cerebral cortex and hippocampus. The prepared slides were stained with Haematoxylin and Eosin and Congo red stains; and were observed under light microscope.

Results: The results revealed that some degree of amyloid deposition in the superior frontal gyrus, (principally in the layer II and III) but well marked in the hippocampus.

Conclusion: It is not surprising that performance on tasks that require information processing in these brain regions decline with age.

Key words: Amyloid. Alzheimer Disease. Brain.

INTRODUCTION

Brain is a complex signaling system much like a computer. Information is coming in, processed and stored in memory; it is subsequently used in the process of recall¹. Dementia, as witnessed in Alzheimer, follows an insidiously progressive course. Its important symptoms are loss of short term memory, eventual disorientation, impaired thinking and changes in personality; the patient usually does not admit that the changes have involved his personality. The most common form of dementia among elderly is a complex neurodegenerative disorder resulting from multiple genetic, non genetic and aging factors. Shakespeare wrote about old age as being a time of "Second childishness and mere oblivion". But it wasn't until 1906 that a German doctor named Alois Alzheimer characterized the structural changes in the brain of people who suffered from loss of recent memory and named the condition Alzheimer's disease¹.

Numerous studies support a central role of β -amyloid (A β) in Alzheimer's disease. The incidence of amyloid deposition increases with age^{2,3}. Alzheimer's

disease or age related memory loss had been reported since long. Amyloid is the general term for protein fragments normally produced by the body. Beta-amyloid is the fragment that is snipped off from amyloid precursor protein. In healthy brain, these proteins are broken down and eliminated but in diseased state, these fragments accumulate to form hard, insoluble plaques and neurofibrillary tangles which keep on accumulating with passage of time and are deposited inside the brain cells^{4,5}.

Amyloidosis is a condition characterized by the deposition in various organs of an amorphous, predominantly extra-cellular, eosinophilic material called amyloid. It is seen as microscopic deposits, in the form of plaques and neurofibrillary tangles which progressively replace the parenchyma of affected organ resulting in gradual loss of function and eventual death^{4,6}.

The key areas of the cerebral cortex involved in memory⁶. Frontal lobe and hippocampus in temporal lobe are more prone to get amyloid deposits. The prefrontal cortex is the commonest site of onset of plaque formation⁷. Amyloid deposition in these areas ultimately results in death of the neurons, which are essential for memory and learning⁸. In all known patients of Alzheimer's disease, some degree of amyloid deposition is seen in the superior frontal gyrus, located principally in layers II and III. These

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changes are particularly pronounced in the right superior frontal and precentral gyri^{7,8}.

One of the important steps in the diagnosis of the specific type of amyloidosis is detection of amyloid deposits in different organs. It is not easy to predict the presence of amyloid deposits in brain on the basis of patients clinical manifestations; the diagnosis is only possible on the basis of histological findings. Amyloidosis could, therefore, be diagnosed only after death⁸. Before the impairment of memory appears, the neuronal degeneration proceeds and amount of tangles increase to produce clinical signs and symptoms. The preclinical period probably starts 20-30 years before the appearance of first symptom⁹.

Congo red is a hydrophilic chemical agent that binds to amyloid in vitro. Apple Green birefringence under polarized light after Congo red staining is the most reliable evidence of the presence of amyloid fibrils in biopsy and/or in autopsy material^{4,8}.

On postmortem examination, cases without clinical evidence of dementia, showed appreciable numbers of β -amyloid plaques in brain parenchyma. These cases were significantly distinct from those with a minimal level of dementia before death in terms of tangles, neuritic plaques, and β -amyloid plaques. Advanced clinical stages of dementia were associated with gradually increasing β -amyloid plaques in hippocampus. Clinical severity was, therefore, strongly correlated with β -amyloid plaques^{6,8}.

The present study was designed to evaluate age related amyloid angiopathy and tangle formation in the brain regions associated with memory obtained from autopsy of local population living in Lahore and its suburbs.

MATERIAL AND METHOD

Sixty brain specimens were obtained from Department of Forensic Medicine and Toxicology, King Edward Medical University, Lahore. Written consent was taken from the close relatives of the deceased and socio-demographic information (age, sex, occupation, place of job, education) was obtained recorded. The cadavers with the history drug intake and any co-morbid condition like diabetes mellitus or hypertension were excluded from the study. The material was obtained within 8 to 20 hours after death and was immersed in 10% formalin at room temperature for one week.

Right and left cerebral hemispheres were sliced in coronal plane and two centimeter thick pieces were obtained; brain tissues were cut perpendicular to the cortical laminae into 4 mm thick pieces from superior

frontal gyrus and hippocampus. However, after three days of fixation, the macroscopic staining was carried out. Sliced pieces of brain were immersed in Lugol's iodine; these were then dipped in 5% solution of Sulphuric acid. The tissue containing sufficient amount of amyloid stained blue¹⁴.

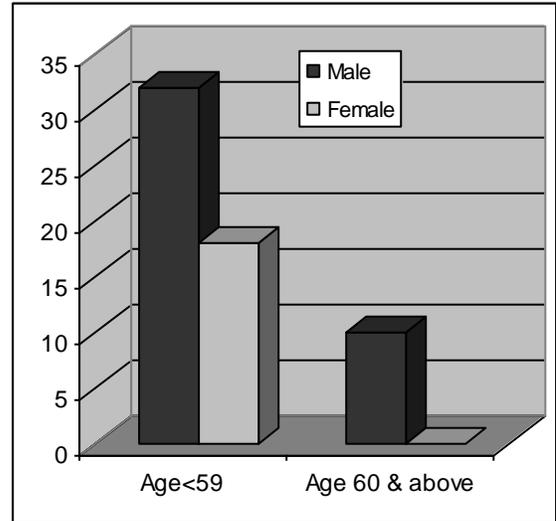


Figure 1: Indicate different age groups and distribution of male to female ratio.

The material was processed in automatic tissue processor. Tissue blocks were prepared. The rotary microtome (Leica RM 2125) was used to slice 10 μ m thick sections. Slides were stained with H and E and congo red stains in the usual way for general histological study; mounted with DPX and examined under Leica 1000 DM microscope.

Statistical analysis: The Statistical Analysis was carried out using computer software Statistical package for social sciences (SPSS). The significance between the two groups was calculated by fisher's exact tests. The tests were applied using SPSS; the difference was regarded statistically significant if the 'p' value was < 0.05.

RESULTS

Table I shows the distribution of amyloid angiopathy in hippocampus and frontal lobe areas. In hippocampus, amyloid angiopathy was seen in 12 out of 18 cases in group II (figure 2). The difference was statistically highly significant ($p=0.013$).

Similarly 8 cases were found to be positive in group II for amyloid angiopathy in frontal lobe; two cases were, however, positive in group I. The difference was statistically significant ($p=0.00095$) when two groups were compared.

Table: I Amyloid Angiopathy in Sixty Autopsy Cases (Hippocampus vs Frontal lobe)

Groups	Hippo-campus		Frontal Lobe	
	+Ve	-Ve	+Ve	-Ve
Group I Age <60 years (42)	0	42	2	40
Group II Age >60 years (18)	12	6	8	10

(Figures in parentheses indicate total number in each group. Fisher's Exact Test was applied)

Hippocampus: Group I vs Group II: $p = 0.013$

Frontal Lobe: Group I vs Group II: $p = 0.00095$

Table II: Angles in Sixty Autopsy Cases (Hippocampus vs Frontal lobe)

Groups	Hippo-campus		Frontal Lobe	
	+Ve	-Ve	+Ve	-Ve
Group I Age < 60 years (42)	4	38	4	38
Group II Age > 60 years (18)	9	9	7	11

(Figures in parentheses indicate total number in each group. Fisher's Exact Test was applied)

Hippocampus: Group I vs Group II: $p = 0.002$

Frontal Lobe: Group I vs Group II : $p = 0.02$

Table II reveals amyloid tangles within the neurons. 4 out of 42 cases of hippocampus and frontal lobe in group I; where as 9 cases out of 18 were positive in group II. Statistical difference was significant when two groups were compared ($p = 0.002$). Tangles observed on H and E stained preparations were confirmed by congo red staining upon examination under polarized light (figure 3).

In frontal lobe, 4 out of 42 cases in group I were observed to be positive for the tangles. 7 out of 18 cases were, however positive for tangle formation in the hippocampus belonging to group II. Statistically the difference was significant when two age groups were compared $p = 0.02$.

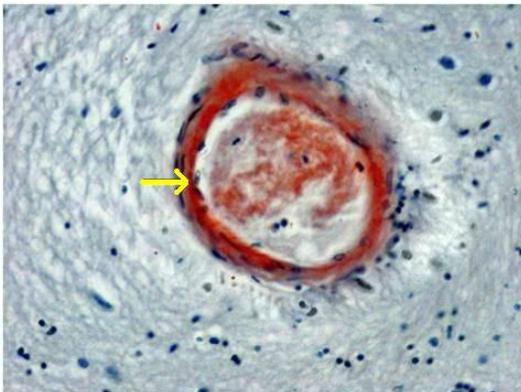


Figure 2: Photomicrograph of hippocampus (age > 60 years) showing amyloid angiopathy (yellow arrow) under light microscopy; stained with Congo red X 400.

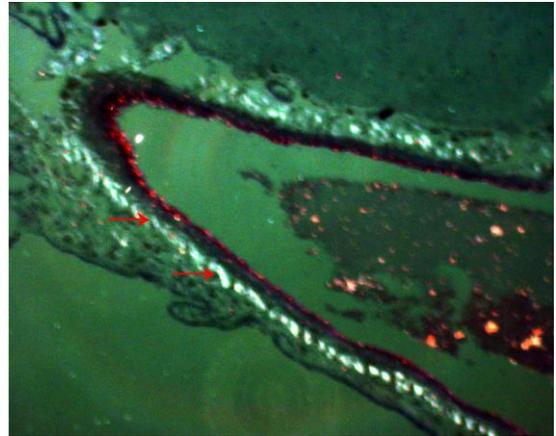


Figure 3: Photomicrograph of cerebral cortex (age > 60 years) showing amyloid angiopathy under polarized light (red arrow). Congo red stained X 400.

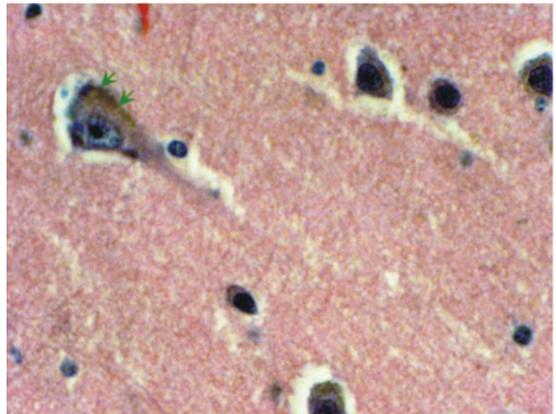


Figure 4: Photomicrograph of pyramidal cell (age > 60 years) of hippocampus showing a tangle extending from the cell body into axon giving it a classical "flame" shape appearance (green arrows) under light microscopy. H and E stained X 400.

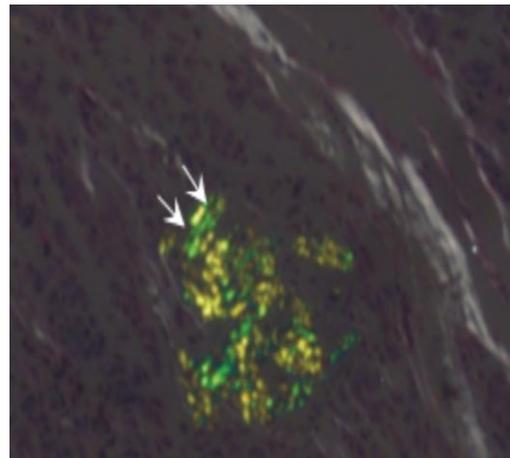


Figure 5: Photomicrograph of (age > 60 years) hippocampus showing apple green birefringence under polarized light after Congo red staining X 400

DISCUSSION

Amyloidosis is a condition characterized by the deposition in various body tissues and organs of an amorphous, predominately extra cellular, eosinophilic material called amyloid^{5,6,7}.

Clinical diagnosis of amyloid deposition in brain during a person's life is only 65 % accurate. Definite diagnosis can only be made by examination of the brain at the time of autopsy^{3,8}.

Thompson et al (2001) focused on human brain to discover the specific pattern of cortical changes in normal and diseased individuals. Results revealed gross and histological variations in all major cortical sulci, callosal and hippocampal and various ventricular regions. They found that grey matter loss was greater in the left cerebral hemisphere¹⁰. Similarly Armstrong (2003) studied the association between several histological features in thin sections of brain tissues of diseased and normal aged individuals. He concluded that there was marked decrease in the volume of the brain; which resulted in enlargement of ventricles¹¹. Dementia is a common neurodegenerative disorder involving as many as 10% of those individuals who were over 65 years of age and substantially reduces life expectancy⁴. The incidence of dementia in developed countries under the age of 65 years is low. The prevalence increases with increasing age, doubling after every 5 years. Alzheimer's disease currently has been estimated to afflict 4 million Americans, a figure which could reach 9 million by 2030. Alzheimer's disease is the 3rd most expansive disease to treat in the United State followed by cancer and heart disease¹². Canadian study on health and aging in 2001 found the incidence of Alzheimer's disease involve 2.52/1000 persons annually (2.3 in women and 2.8 % in men). Further, it was observed that the increase in the incidence of the condition was age related but there was no significant difference in the incidence of the condition between male and female¹³.

Cerebral amyloid is also known as congophilic angiopathy or cerebrovascular amyloidosis¹. It is a disease of small blood vessels of the brain in which deposits of amyloid protein in the vessel walls lead to stroke, brain hemorrhage, or dementia. Cerebrovascular amyloid angiopathy usually affects patients over 45 years of age, and its incidence increases rapidly towards the 7th decade of life⁴. Weller et al in 1998 performed a study on six human brains from those who were clinically not demented, with an average age of seventy nine years. They found that in the leptomeninges, small arteries were most severely affected by amyloid β deposition than large arteries and veins; small deposits of amyloid present in the connective tissue of the arachnoid

matter². Takeda et al (2003) studied six autopsy cases of subcortical hematoma; they reported that cerebral amyloid angiopathy was characterized by amyloid deposition not only in cerebral blood vessels but also in the meningeal vessels and especially those at the depth of the cerebral sulci. Arteries which were heavily deposited with amyloid show lack of immunostaining for collagen type I, III, V and VI in the amyloid containing parts of the vessel wall¹⁴. In an autopsy study of twenty individuals, between the ages of twenty to ninety years, Shinkai (1995) reported cerebral amyloid angiopathy in intracranial blood vessels and leptomeninges. An amyloid deposition in the leptomeninges was reported to increase sharply from fifty to seventy years¹⁵. In 2004, a study on three autopsy specimen was conducted by Wisniewski and Wegiel which revealed that amyloid deposits were present in smooth muscle cells of the media of arteries of the brain; it was further stated that amyloid deposition initially started in smaller blood vessel and subsequently extended to involve the larger meningeal vessels¹⁶. Further Skoog et al investigated the brain by CT scan and examined CSF of individuals of 85 years old; reported that both isoform of amyloid (A β 40 and A β 42) were found in varying concentrations in the cerebrospinal fluid; affected persons had history of recurrent hemorrhagic stroke between the ages of 45 and 65 years; both morbid conditions could be regarded as a presumable cause of cognitive deterioration and appeared as the first symptom of the disease. Further, they also found cerebral amyloid angiopathy in several familial conditions. Several mutations in the APP gene located at chromosome 21 were said to be associated with severe cerebral amyloid angiopathy¹⁷.

Present study is in conformity with the previous studies. In hippocampus, 12 cases were found to be positive for amyloid angiopathy in age group of sixty years and above, involving media of the small arteries (Figure 2-3). Statistical calculation using Fisher exact test indicated that the difference between two groups was significant $p = 0.013$ (Table I). Similarly in cerebral cortex two cases showed amyloid angiopathy in age group less than sixty years, whereas eight cases found to be positive for amyloid angiopathy in age group sixty years and above. The findings in the current project were statistically significant ($p = 0.00095$) when two groups were compared. Thus the present study is in agreement with earlier works that reported a sharp increase in number of positive cases with advancing age. Changes in the brain vascular system, which occur with increasing age, accompanied with neuronal degeneration. Although, all elderly cases did not show same degree of age related amyloid

angiopathy yet, histological changes in the brain occurred, even when the vessels were strikingly normal.

Giulio Cesare Aranzi (circa 1564) was the first anatomist who used the term hippocampus to describe part of brain because of its resemblance to a sea horse; it was initially thought that this organ is concerned with olfaction. A Russian anatomist Vladimir Bekhterev in 1900 noted the role of the hippocampus in memory; his findings were, based on observations on a patient with profound memory disturbances. However, for many years, the conventional view of the hippocampal function continued and was thought to be comparable relating to emotion¹⁵.

From the available literature we conclude that frontal lobe of cerebral cortex and hippocampus are the areas which are concerned with the memory and are sensitive for the age related changes.

Aggregation of amyloid beta peptide in extracellular plaques and the presence of abnormally phosphorylated neurofibrillary tangles in regions of the central nervous system, concerned with learning and memory, are typical pathological features of Alzheimer's disease in brain. Although, the present study did not aim at any molecular mechanism involved, yet the earlier works on the subject suggested that the abnormal processing of the transmembrane amyloid precursor protein leads to the formation of amyloid β which shows high neuronal toxicity and accumulates in plaques or as soluble oligomers, eventually causing neurodegeneration and memory dysfunction.

CONCLUSION

The result of this study suggest that the deposition of amyloid starts at much earlier age like thirty five years of age which is further potentiated by various age related changes in brain. Of the brain regions affected by aging, the hippocampus and the prefrontal cortex seem to be particularly vulnerable, but even within and between these regions the impact of aging on neuronal function can differ. These changes initially start at the microscopic level then with the passage of time they become grossly apparent.

For the detection of early cell changes of Alzheimer's disease; it is hoped that the technique shall be available to identify location and extent of amyloidosis in specific area of the living individual. At present the investigated technique in living is not available. We have to wait till some markers for amyloid are available which could be detected by suitable imaging technique.

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