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MOUSE CEREBRAL CORTEX IS MORE VULNERABLE TO AGE RELATED DEPOSITION OF SENILE PLAQUES THAN THE HIPPOCAMPUS: A NEUROPATHOLOGICAL STUDY OF AMYLOID PLAQUES IN THE NORMAL PROCESS OF BRAIN AGING

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ABSTRACT
The amyloid β (Aβ) is a protein, deposited on the neuronal plasma membrane in the patients of Alzheimer's disease (AD). It is considered as the hallmark of AD. It is generated due to the abnormal processing of amyloid precursor protein (APP). It exists in the isoforms like Aβ40 and Aβ42 that get deposited in the brain as senile plaques. Deposition of senile plaques leads to dementia. Enormous literature speculates the neuropathology of amyloidosis in AD. However, there are hardly any reports speculating the deposition of amyloid plaques in the normal process of aging. In the present investigation, we have studied the deposition of senile plaques by Congo red method in the cerebral cortex and hippocampus of Swiss albino mouse *Mus musculus* of 12, 18 and 24 months of age. As compared to 12 and 18 months, at 24 months of age the cortical and hippocampal region showed a highly significant increase in the deposition of senile plaques. Among the cerebral cortex and hippocampus, the cerebral cortex showed higher deposition of senile plaques. These results demonstrate the progressive deposition of senile plaques in the normal course of aging, i.e. in the absence of neurological disorder such as AD.

INTRODUCTION

Alzheimer's disease (AD) is a neurological disorder in which patients experience serious dementia i.e. loss of memory. This is marked by the extracellular deposition of β- Amyloid (Aβ) and intracellular aggregation of neurofibrillary tangles in the neurons [1]. Aβ is produced from the membrane bound Amyloid Precursor Protein (APP). The APP is involved in the formation of synapses and their repair [2]. The "amyloid cascade hypothesis" [3-4] states that abnormal metabolism of Amyloid Precursor Protein (APP) in the brain is a primary event in the pathogenesis of AD. The Aβ40 and Aβ42 are the most common isoforms of amyloid beta that are produced as monomers. These monomers aggregate into dimers, trimers, oligomers, protofibrils and fibrils to form amyloid plaque [5]. Aβ oligomers are toxic forms of the Aβ peptide [6]. The amyloid β interacts with neurons and glial cells, and triggers the inflammatory process resulting in oxidative stress [7]. The above cited literature emphasizes amyloid deposition in AD. Per se, there are seldom reports describing the process of amyloid deposition in the normal process of aging. The present investigation is an attempt to understand the extent of deposition of senile plaques in the cerebral cortex and

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hippocampus of mouse at 12, 18 and 24 months of age in the absence of neurological disorder such as Alzheimer's or Parkinson's disease.

MATERIAL AND METHODS

For the present investigation Swiss male albino mice *Mus musculus* were used as an experimental animal. The work was carried out with the due permission of the Institutional Animal Ethics Committee. The animals were reared in the departmental Animal house and pelleted animal chaw (Pranav Agro Industries, Sangli) and water were provided *ad libitum.* The animals were exposed to 12hr-12hr light dark cycle. Animals were sacrificed by deep ether anesthesia after 12, 18 and 24 months of age. Six animals were used in each age group. The brain was gently excised on prechilled platform and longitudinally cut into two equal halves. The longitudinal halves were fixed in neutral buffered formalin for 24 hrs, washed under running tap water for 24 hrs and dehydrated through ascending grades of ethanol viz. 30%, 50%, 70%, 90% and 100% each for 2hrs duration. Then the halves were cleared in 1:1 ethanol-xylene mixture and finally into xylene for 15 min, finally embedded in molten paraffin wax at 60^{o}C for 90 min, initial wash was about 30 min followed by impregnation for 60min. The rectangular paraffin blocks were made, trimmed and cut into 6µm thick sections. The paraffin sections were spread on the micro slides, which were further used for histochemical studies.

Histochemical studies of β amyloid

Staining of β amyloid was carried out by modified Congo red method described by Puchtler *et al.* [8]. Sections were deparaffinized and hydrated through descending grades of ethanol and were stained with Mayer's haematoxylin for 10 min. Then sections were rinsed in distilled water and treated with 80% ethanol saturated with NaCl followed by staining with Congo red for 1 hour at 37^{0}C. Thereafter sections were dehydrated rapidly in 90% and absolute alcohol and cleared in xylene and mounted in D.P.X. and observed under microscope. The amyloid positive areas were measured in the micrometer square (µm^2) under oil immersion lens i.e. at 1000X magnification.

Statistical analysis

The results were calculated as the arithmetic mean of six animals ± standard deviation. To study the level of significance in three age groups, the amyloid positive areas from 18 months were compared with 12 months and area of amyloid plaques from 24 months were compared with 18 months in both the regions of the brain. To know the level of significance among the cerebral cortex and the hippocampus, the areas of amyloid plaques from the both the regions were compared with each other at respective ages.

RESULTS AND DISCUSSION

The photomicrographs of cerebral cortex and hippocampus are displayed in figure 1and 2 respectively. The cerebral cortex of 12 months old mice showed 382.0 ± 10.81 µm^2 area positive for senile plaques (Figure 1A); whereas, the hippocampal region showed 337.66 ± 9.81 µm^2 area positive for senile plaques (Figure 2A). In the cerebral cortex of 18 months old mice (Figure 1B)the amyloid positive area was 672.0 ± 22.2 µm^2, while in the hippocampal region it was 429.5 ± 21.51 µm^2 (Figure 2B). At the age of 24 months in the cerebral cortex, the amyloid positive area was 2990.33 ± 88.22 µm^2 (Figure 1C) and in the hippocampus it was 1418.66 ± 84.83 µm^2 (Figure 2C). There was age related increase in the amyloid positive area in both the cerebral cortex and the hippocampus from12 to 18 months and from 18 to 24months. This increase was highly significant ($p<0.001$). When an amyloid positive area in the hippocampus was compared with the amyloid positive area in the cerebral cortex, it is observed that cerebral cortex contains greater amyloid positive area. This difference was highly significant at $p<0.001$. At the age of 24 months the amyloid positive area in the hippocampus was 50% smaller than the amyloid positive area from the cerebral cortex.

The results demonstrate age related increase in the amyloid positive area in the mouse cerebral cortex and the hippocampus. Under pathological conditions APP is metabolized by the amyloidogenic pathway and gets accumulated due to the reduction in the clearance of Aβ peptides [9]. However, the present study depicts that amyloid deposition also occurs in nonpathological condition. Rhein and Eckert (2007) described that the accumulation of Aβ in the brain causes mitochondrial dysfunction, increased oxidative stress, abnormal neuroinflammatory response, decreased neuroplasticity and increased tau phosphorylation that finally leads to neuronal death [7]. Recently we found the abnormal mitochondria and degenerative ultrastructural changes in the cerebrocortical neurons in mice during aging [10].

The age related increase in the deposition of amyloid plaques in the cerebral cortex and hippocampus observed in this study may be due to declined autophagic clearance of amyloid β in the neurons of cerebral cortex and hippocampus. Dice [11] and Martinez-Vicente *et al.* [12] found that there is an age related decline in lysosomal proteolysis. Another reason may be altered processing of amyloid precursor protein at an advanced age.

Table 1. Showing the Amyloid positive area in µm^2 in the cerebral cortex and hippocampus of mouse brain. Results are expressed as mean ± SD of 6 animals.

Age of mouse	Amyloid positive area stained by Congo red in µm^2	
	Cerebral cortex	Hippocampus
12 months	382.0 ± 10.81	337.66 ± 9.81 ^{b***}
18 months	672.0 ± 22.2 ^{a***}	429.5 ± 21.51 $^{a*** b***}$
24 months	2990.33 ± 88.22 ^{a***}	1418.66 ± 84.83 $^{a*** b***}$

a*** indicates p<0.001 i.e. highly significant when area of amyloid plaques from 18 months were compared with 12 months and area of amyloid plaques from 24 months were compared with 18 months. b*** indicates p<0.001 i.e. highly significant when area of amyloid plaques from hippocampus were compared to area of amyloid plaques from cerebral cortex at respective ages

Figure 1. Photomicrographs exhibiting the deposition of Congo red positive Senile Plaques in the mouse cerebral cortex (400X) (1000X)

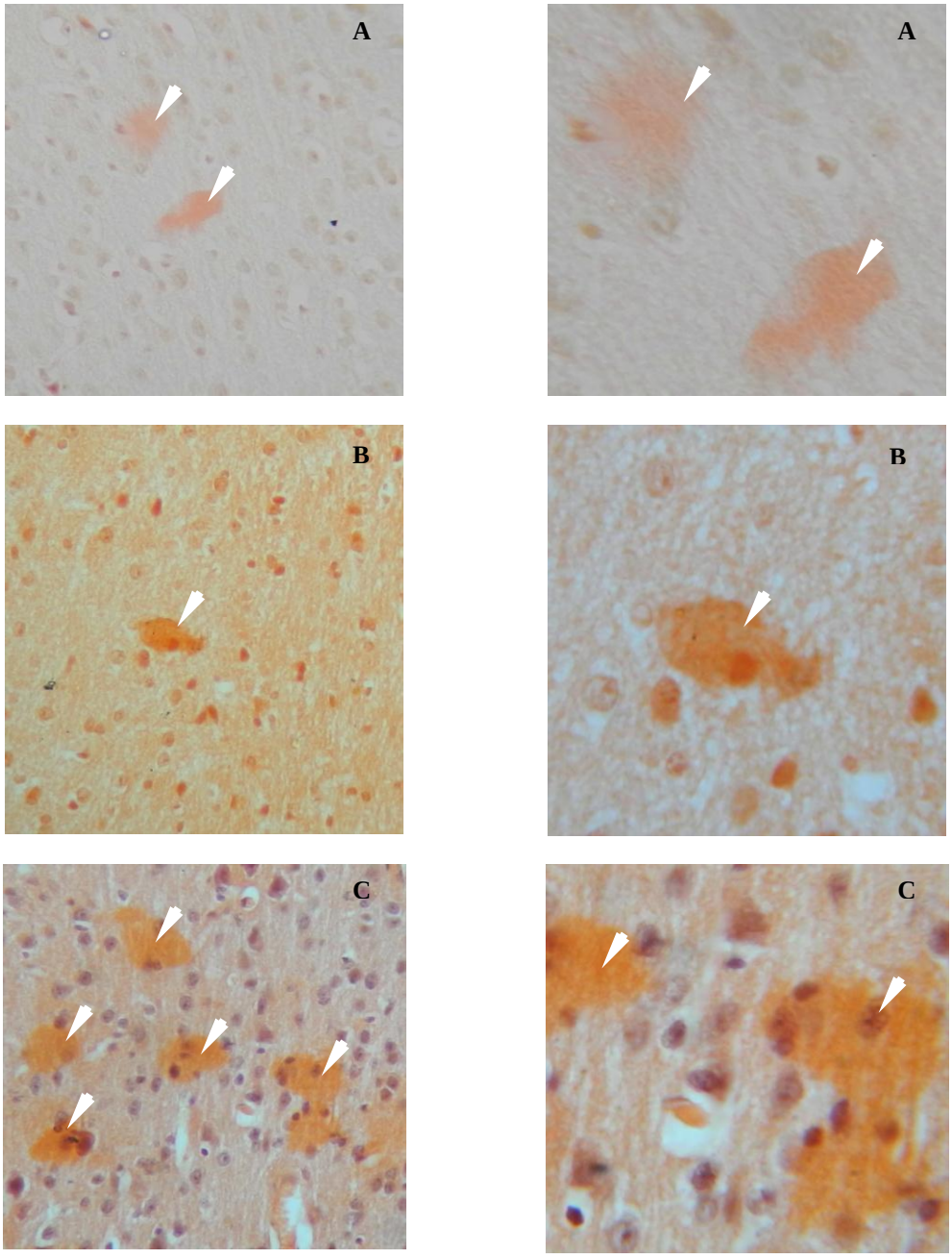
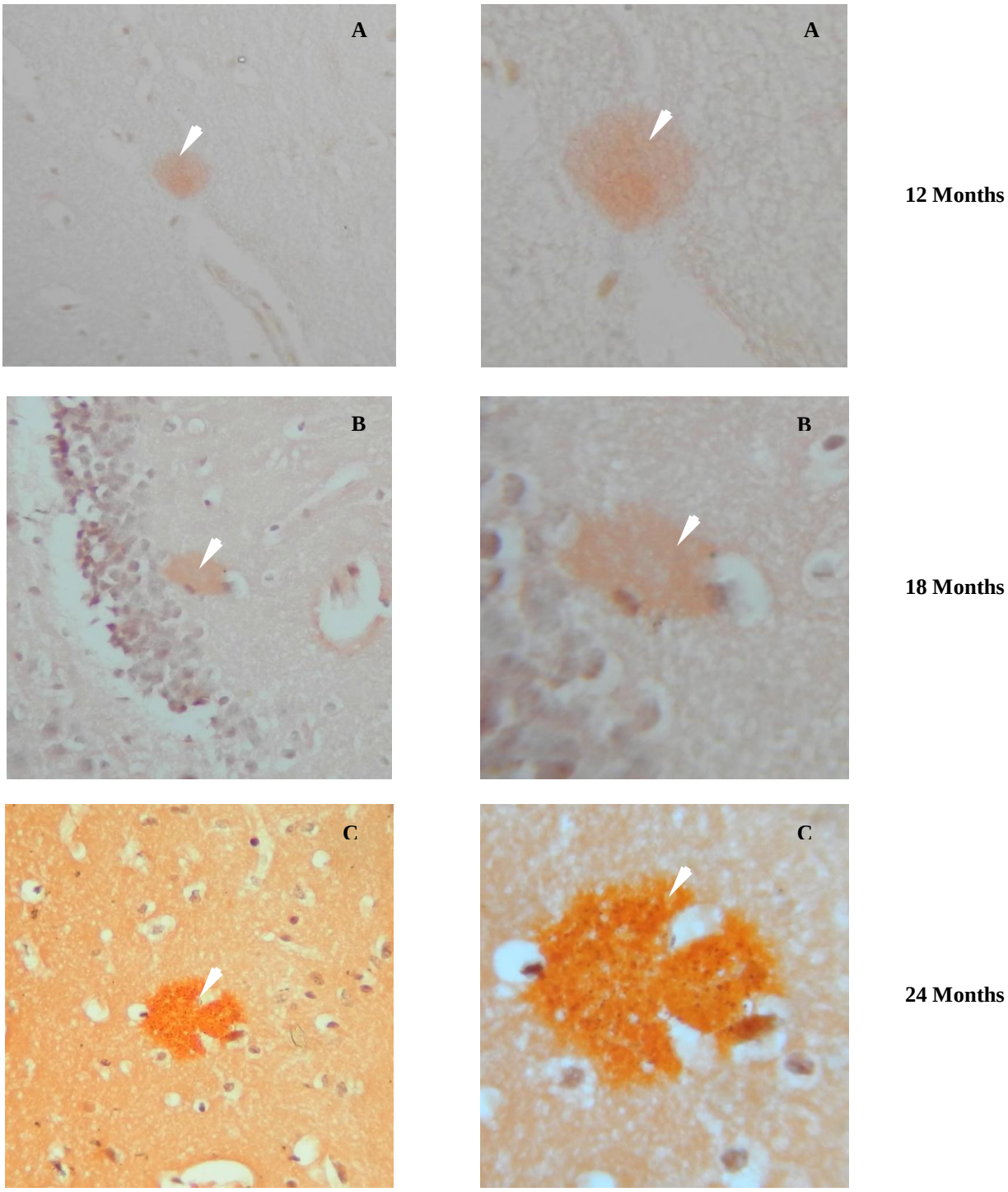
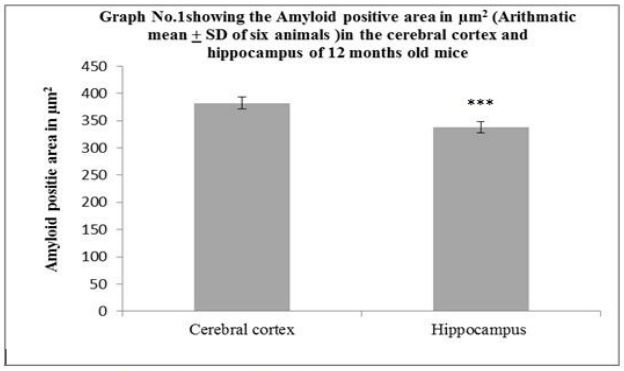


Figure 2. Photomicrographs exhibiting the deposition of Congo red positive Senile Plaques in the mouse hippocampus

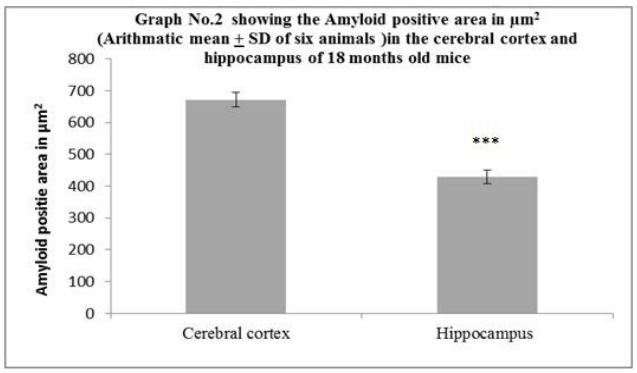
(400X) (1000X)



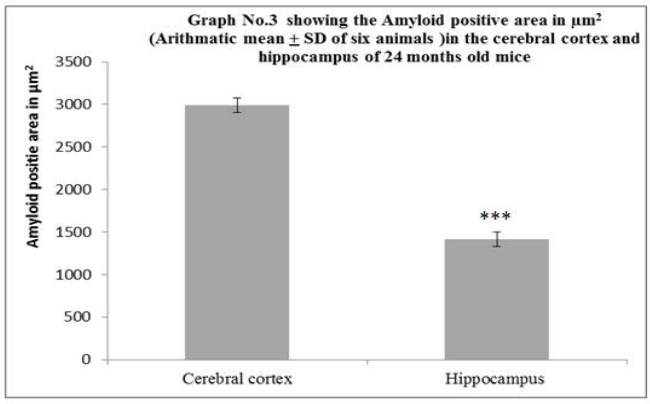
White arrow indicates Senile plaque



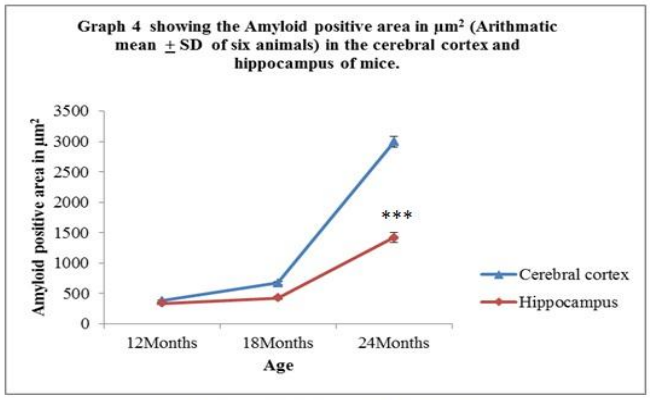
*** indicates p<0.001 i.e. highly significant



*** indicates p<0.001 i.e. highly significant



*** indicates p<0.001 i.e. highly significant



*** indicates p<0.001 i.e. highly significant

Mattson [13] and Tanzi *et al.* [14] found that increase in Aβ levels is due to the altered proteolytic processing of the amyloid precursor protein (APP) and decline in the lysosomal clearance. The increasing number of Aβ peptides forms the oligomers of amyloid beta that leads to neuronal toxicity via generating oxidative stress [15]. Pathan *et al.* [10] demonstrated the age related increase in the lipid peroxidation in the cerebral cortex and the hippocampus of mice. Neurons are susceptible to damage as revealed by Bele *et al.* (2015) in primary culture of mouse hippocampal pyramidal neurons [16]. In our earlier study we found that the cerebral cortex exhibits more aggregation of neurofibrillary tangles than the hippocampus [17].

The findings of the present study further strengthen these observations which may be involvement of hippocampus in adult neurogenesis and thus the cells may be occasionally rejuvenating unlike cerebrocortical neurons. Aβ is a suspected culprit in causing dementia [18]. Age related dementia is a common problem in the elderly, which may be due to a gradual increase in the deposition of amyloid plaques during aging.

CONCLUSION

The present findings unravel one of the reasons of senile dementia and emphasize the progressive deposition of Aβ during advancing age.

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CONFLICTS OF INTEREST: None

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