Prevalence of β-amyloid protein deposition in normal aging non-demented brains in a Malaysian population

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Abstract

It is well known that β-amyloid is pathologically accumulated in the brains of patients with Alzheimer’s disease. In addition, it is also present in smaller quantities in the non-demented normal aging brain. In this study, we attempted to establish the prevalence of β-amyloid deposition in non-demented subjects according to age, sex and ethnic group. 200 consecutive retrospective autopsy cases prior to 1993, whose causes of death were unrelated to dementia or mental retardation and who were 40 years of age and above, were obtained from the files of the Department of Pathology, University of Malaya Medical Centre. Histological sections of the brain were screened with alkaline Congo red for amyloid and positive cases were stained by a standard immunoperoxidase method for β-amyloid. The results were then correlated against age, sex and ethnicity of the subjects. β-amyloid deposition was observed only in individuals above the age of 50 years, at the rate of 2.0% (1 positive case / 49 cases screened) in the 50 - 59 years age group, 5.4% (2 / 37) in the 60 - 69 years age group, 6.7% (1 / 15) in the 70 - 79 years age group and 38.5% (5 / 13) in the 80 - 89 years age group. There appears to be an increased prevalence of β-amyloid deposition in the brain with increasing age but we did not observe any significant differences according to sex or ethnicity.

Key words: β-amyloid, aging, Alzheimer’s disease, Congo red, immunoperoxidase

INTRODUCTION

Amyloid is a β-pleated fibrillary proteinaceous substance which is deposited in intercellular spaces in various tissues in different pathological states and in association with senile degenerations. Senile deposits have been documented in the pancreas, heart, brain and even seminal vesicles. Studies have shown that in senile amyloidosis, the deposits are characterised by different amyloid fibril proteins, which have their own unique structural, biochemical and immunological characteristics although they share similar histochemical and morphological features. Recently, there has been an explosion of literature on amyloid deposition in the brain. This renewed interest is related to the emergence of Alzheimer’s disease as a major health problem and partly to the rising proportion of the aged in the population as a result of improving medical care.

In the brain, four distinct types of pathological β-pleated fibrillary deposits are recognised. The first, described as cerebral amyloid angiopathy (congophilic amyloid angiopathy), is characterised by amyloid deposits in the media of blood vessels of the brain, particularly around the small arteries and arterioles of the meninges and superficial cortex of the cerebrum. The second type consists of extracellular amyloid deposited in the core of classical senile plaques (mature plaques). The senile plaque consists of an acellular central core of amyloid surrounded by a peripheral zone of neuronal processes and glial cells. The third type is the intercellular paired helical filaments of neurofibrillary tangles, most frequently seen in Alzheimer’s disease. The fourth type of amyloid deposit is limited to the spongiform encephalopathies of Kuru and Creutzfeldt-Jakob disease, where the deposit consists of stellate amyloid cores probably derived from the prion protein. The two main types of amyloid deposits found in normal aging brains are those of senile plaques and congophilic amyloid angiopathy. The amyloid protein involved, named β-amyloid protein or A4 protein, is antigenically similar to that in amyloid plaques of Alzheimer’s disease.

The age of onset of senile plaques and amyloid angiopathy is still not well-established. Rumble et al reported a subject as young as 13 years to have β-amyloid cerebral plaques but most investigators agree that the prevalence of β-amyloid deposition increases rapidly after the sixth decade and by the end of the eight decade, about 80% of normal aging brains show amyloid...
deposition detectable by immunohistochemical methods. However, β-amyloid deposition is known to be less extensive in normal aging brains compared to patients with Alzheimer’s disease or Down’s syndrome.

While the prevalence and pattern of systemic amyloidosis and several types of localised amyloidosis of the heart, skin, kidneys and tumours in the Malaysian population have been documented, there is a lack of information on the prevalence of cerebral amyloid deposition, particularly of β-amyloid protein in the Malaysian population. The objective of this study is to gain basic information on the prevalence of amyloid in the normal aging brain in the Malaysian population based on autopsy material at the University Hospital, Kuala Lumpur. We are not aware of any study of similar nature in an Asian population.

MATERIALS AND METHODS

A retrospective study was carried out on archival paraffin-embedded cerebral tissue from 200 consecutive individuals above the age of 40 years who have been autopsied at the University Hospital, Kuala Lumpur. Cases with a history of dementia, mental retardation or Down’s syndrome were excluded. A maximum of 3 different cerebral sections per case were chosen. 6 μm thick sections were cut, stained with alkaline Congo red and screened histologically for the presence of amyloid deposits, defined as congophilic rose-pink deposits which also exhibited green birefringence. Additional sections were then cut from amyloid positive blocks, placed upon poly L-lysine (PLL) coated slides, and stained by the standard peroxidase-antiperoxidase (PAP) immunoperoxidase method using a polyclonal antibody against β-amyloid protein (courtesy of Professor Colin Masters, University of Melbourne). Sections from the brain of a confirmed case of Alzheimer’s disease were used as positive control.

The histomorphological details of the detected β-amyloid deposits were carefully recorded. Demographic and clinical information on the screened subjects were obtained from autopsy records.

RESULTS

Demographic characteristics

Table 1 shows the demographic profile of the subjects of this study. Of the 200 subjects studied, 90 were Chinese, 19 Malay, 72 Indian and 19 were of other ethnicity namely Indonesian (13), Caucasian (3), Thai (1), Japanese (1) and Myanmarese (1). Irrespective of ethnicity, less subjects were available for study with ascending age. At age group 70 years and above, there was a bias towards the Chinese ethnic group, i.e. there were 25 Chinese while the other ethnic groups totalled only 4.

There were 153 males and 47 females in this study. A strong bias towards male subjects was observed in the younger age groups, however, the sex ratio was more balanced in the older age groups (Fig. 1). In the age group 80 - 89 years, there were 7 males and 6 females in contrast to the age group 40 - 49 years where there were 73 males and only 13 females.

β-amyloid deposition

β-amyloid was detected in 9 (4.5 %) subjects. Two morphological types of β-amyloid deposits were observed. One was in the form of cerebral cortical plaques. Under polarised light, the congophilic plaques exhibited an apple-green birefringence with a Maltese cross appearance (Fig. 2). It was organised into a central core of compact amyloid surrounded by fibrillary deposits occasionally associated with microglial cells and astrocytes. It was mainly found in the superficial cortex. The second morphological
type of β-amyloid deposition consisted of deposits in the media of small vessels of the cerebrum and meninges (Figure 3).

Both types of deposits were immunoreactive for β-amyloid by immunoperoxidase staining (Figure 4). However, the intensity of staining was mild to moderate compared to the strong reaction observed in the positive control.

Two subjects showed β-amyloid deposition in blood vessels only. Five revealed senile cortical plaques only while another two cases revealed both vascular deposits and cortical plaques (Table 3).

**Demographic profile of amyloid-positive subjects**

**Age distribution**
The prevalence of β-amyloid cerebral deposition increased with age (Table 2). There were no cases positive for amyloid below 50 years of age. In contrast, there was an abrupt rise in prevalence from 6.7% in the age group 70 - 79 years to 38.5% in age group 80 - 89 years.

**Ethnic distribution**
Of the 9 subjects positive for amyloid (Table 3), 8 were Chinese and 1 was Sikh (Indian). They represented 8.9% and 1.4% of Chinese and Indian subjects respectively. There were no amyloid-positive subjects from the Malay and ‘others’ ethnic groups.

**Sex distribution**
There were 4 male and 5 female subjects who were positive for amyloid (Table 3). They represented 2.6% and 10.6% of the subjects in the male and female groups respectively.

**Cause of death**
The causes of death of amyloid-positive subjects are listed in Table 3. None of the cases had physical evidence or an obtainable history of organic neurological disease, mental retardation or dementia.

**DISCUSSION**
Our study showed an increase in prevalence of cerebral β-amyloid deposition with increasing age. Below the age of 50 years, there were no cases exhibiting cerebral β-amyloid deposition.
FIG. 2: Photomicrograph showing cerebral amyloid plaque with central core of fibrillary amyloid deposit displaying apple-green birefringence with a Maltese-cross configuration. Congo red stained section viewed under cross polarised light x100

FIG. 3: Photomicrograph showing amyloid in the media of small vessels of cerebral cortex characterised by typical congophilia and apple-green birefringence under cross-polarised light x500

FIG. 4: Photomicrograph showing blood vessels with amyloid deposits and adjacent cerebral plaques (arrow) exhibiting immunopositivity for β-amyloid protein. PAP method using Ab to β-amyloid protein x500
TABLE 3: Age, sex, ethnic origin, cause of death and histomorphological types of β-amyloid deposition in the nine positive subjects.

<table>
<thead>
<tr>
<th>No</th>
<th>Age (yrs)</th>
<th>Ethnic group</th>
<th>Cause of death</th>
<th>Types of amyloid deposits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>85</td>
<td>Chinese</td>
<td>Hanging</td>
<td>Blood vessels</td>
</tr>
<tr>
<td>2.</td>
<td>82</td>
<td>Chinese</td>
<td>Abdominal and pelvic injuries</td>
<td>Senile plaques</td>
</tr>
<tr>
<td>3.</td>
<td>56</td>
<td>Chinese</td>
<td>Corrosive perforation of stomach</td>
<td>Blood vessels</td>
</tr>
<tr>
<td>4.</td>
<td>68</td>
<td>Sikh</td>
<td>Undetermined</td>
<td>Senile plaques</td>
</tr>
<tr>
<td>5.</td>
<td>75</td>
<td>Chinese</td>
<td>Multiple injuries</td>
<td>Senile plaques and blood vessels</td>
</tr>
<tr>
<td>6.</td>
<td>69</td>
<td>Chinese</td>
<td>Hanging</td>
<td>Senile plaques</td>
</tr>
<tr>
<td>7.</td>
<td>85</td>
<td>Chinese</td>
<td>Hypovolaemic shock due to multiple intraabdominal injuries</td>
<td>Senile plaques</td>
</tr>
<tr>
<td>8.</td>
<td>83</td>
<td>Chinese</td>
<td>Multiple injuries</td>
<td>Senile plaques</td>
</tr>
<tr>
<td>9.</td>
<td>85</td>
<td>Chinese</td>
<td>Multiple injuries</td>
<td>Senile plaques and blood vessels</td>
</tr>
</tbody>
</table>

This was despite the fact that 40% of the study subjects were below the age of 50 years. In contrast, the 80-89 years age group contributed 5 amyloid positive cases (55.6%) despite representing only 6.5% of the study subjects.

No conclusions could be drawn regarding ethnic predilection although there appeared to be more amyloid-positive subjects in the Chinese ethnic group. However, the study was limited by the small numbers of subjects of other ethnicity, especially in the older age groups, where the prevalence of β-amyloid deposition was higher. As a result, we could not draw any statistical significance to the findings of prevalence of β-amyloid deposits in relation to ethnicity.

About 10% of female subjects were positive for β-amyloid while only 2.5% of male subjects were positive. However, we noted that in the younger age groups, females made up only a small proportion of subjects when compared to males but this sex ratio evened out with increasing age (Figure 2). As such, the higher amyloid positive percentage in females is skewed by the smaller numbers of female subjects in the younger age groups. Therefore, it is not possible to conclude if there was any true difference in the prevalence of β-amyloid deposition between the sexes.

The findings of increasing prevalence with increasing age are in keeping with other studies. Mann et al. found 60 non-demented brains found only 1 case with senile amyloid plaques in the brain below the age of 50 years and also observed an increasing prevalence in the older age groups. Davies et al. found all 20 patients who were younger than 50 years of age to be negative for cerebral amyloid. This suggested that detectable β-amyloid deposition are usually found in normal brains only after about 50 years of age.

However, in comparison with most other studies, the prevalence of β-amyloid positivity found in this study is low. Davies et al. found that the prevalence of β-amyloid deposition in the brain to be as high as 80% after the age of eighty years. Ogomori et al. found β-amyloid deposits in 12 out of 20 (60%) non-demented normally aged brains at 50 years of age and above. While we did not attempt to quantify the amount of amyloid present in our study, it is known from other studies that there is also an increased density of β-amyloid deposition with age.⁷,⁸

One of the reasons for the lower prevalence in this study could be related to study design. As this was a retrospective study of autopsy cases, the brains of most of the cases, especially those without neurological injury, were not sampled widely for histology. In addition, the anatomical location of the histological samples taken were not standardised. Many other studies involved screening more than 3 blocks from different anatomical locations of the brain, hence allowing a higher pick-up rate. Another reason for our lower prevalence could be a higher false negative rate due to using the Congo red stain to screen for amyloid in contrast to immunohistochemical or fluorescent (thioflavin) methods.⁹ Although specific for amyloid, Congo red positively is known to be less sensitive than immunostaining. Rumble et al. found that all cases under 40 years old in his study were negative for amyloid by conventional
histological methods (Bodian silver impregnation and Congo red with polarization microscopy) but on using immunocytochemistry, he detected 5 cases in the same group. Our study also has a high proportion of subjects in the younger age group (Table 2). Hence, it is not surprising that this study yielded a lower overall prevalence than other studies in which a larger proportion of the study population was of an older age group. Furthermore, epidemiological studies have shown that there is a lower prevalence rate of dementia in the Asian population, especially in the Chinese ethnic group when compared with the Western population. As the presence of β-amyloid is linked to the prevalence of dementia, the lower prevalence rate in this study could be attributed to true lower prevalence of β-amyloid protein in the brain of Asian population.

Our study could not draw any significant difference in the prevalence of cerebral β-amyloid deposition in the brain between sexes. Other investigators have found no significant difference in the two sexes. To our knowledge, there is no published information on the prevalence of β-amyloid deposition in different ethnic groups. We attempted to study this aspect, taking advantage of Malaysia’s unique multiracial population where the individual ethnic groups are still relatively homogenous. Unfortunately, the imbalance in the distribution of the ethnic groups especially of the older age group in our study prevented any conclusions. Further studies will have to be conducted before any conclusions can be drawn.

The physiological and pathological roles of β-amyloid in the brain have been the subject of numerous studies. β-amyloid is linked to Alzheimer’s disease and is hypothesized to be a major pathogenic factor in the disease. It has been shown that a gene in the long arm of chromosome 21 encodes for β-amyloid precursor protein (BAPP). BAPP has been shown to be ubiquitously expressed physiologically in all the cells of the body, including neurons, astrocytes and endothelial cells and is also found in skin, subcutaneous tissue and intestine. It is metabolised via 2 different pathways, the exocytotic pathway, where a soluble portion of BAPP is released and the endocytotic pathway where a more ‘amyloidogenic’ protein is released. The endocytotic pathway involves the trafficking of part of BAPP including the amyloidogenic portion in an acidic intracellular compartment where further processing releases β-amyloid into the extracellular compartment.

Hence, there is increased release and deposition of β-amyloid with time. Therefore, it is not surprising that there is an increase in β-amyloid deposition with age in normal non-demented individuals as found in our study. In the aging brain, local changes in the pH or the influence of free radicals is believed to create a milieu that favours formation of β-amyloid. In Down’s syndrome, cerebral β-amyloid deposition has been demonstrated at an earlier age than in both Alzheimer’s disease and non-demented aged brains. This is believed to be due to overexpression of the BAPP gene in Trisomy 21 causing increased levels of BAPP and hence β-amyloid, leading to an increased deposition in the brain. However, in Alzheimer’s disease, disordered BAPP metabolism is believed to initiate the neuropathology. A gene in an unknown locus in chromosome 14 appears to have a role in expressing or processing BAPP or metabolism of β-amyloid peptide. An accumulation of β-amyloid due to defective expression appears to be an early and necessary event in the pathogenesis of Alzheimer’s disease. Recently it has been shown that the presence of E4 allele in chromosome 19 increased the risk of developing Alzheimer’s disease.

β-amyloid is deposited in the brain in two forms: senile cortical plaques and cerebrovascular amyloid. In the pathogenesis of senile plaques, BAPP is deposited in synaptic endings and β-amyloid is generated locally by aberrant proteolysis. The earliest form comprises amorphous or diffuse plaques with amyloid only detectable by immunohistochemical techniques. The β-amyloid deposited accumulates slowly over time and soon becomes denser deposits. It is known presently that only aggregated amyloid is neurotoxic. Consequently, they exert their neurotrophic effects on local neurons and their processes, causing swelling and distension of neurites, manifesting morphologically as senile plaques. The close association of microglial cells and astrocytes with the plaques is believed to be a reactive process.

The pathogenesis of cerebrovascular amyloid is slightly different. An increased leakiness of the microvasculature causes circulating β-amyloid precursor protein to pass through the endothelium to be deposited in the media. The mechanism of β-amyloid toxicity depends on the quantity of the protein deposited over time, the physicochemical state and presence of β-amyloid associated proteins such as heparan sulfate, α1-antitrypsin, components of the
classical complement pathway and apolipoprotein E. As described earlier, only aggregated amyloid is neurotoxic. ApoE4 appears to form aggregates with β-amyloid more readily and hence imposes an increased risk of neuronal damage. β-amyloid may also induce the neurotrophic effects directly or enhance the vulnerability of neurons to other common insults. The increased vulnerability is mediated by an increase in intracellular free calcium ions which is induced by β-amyloid protein. As a result, the neurons succumb more readily to other insults which result in toxic elevated calcium levels. Another mechanism proposes that β-amyloid exerts a trophic effect by competitively inhibiting a receptor of serpin protease inhibitor-enzyme complex (SEC). The function of the SEC receptor is to clear extracellular protease. As a result, chronic proteolytic damage to cell membranes and cytoskeleton occurs and plaques result. Another hypothesis is that β-amyloid induces apoptosis. Other mechanisms imply that β-amyloid deposition is not the pathogenic mechanism. Instead the senile plaques are a marker of impaired βAPP function which appears to be neuroprotective and necessary for neuronal survival.

As a result of the neurotrophic effect, there is cholinergic loss in the cerebral cortex. It is known that the cholinergic system is important for memory. Hence there seems to be a selective vulnerability of systems subserving memory, learning and cognition. In addition, there is also decreased or abnormality of noradrenaline, 5-hydroxytryptamine, substance P, neuregulin and somatostatin systems in the cerebral cortex especially in the region of high plaque density though not in the same intensity as the cholinergic system. However, the physiology of the function of these transmitters is unclear.

Huber et al using mice models noted that memory function was impaired when anti-βAPP was injected into the body. They concluded that βAPP may be essential in memory function by stabilising synapses of neuronal processes. They further concluded that in Alzheimer’s disease, instability of synapses lead to decline in cognitive function and massive loss of neurons may represent a reason for the dementia. However, Benzing et al concluded that it is the loss of synapses and not β-amyloid deposition per se, that underlies the dementia. In normal aging, it is presumed that a similar pathophysiological pathway, occurring at a lower level of activity, contributes to memory deterioration.

ACKNOWLEDGMENT
We would like to thank Professor Colin Masters of the University of Melbourne, Australia, for his kind donation of both the antibody against β-amyloid protein and positive control material, and Mr. M.Y.Lim of the University of Malaya for technical assistance.

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