



Importance of β -APP, ADAM9, 10, 17 in Alzheimer Disease: Preliminary Autopsy Study with Immunohistochemical Expression in Human Brain

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ABSTRACT

Alzheimer's disease (AD) is encountered as an important health problem. It was exposed that in the pathophysiology of AD, formation, and aggregation of amyloid β from amyloid precursor protein (APP), was restrained by α -secretase group, ADAM (a disintegrin and metalloproteinase) enzymes. From this perspective, ADAM group of enzymes can be presumably used in the future both as a diagnostic marker, and potential treatment modality. In our study, 9 cases with or without AD in different age groups with various causes of death who were autopsied in the Bursa Morgue Department of the Council of Forensic Medicine of Turkey were included in the study. Tissue samples harvested from temporal regions of the brains of the cases were immunohistochemically stained with β -amyloid precursor protein (APP), ADAM9, ADAM10, and ADAM17. The specimens were evaluated as for distribution, and intensity of staining. The lowest mean distribution score of immunohistochemical staining (2.44) was detected for β -APP, and ADAM 9, while it was 3 for ADAM10. The highest distribution score (3.11) belonged to ADAM17. Our aim was to analyze histochemically cerebral β -APP and ADAM9, ADAM10, ADAM17 expressions in cases with and without a clinical diagnosis of Alzheimer's disease. Based on their staining patterns, we revealed their characteristic features, compared our study results with those of the scarce number of studies in the literature, and despite our limited number of cases, we intended to contribute to the future studies.

Keywords: APP, ADAM, Alzheimer, Immunohistochemical, Expression, Autopsy

INTRODUCTION

In our modern world, with an aging population, Alzheimer's disease (AD) is encountered as an extremely important health problem [1-4]. In AD, β -amyloid ($A\beta$) accumulates in tissues as a result of degradation of amyloid precursor protein (APP) which is a transmembrane glycoprotein [1,5]. The important roles of cell membrane-bound enzymes i.e. disintegrins and metalloproteinase domain-containing proteins (ADAM9, ADAM10, ADAM17) in these processes, APP amyloid cascade, the functioning of pathogenetic processes, and subtypes of amyloid proteins are already known [1,5-7]. In various studies, ADAM group of enzymes are termed as α -group secretases. Contrary to β , and γ -secretases which induce production of amyloid from APP, they prevent production and accumulation of $A\beta$ proteins. Besides, some authors have reported that they antagonized the activities of amyloids via various mechanisms with potential trophic effects on nerve tissue [1,6,8]. In addition, their roles in the reinforcement of intellectual functions have been also claimed [9]. In transgenic animal models, α -secretase effects of ADAM10 have been also demonstrated [7,10]. In cases with AD, marked decreases in the levels of ADAM10, which is known to be especially effective on proteolytic processes involving APP, have been also reported [3]. In this pilot study, our aim was to histochemically analyze cerebral β -APP, ADAM9, ADAM10, ADAM17 expressions in autopsy cases with and without AD, reveal their

characteristic features, and compare our study results with those of the scarce number of studies in the literature. We believe that in the future, enzymes of the ADAM family can be thought of as a diagnostic and therapeutic modality regarding elucidation of AD, and important pathophysiological processes. Within this context, despite our limited number of the patient population, this preliminary study will make useful contributions to prospective studies.

MATERIALS AND METHODS

A total of 9 cases with different causes of death and age groups autopsied in the year 2013 in the Bursa Morgue Department within 8 hours of their death, in whom tissue specimens were retrieved for histopathological analysis were included in the study. The cases included 2 cases with AD, and 7 control cases who belonged to different age groups [age groups in years and the respective number of cases were as follows: 25-35 years (n=1); 35-45 years (n=1); 45-55 years (n=1); 55-65 years (n=1); 65-75 years (n=1); 75-85 years (n=1), and >85 years (n=1)] were analyzed as for their ages, causes of death, and cerebral macroscopic, and microscopic findings. Tissue samples of a total of 9 cases, harvested from temporal region of the brain were fixed in 10% formalin and embedded in paraffin wax. Sections 3-4 μ m thickness were cut and stained with hematoxylin and eosin.

Immunohistochemistry

Following fixation with 10% formaldehyde, the samples were embedded in paraffin blocks and 5 μ m-thickness sections were obtained from the selected paraffin blocks and placed on APTES-coated slides. The sections were deparaffinized in xylene, rehydrated through graded alcohols. Rehydration was followed by incubation with 2% hydrogen peroxide and methanol for 5 min to prevent intrinsic peroxidase activity. After the samples were washed 3 times using PBS (phosphate-buffered saline, pH: 7.4), they were warmed in a microwave oven in 0.1 nM sodium citrate for 10 min and the antigen retrieval procedure was completed. Following the incubation and blocking of the non-immune serum at room temperature for 20 min. Then the preparations were placed on polylysine coated slides and stained for histochemical analysis of β -APP, ADAM9, ADAM10, and ADAM17. The procedures were processed according to the protocols recommended for the anti-beta amyloid antibody, anti-human ADAM9, ADAM10 and ADAM17 antibodies (Abcam). After being deparaffinated at 65°C in the heating chamber and rehydrated, sections were subjected to epitope retrieval in 10X EDTA buffer (pH: 8.0) at 110°C for 30 min. Subsequently, the sections were exposed to 3% H₂O₂ for 20 min to bleach endogenous peroxidases and then rinsed 3 times in PBS for 10 min. Sections were respectively incubated with a rabbit anti-beta amyloid antibody and anti-human ADAM9, ADAM10 and ADAM17 antibodies (all 1:250 in BSA) for 1 hour at 37°C, washed 3 times in PBS and incubated in a biotinylated goat secondary anti-mouse polyclonal antibody (Abcam) for 15 min at 37°C. The specificity of the antibodies was examined by the omission of the primary antibodies. Following rinsing in PBS, the tissues were made visible with 3,3'-diaminobenzidine tetrahydrochloride (DAB chromogen, Abcam) and counterstained with hematoxylin.

Finally, the sections were dehydrated in graded ethanol, immersed in xylene and coverslipped. All preparation was examined microscopically under 40X magnification (Olympus BX53). Regarding extensity, we determined the staining score for each section: 0: if it showed no staining; 1 (1%-25%): occasional staining with most fields negative; 2 (26%-50%): focally abundant staining with most fields having no staining; 3 (51%-75%): focally abundant staining with most fields showing positive staining; or 4 (76%-100%): prominent staining throughout the section. The staining intensity was recorded using a semi-quantitative scoring system: 0: absent; 1: weak staining; 2: accumulations with greater staining intensity; 3: strong and dark staining; 4: very strong and, the darkest observed staining. For all markers, cerebral intraparenchymal, and membrane staining was performed.

RESULTS

Descriptive characteristics of all cases, extensity, and intensity of immunohistochemical staining patterns are summarized in Table 1. The median age of the 7 male, and 2 female patients was 64.8 years (min.=30 years, and max.=89 years). Causes of death of the patients included head trauma (n=3), burn accident, carbon monoxide poisoning (n=1), congenital coronary artery anomaly (n=1), gastric cancer (n=1), heart failure (n=2), acute myocardial infarction (n=1).

Table 1 Descriptive characteristics of the cases (age, gender, cause of death, cerebral macroscopic, and microscopic findings), staining extensity/intensity scores of β -APP, ADAM9,10,17

Cases	Age	Gender	Cause of Death	Histopathology Findings of the Cerebral	Macroscopic Findings of the Cerebral	Staining extensity	Staining intensity						
						β -APP	β -APP	ADAM9	ADAM9	ADAM10	ADAM10	ADAM17	ADAM17
AD Case 1	81	Male	Blunt head trauma, brain hemorrhage	Contusion, subarachnoidal bleeding	Subarachnoidal bleeding	3	2	3	3	4	4	4	3
AD Case 2	89	Male	Blunt head trauma, brain hemorrhage	Subdural, subarachnoidal bleeding	Subarachnoidal intraparenchymal bleeding	3	1	0.2	1	1	1	0	0
Case 3	30	Female	Coronary artery anomaly	Normal	Congestion	3	3	3	2	3	4	3	3
Case 4	41	Male	Heart failure	Normal	Congestion	2	2	3	2	3	2	3	2
Case 5	52	Male	Heart failure	Normal	Congestion	3	2	3	1	3	1	4	4
Case 6	56	Male	Gastric cancer	Normal	Congestion	2	1	2	1	3	2	3	2
Case 7	67	Male	Blunt head trauma, brain hemorrhage	Subdural, subarachnoidal bleeding	Subarachnoidal bleeding, liquefaction necrosis	2	1	2	1	3	2	3	1
Case 8	80	Female	Acute myocardial infarction	Normal	Normal	3	1	2	1	4	3	4	4
Case 9	88	Male	Burning, CO poisoning	Normal	Normal	1	1	2	1	3	1	4	2

All specimens were stained by immunohistochemical methods, and staining scores were determined. Median extensity scores of immunohistochemical staining were estimated as 2.44 for β -APP, and ADAM9 (Figures 1 and 2), 3 for ADAM10 (Figure 3), and the highest, 3.11 for ADAM17.



Figure 1 β -APP immunostaining in postmortem brain parenchyma. Immunostaining for APP shows focal staining of the neuron cells, glial cells, and neutrophils. The vascular endothelium shows strong expression and the vascular wall (original magnification 200X)

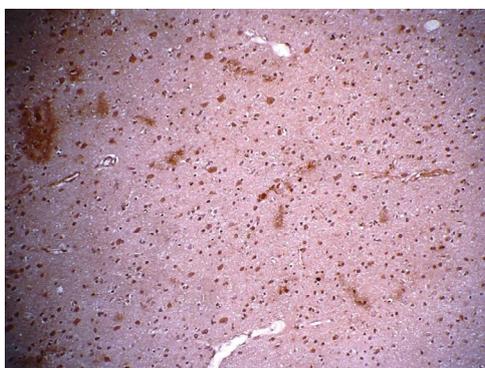


Figure 2 Immunostaining for ADAM 9 in postmortem brain parenchyma. Diffuse and strongly expressed ADAM 9 (+++), cytoplasmic staining of glial cell and neuron cells was noted in the brain tissue (original magnification 200X)

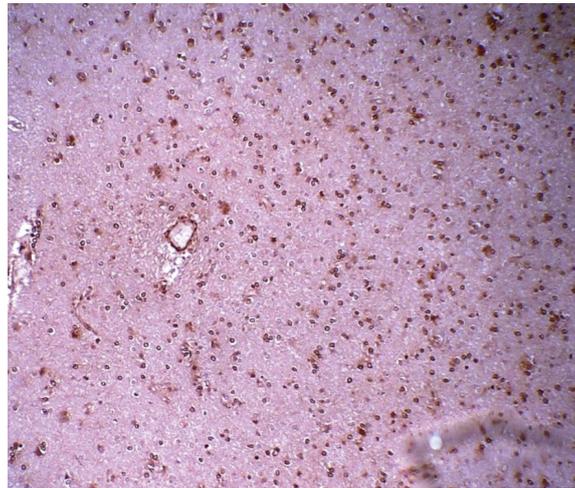


Figure 3 Diffuse staining for ADAM 10 in the brain parenchyma. Immunostaining for ADAM 10 (+++) shows diffuse and strong expression of the glial cells, most likely astrocytes (original magnification 200X)

Intensity scores were also estimated as 1.44 (ADAM9), 1.55 (β -APP), 2.22 (ADAM10), and 2.33 (ADAM17) for respective histochemical markers (Figure 4).

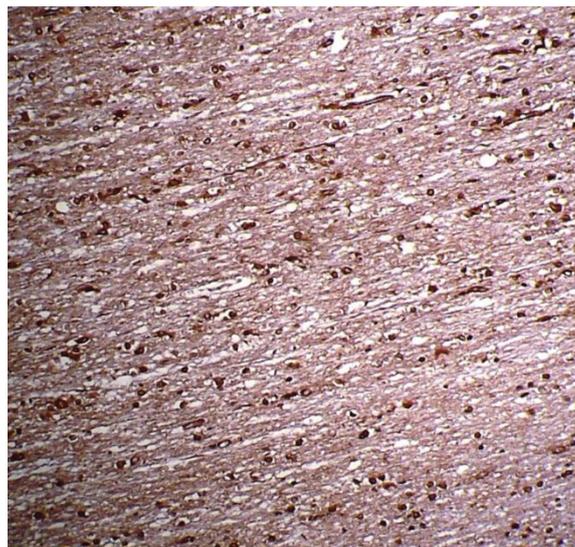


Figure 4 Diffuse and intensive staining for ADAM 17 (++++ of glial cells in a fibrillar background in the brain parenchyma (original magnification 200X)

DISCUSSION

As a transmembrane glycoprotein, APP is known to play roles in diverse biological processes [5]. Among biological processes, different enzymes as α -secretases, which are functional in amyloid cascade including ADAM9, ADAM10 ADAM17, are known to play roles in proteolysis [1,6]. Important roles of these enzymes together with APP in the pathophysiology of neurodegenerative diseases as AD in addition to their critical functions in malignancies and many inflammatory processes have been revealed [4,5]. The researchers detected that in AD, α -secretases exerted proteolytic effects on APP preventing production, and accumulation of A β proteins [1,6,7,11]. Secretase-like effects of ADAM10 on APP were detected [7,10]. Besides, in various studies marked decreases have been reported in the levels of ADAM10, which is especially effective on proteolytic processes involving APP [3,11,12]. Colliaghi, et al., indicated that since in cases with AD, similar comparable changes in platelets, cerebrospinal fluid, and a soluble amyloid precursor protein (APP) alpha (sAPP α) concentrations have been observed, this substance could be used as a marker in cases with AD. However, they also detected that the correlation between peripheral, and central

compartments is extremely important. Additionally, they demonstrated a decrease in the levels of ADAM10 and indicated that ADAM10 plays an important *in vivo* role in the molecular pathogenesis of AD, and asserted that if its levels could be increased then important advances could be achieved in the treatment of AD [3]. Similarly, Bekris, et al., claimed that they had disclosed a small number of amyloid plaques in patients with increased hippocampal ADAM10 concentrations. They also reported that patients with higher sAPP α concentrations in their cerebrospinal fluid had better cognitive scores [11]. In a different study, the authors detected that proteolytic processes effective on blood platelet APP function were similar to those observed in AD brain tissues. In cases with lower ADAM10 levels, higher A β levels were detected contrary to lower APP isoform levels [12]. However in our cases with Alzheimer, extensity scores of immunohistochemical staining for β -APP (3 vs 3), ADAM9 (3 vs 2), ADAM10 (4 vs 1), and ADAM17 (4 vs 0) were detected as indicated in parentheses. Moss, et al., revealed strong correlations between effects of ADAM10, and ADAM9. They emphasized that inhibition of ADAM9 induce degradation of APP with potentially important outcomes for AD [1]. However, in our cases especially with lower intensity scores for ADAM9 expression, relatively higher ADAM10 scores were noted in Table 1. The lowest, and the highest mean staining intensity scores belonged to ADAM9 (1.44), and ADAM10 (2.22), respectively. Besides, in a different study, the authors revealed that ADAM9, and ADAM15 induced release of ADAM10 ectodomain. The authors also disclosed the impact of ADAM9 and ADAM15 in the degradation process of ADAM10 which enabled ADAM10 to function as a signal protein [13]. Prox, et al., revealed that ADAM10 is instrumental for synaptic and neuronal network function in the adult mice brain [14]. In an animal model of AD, it was also demonstrated that overexpression of ADAM10 increased sAPP α concentrations which resulted in the prevention of A β , and its accumulation in plaques, and thus emphasized potentially important roles of α secretases in the treatment of AD [2,4]. However, in our study, extensity scores of staining for β -APP, and ADAM10 were detected as 2.44 and 3, respectively. Zhang, et al., performed a model study with recent immunosuppressive agent rapamycin, and disclosed that rapamycin which inhibits ADAM10, enhanced the formation of A β , and caused a decrease in sAPP α levels. Certain risks of rapamycin use in cases with AD have been proposed [15]. In our investigation highest mean extensity, and intensity expression score (3.11) belonged to ADAM17, which is nearly equal to mean staying extensity expression score for ADAM 10 (3.00). However in an *in vitro* study by Allison, et al., the investigators indicated crucial importance of an α -secretase (ie. ADAM10) in the degradation process of APP, however, ADAM17 could have a minor impact on this process [9]. As demonstrated in the same study, ADAM10 and ADAM17 had no effect on angiotensin-converting enzyme (ACE), which is a transmembrane glycoprotein, which regulates blood pressure [9]. On the other hand, Japanese investigators also detected that ADAM10 and ADAM17 exert their combined impact on the degradation process of a transmembrane protein termed as alcadein, which is co-located in the cell membrane with APP having similar characteristics [8]. In their studies, Lammich, et al., revealed postscriptonal ADAM10 expression from the 5'-UTR region related to the ADAM10 gene, and its relation with A β production from APP [16]. However various studies have demonstrated that variations in ADAM10 gene induced marked changes in the cerebrospinal fluid sAPP α concentrations, and ADAM group enzymes was advocated to be potentially useful genetic markers [17]. As has been revealed in studies performed with blood samples, the researchers indicated that in cases with AD amyloidogenic pathways in platelets were similar to those found in the central nervous system, and they also stated that blood sAPP α and ADAM levels could be used in the treatment and monitorization of patients with AD [3,9,12].

In our study, β -APP, and ADAM9, ADAM10, ADAM17 expressions were evaluated as for their staining extensities, and intensities. The lowest mean extensity score was detected as 2.44 for β -APP, and ADAM9, while the highest score (3.11) belonged to ADAM17. The lowest, and the highest intensity scores belonged to ADAM9 (1.44), and ADAM17 (2.33), respectively. In our cases with Alzheimer's disease, extensity scores were also estimated for β -APP (3 vs 3), ADAM9 (3 vs 2), ADAM10 (4 vs 1), ADAM17 (4 vs 0).

CONCLUSION

Despite a limited number of autopsies performed, we think that our pilot study will make favorable contributions to future studies, and thanks to investigations revealing characteristic features of ADAM group enzymes, diagnostic, and potential treatment modalities of AD will be improved in time at all.

DECLARATIONS**Conflict of Interest**

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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