

A Histochemical Analysis of Neurofibrillary Tangles in Olfactory Epithelium, a Study Based on an Autopsy Case of Juvenile Alzheimer's Disease

Shino Shimizu¹, Ichiro Tojima¹, Keigo Nakamura¹, Hideaki Kouzaki¹, Takeshi Kanetsaka², Norihiro Ogawa², Yoshio Hashizume², Hiroyasu Akatsu³, Akira Hori⁴, Ikuo Tooyama⁵ and Takeshi Shimizu¹

¹Department of Otorhinolaryngology-Head and Neck Surgery, Shiga University of Medical Science, Otsu, Shiga, Japan, ²Choku Medical Institute, Fukushima Hospital, Toyohashi, Aichi, Japan, ³Department of Community-Based Medical Education, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Aichi, Japan, ⁴Center of Pathology, Forensics and Genetics, Hannover Medical School, Hannover, Germany and ⁵Molecular Neuroscience Research Center, Shiga University of Medical Science, Otsu, Shiga, Japan

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The pathological changes of Alzheimer's disease (AD) begin 10–20 years before clinical onset, and it is therefore desirable to identify effective methods for early diagnosis. The nasal mucosa is a target tissue for measuring AD-related biomarkers because the olfactory nerve is the only cranial nerve that is exposed to the external environment. We describe an autopsy case of rapidly advanced juvenile AD (JAD), focusing on the olfactory system. The formation of senile plaques, neurofibrillary tangles (NFTs), and neuropil threads was examined in the temporal cortex, hippocampus, olfactory bulb, and olfactory and respiratory epithelia in the bilateral olfactory clefts. Neurodegenerative changes in the olfactory and respiratory epithelia and the pathological deposition of amyloid β 42 (A β 42) and phosphorylated tau were also examined. As a result, senile plaques, NFTs, and neuropil threads were found in the temporal cortex, hippocampus, and olfactory bulb. NFTs were also found in the olfactory epithelium. Degenerated olfactory cells and their axons stained positive for phosphorylated tau. Supporting cells in the degenerated olfactory epithelium stained positive for A β 42. In conclusion, pathological biomarkers of AD were expressed in the degenerated olfactory epithelium of this JAD patient. This observation suggests that nasal samples may be useful for the diagnosis of AD.

Key words: Alzheimer's disease, olfactory bulb, olfactory epithelium, amyloid β 42, phosphorylated tau

I. Introduction

Dementia is the collective name for a group of progressive brain syndromes that affect memory, thinking, behavior, and emotion. The most well-known form of dementia is Alzheimer's disease (AD), which accounts for

50–60% of all cases. The histopathological hallmarks of AD are senile plaques, neurofibrillary tangles (NFTs), and neuropil threads. Senile plaques are extracellular deposits of amyloid- β (A β) protein, and NFTs are aggregates of abnormally phosphorylated tau protein within the cytoplasm of neurons [4]. Neuropil threads are abnormal neurites containing straight and paired helical filaments with phosphorylated tau protein. Recent advances in AD research have revealed that these pathological changes begin as many as 10–20 years before the clinical onset of AD [3, 8, 14]. Therefore, it is important to identify effec-

Correspondence to: Shino Shimizu, Department of Otorhinolaryngology-Head and Neck Surgery, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga 520–2192, Japan.
E-mail: sshino@belle.shiga-med.ac.jp

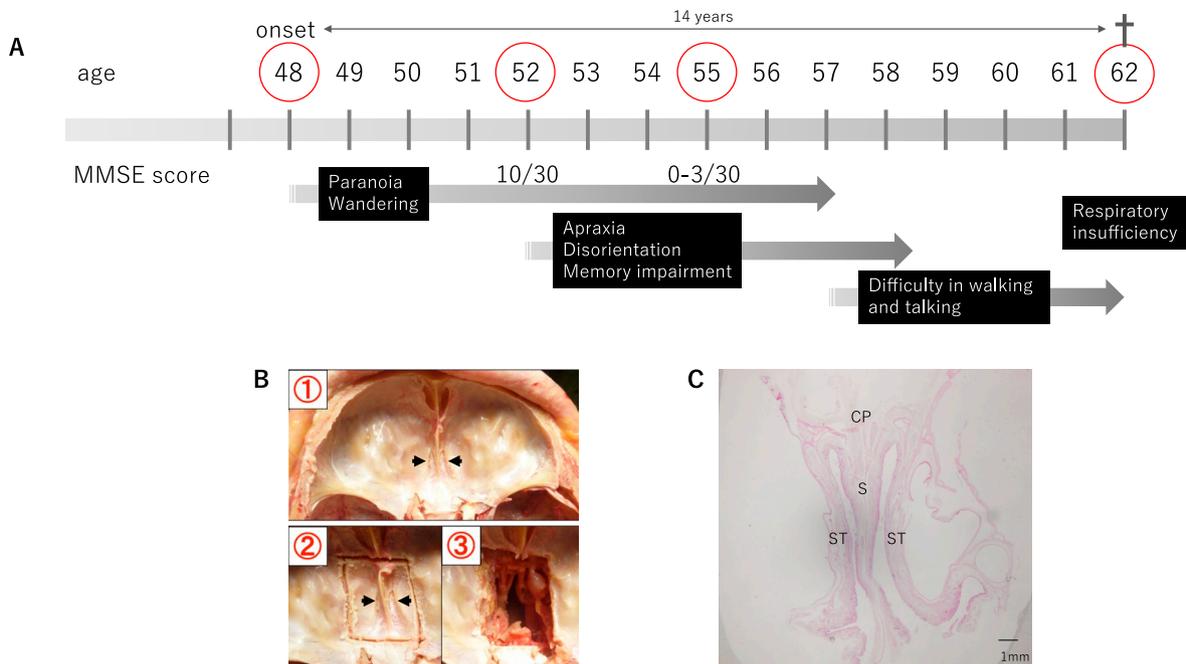


Fig. 1. Clinical course of the JAD patient in the present case (A). Cribriform plate and olfactory clefts (B and C). B: Frontal skull base (1) was cut around the cribriform plate (arrows) (2). Nasal tissue of the bilateral olfactory clefts was removed with the skull base (3). C: Bilateral olfactory clefts stained with HE. S, nasal septum; CP, cribriform plate; ST, superior turbinate. Bar = 1 mm. MMSE, Mini-Mental State Examination.

tive methods for the early diagnosis of AD using not only clinical assessments but also biomarkers [1].

The nasal mucosa is a target tissue for measuring AD-related biomarkers such as A β 42 and phosphorylated tau. Olfactory dysfunction is an early sign of AD, as most patients suffer from anosmia [6]. A recent study found that A β deposition in the olfactory epithelium (OE) can predict mild cognitive impairment and is associated with AD and dementia [5]. The olfactory bulb is often severely affected at an early stage in AD patients [15]. In addition, A β and tau deposits have been found in the OE of AD patients at autopsy [2, 11]. Animal experiments have shown that isotope-labeled A β peptides (^{125}I -A β 40) is transported to the nasal cavity via a non-hematogenous pathway [10]. Deposits of A β peptides in the nasal mucosa in a transgenic mouse model of AD were found to be well correlated with the amount of A β deposited in the brain [9]. These results suggest that the nasal cavity may be a useful site to measure biomarkers enabling the early diagnosis of AD. We recently reported a highly sensitive method for measuring A β 42 and phosphorylated tau in human nasal smears [12].

In the present study, we describe an autopsy case of progressive juvenile AD (JAD). A series of tissues including the temporal cortex, hippocampus, olfactory bulbs, and nasal epithelium were examined by immunohistochemical staining. To examine the clinical usefulness of nasal samples from the OE for the diagnosis of AD, we focused on 1) morphological changes in the olfactory and respiratory epithelia, 2) the distribution of senile plaques, NFTs, and neuropil threads, and 3) expression of the AD-related

biomarkers A β 42 and phosphorylated tau in the bilateral olfactory clefts.

II. Materials and Methods

Clinical course

The clinical course of a Japanese female patient with JAD is shown in Figure 1A. The patient began to wander and exhibit signs of paranoia at the age of 48 years. At the age of 52 years, she first visited Fukushima Hospital, Aichi, Japan, suffering from apraxia, disorientation, and memory disorder. Her Mini-Mental State Examination (MMSE) score was 10/30, and computed tomography scan showed significant brain atrophy. She was clinically diagnosed with AD. Her MMSE scores continued to worsen (8/30 [age 53 years], 3/30 [54 years], and 0–3/30 [55 years]), and she was admitted to the hospital at the age of 55 years. At the age of 57 years, she was unable to walk nor carry on a conversation. At age 61 years, she developed aspiration pneumonia and died of respiratory failure at the age of 62 years.

Brain, olfactory bulb, and nasal epithelium

The patient's brain was removed 1 hr after death and immersed in 10% neutral buffered formalin. The patient's nasal tissue, containing the bilateral olfactory clefts and cribriform plate, was placed in 10% neutral buffered formalin. The nasal tissues were then decalcified with 5% formic acid and 5% formalin in water for 3–4 days. The brain and nasal tissues were then embedded in paraffin. As shown in

Figure 1B, the frontal skull base was cut around the cribriform plate, and nasal tissue of the bilateral olfactory clefts was removed with the skull base. The bilateral olfactory clefts are shown in Figure 1C. For neuropathological analyses, sections were stained with hematoxylin-eosin (HE) and Gallyas-Braak (GB) silver stain.

Immunohistochemistry

Tissue sections were deparaffinized in xylene and rehydrated. After washing with tap water, samples were incubated with formic acid for 5 min at room temperature (RT). After heat-induced antigen retrieval with 99% formic acid, and quenching with 3% hydrogen peroxide, samples were incubated with primary antibody in phosphate-buffered saline (PBS) containing 1% bovine serum albumin overnight at 4°C. The primary antibodies used in this study were mouse monoclonal antibody against phosphorylated tau (AT-8, 1:1,000, Innogenetics, Ghent, Belgium) and rabbit polyclonal antibody against A β 42 (#18582, 1:200, Immuno-Biological Laboratories Co. Ltd.). The next day, samples were incubated for 30 min with Histofine simple stain MAX-PO (MULTI) (Nichirei Co. Ltd., Japan) at RT and then washed with PBS for 5 min, PBS containing 3% Triton-X100 for 5 min, and PBS for 5 min. Peroxidase labeling was colorized with 3,3-diaminobenzidine tetrahydrochloride or 3-amino-9-ethylcarbazole as a chromogen. The immunolabeled sections were counterstained lightly with hematoxylin.

III. Results

Hippocampus and temporal cortex

Numerous A β 42-positive senile plaques were found in the hippocampus and temporal cortex. GB silver staining also revealed NFTs and neuropil threads in the hippocampus and temporal cortex, and these are immunohistochemically positive with phosphorylated tau (Fig. 2A–H).

Olfactory bulb

The glomerulus is a round structure located in the olfactory bulb, in which synapses form between the olfactory nerve terminals and the dendrites of mitral, periglomerular, and tufted cells. A β 42-positive senile plaques were found around the glomerulus. NFTs and neuropil threads were also found and stained positive for phosphorylated tau and GB silver (Fig. 2I–L). The number and amount of senile plaques, NFTs, and neuropil threads in the olfactory bulb were lower than those in the hippocampus and temporal cortex.

Olfactory and respiratory epithelia in the olfactory clefts

The cranial area of the bilateral olfactory clefts was covered by OE, and the transition area between the olfactory and respiratory epithelia was observed in the inferior part of the nasal septum and superior turbinate. The OE was composed of three distinct cell types: basal cells, olfac-

tory cells, and supporting cells. The structure of the OE in this JAD patient exhibited notable changes. Representative photographs of typical OE, OE with fibrous changes, and OE with few olfactory cells are shown in Figure 3. Typical OE was observed mostly in the cranial area of the olfactory cleft. Olfactory cells were present in two to four layers on the basal cells, and supporting cells with round nuclei were located in the outermost layer of the OE (Fig. 3A). OE with fibrous changes was found mostly in the septum area, where the layer structure of the olfactory cells was disturbed by fibrous structures (Fig. 3B). The number of olfactory cells was decreased, and some olfactory cells were located on the lumen side of the supporting cells. OE with few olfactory cells was mostly observed in the superior turbinate, where there were very few olfactory cells and the OE was composed of basal cells and supporting cells (Fig. 3C).

In typical OE, the cytoplasm of supporting cells stained faintly positive for A β 42 (Fig. 3D). However, immunostaining and GB silver staining did not reveal any senile plaques, NFTs, or neuropil threads (Fig. 3D, G, J). In OE with fibrous changes, fibrous structures were formed above the basal layer; this fibrous structure was thought to be NFTs because it stained strongly positive for phosphorylated tau and partially positive by GB silver staining (Fig. 3H, K). Some degenerated olfactory cells and their axons in the lamina propria of this epithelium stained positive for phosphorylated tau (Fig. 4A–C). Olfactory fascicles also stained partially positive for phosphorylated tau (Fig. 4D). The cytoplasm of supporting cells stained faintly positive for A β 42 (Fig. 3E). In the OE with few olfactory cells, the cytoplasm of supporting cells stained positive for A β 42 (Fig. 3F). No olfactory cells were present, and the OE was negative in phosphorylated tau and GB silver staining (Fig. 3I, L).

The respiratory epithelium, composed of basal cells, secretory cells, and ciliated cells, was observed in the inferior part of the nasal septum and superior turbinate. The neurodegenerative changes did not extend into this epithelium, and neither A β 42 nor phosphorylated tau was detected (data not shown).

IV. Discussion

Olfactory dysfunction is an early and common symptom of AD [5], and neuropathological changes such as deposition of A β 42 and phosphorylated tau are detected in the OE. Because the olfactory cells are part of the only cranial nerve that is exposed to the external environment, various methods for the early diagnosis of AD using nasal specimens such as biopsy material, discharges, smears, and lavage fluids have been proposed [2, 9, 12, 15]. In our previous study, we developed a highly sensitive method for the measurement of A β 42 and phosphorylated tau using nasal smears and found that the ratio of phosphorylated tau to total tau is significantly higher in AD patients than healthy

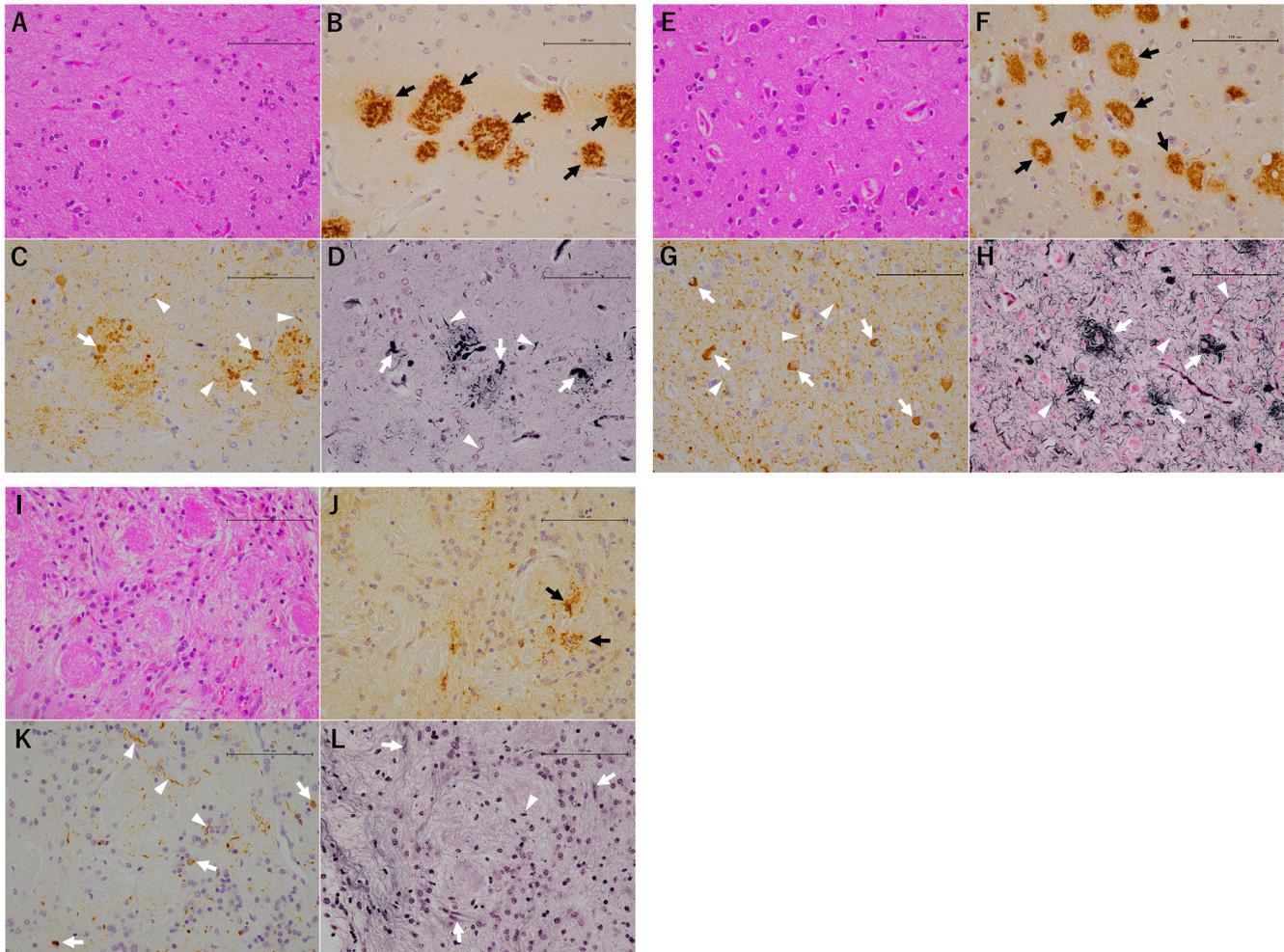


Fig. 2. Microscopic images of the hippocampus (A–D), temporal cortex (E–H), olfactory bulb (I–L). A, E, and I, HE staining; B, F, and J, A β 42 immunostaining; C, G, and K, phosphorylated tau (AT8) immunostaining; D, H, and L, GB silver staining. A β 42-positive senile plaques (black arrows) were found in the hippocampus (B) and temporal cortex (F). NFTs (white arrows) and neuropil threads (white arrowheads) were also revealed in the hippocampus and temporal cortex by GB silver staining (D, H), and these threads stained positive for phosphorylated tau by immunohistochemistry (C, G). In the olfactory bulb, the glomerulus is the round structure in which synapses form between the olfactory nerve terminals and the dendrites of mitral, periglomerular, and tufted cells (I). A β 42-positive senile plaques (black arrows) were found around the glomeruli (J). NFTs (white arrows) and neuropil threads (white arrowheads) exhibited positive phosphorylated tau and GB silver staining (K, L). Bar = 100 μ m.

controls [12]. However, the sources of these AD-related biomarkers in nasal smears have not been clearly identified. In the present autopsy case of a JAD patient, we examined the neurodegenerative changes in the olfactory and respiratory epithelia and the pathological deposition of A β 42 and phosphorylated tau in the bilateral olfactory clefts. Nasal tissue was removed with cribriform plate, and coronary section was used to examine all of the olfactory clefts.

Morphologically, the structure of the OE was markedly altered, and neurodegenerative changes were observed at different levels. Normal OE was observed primarily in the cranial area of the olfactory clefts. NFTs and degenerated olfactory cells positive for phosphorylated tau were observed primarily in the septum area. Axons of olfactory neurons and olfactory fascicles in the lamina propria under this epithelium stained positive for phosphory-

lated tau. These findings agree with those of the previous report showing that phosphorylated tau is particularly abundant in NFTs and dystrophic neurites coursing through the lamina propria of the OE [2]. These results indicate that olfactory dysfunction in AD patients may be caused in part by neurodegenerative changes in olfactory neurons in the OE, although the olfactory bulb and olfaction-related cerebral cortex are also impaired. Phosphorylated tau was not detected in OE exhibiting advanced neurodegenerative changes such as the disappearance of olfactory cells. However, A β 42 was detected in the cytoplasm of supporting cells in this epithelium. It has been reported that supporting cells exhibit phagocytic activity that functions in removing apoptotic olfactory neurons [16, 17]. In addition, the number of phagocytic supporting cells is increased after bulbectomy in newborn mice [16]. Cytoplasmic aggrega-

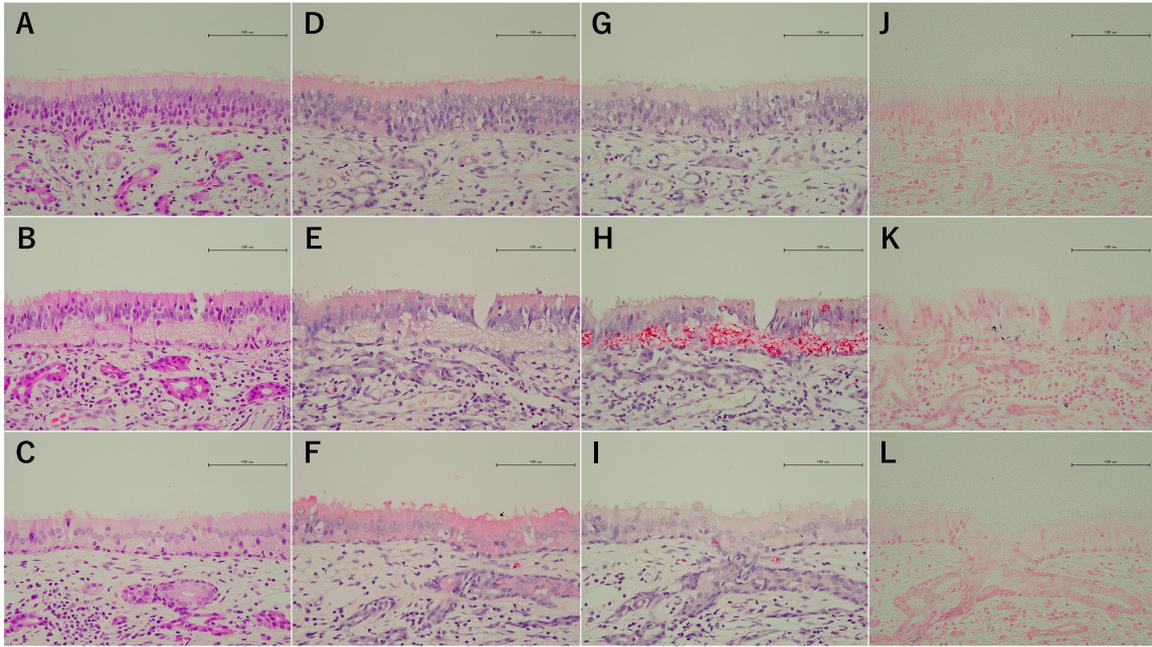


Fig. 3. Representative microscopic images of the OE; typical OE (A, D, G, J), OE with fibrous changes (B, E, H, K), and OE with few olfactory cells (C, F, I, L). HE staining (A, B, C), amyloid β 42 immunostaining (D, E, F), phosphorylated tau (AT8) immunostaining (G, H, I), and GB silver staining (J, K, L). Bar = 100 μ m.

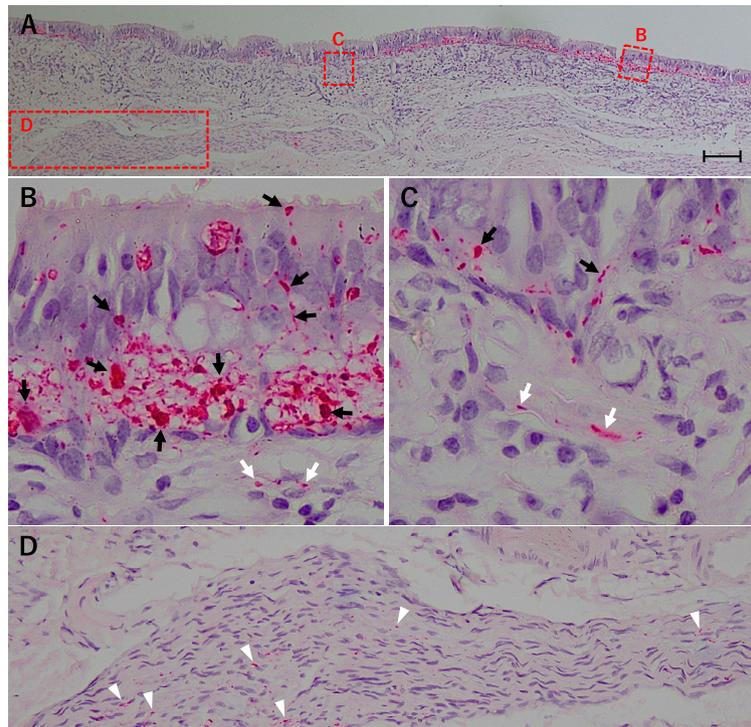


Fig. 4. Microscopic images of OE with fibrous changes in the septum area of the olfactory clefts, immunostained for phosphorylated tau (AT8) (A). Some degenerated olfactory cells (black arrows) and their axons (white arrows) in the lamina propria of this epithelium stained positive for phosphorylated tau. Olfactory fascicles also stained partially positive for phosphorylated tau (white arrowheads) (D). Bar = 100 μ m.

tion of A β 42 in the supporting cells may thus be related to the phagocytosis of degenerated olfactory neurons.

AD is known to target the hippocampus and temporal cortex, which are involved in storing and retrieving new information, and targeting of this area is responsible for the first symptom of memory impairment. The accumulation of A β 42 in senile plaque and phosphorylated tau in the cytoplasm of NFTs and neuropil threads was confirmed in the hippocampus and temporal cortex, and these changes were also observed in the olfactory bulb. However, no senile plaques were found in the OE. Senile plaques, extracellular depositions of A β protein, were not found in the olfactory glomerulus of the olfactory bulb, in which olfactory neurons form synapses. Olfactory neurons regenerate continuously, and extracellular A β protein may be eliminated with degenerated neurons via phagocytosis by macrophages or supporting cells in the OE.

Biopsies of OE tissue could potentially be used for the early diagnosis of AD. It is crucial to obtain the OE, as in the present case neurodegenerative changes did not extend to the adjacent respiratory epithelium, where no A β 42 or phosphorylated tau were detected. However, it is difficult to obtain human OE *in vivo*. The success rates of OE biopsy ranges from 16 to 100% depending on the technique used and region targeted [7]. Autopsy data indicate that OE is distributed throughout approximately 30% of the olfactory cleft and gradually replaced by respiratory epithelium with aging. When tissue is taken at the nasal septum 8 mm below the cribriform plate, 61% of tissues of individuals up to the age of 60 years have olfactory neurons, but only 31% of tissues of individuals over the age of 60 years have olfactory neurons [13]. Thus, more effective and practical methods for the early diagnosis of AD using nasal specimens are needed.

In conclusion, the present autopsy case demonstrated for the first time that varying neurodegenerative changes occur in the OE in the olfactory clefts in patients with JAD. The OE thus has the potential to provide AD-related biomarkers useful in the diagnosis of AD. The present case involved progressive JAD, and therefore, the neurodegenerative changes and deposition of biomarkers in the OE during the early stages of AD will be examined in future studies.

V. Conflicts of Interest

The authors declare that there are no conflicts of interest.

VI. Acknowledgments

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