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Quantification of immunohistochemical findings of neurofibrillary tangles and senile plaques for a diagnosis of dementia in forensic autopsy cases

Mio Takayama, Masayuki Kashiwagi, Aya Matsusue, Brian Waters, Kenji Hara, Natsuki Ikematsu, Shin-ichi Kubo *

Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, Japan

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ABSTRACT

We report the quantification of immunohistochemical findings for a diagnosis of dementia in autopsy cases among older decedents. Autopsy cases were selected with the following requirements: >65 yo; no head injuries, thermal injuries, or heat stroke; no intracranial lesions; and within 48 h of death. Among cases that met all requirements, 10 had a clinical diagnosis of dementia were included in dementia group. Non-dementia group consisted of 38 cases without any record of dementia. To compare these groups, immunohistochemically, beta-amyloid, tau protein, gephyrin, and IL-33 were examined in five regions. Quantitative analysis was performed by collecting with image data analyzed using analysis software. Image data on tau-immunopositive neurofibrillary tangles (NFT) and beta-amyloid-positive senile plaques (SP) were photographed. Criteria for dementia were made by counting and measuring NFT and SP from image data using software. Differences in SP and NFT were effective for discriminating between the two groups. These criteria may reveal the presence and progression of dementia. Total of tau-positive NFT in Ammon's horn (AH) may be useful for diagnosing dementia. When the total is more than 41 in approximately 6 mm^2 of AH, the possibility of dementia is considered. Total of beta-amyloid-positive SP in the parahippocampal gyrus (PHG) may be useful for diagnosing dementia. When the total in approximately 5 mm^2 of PHG is more than 47, the possibility of dementia is considered. Immunohistochemical staining may be more useful for obtaining image data for quantification than conventional staining techniques, such as Bielschowsky-Hirano's silver staining.

1. Introduction

The diagnosis of dementia in forensic autopsy cases has become more important as Japanese society progressively ages. In forensic autopsy cases of elderly decedents where the circumstances leading to their deaths are difficult to assess, such as driving the wrong way on the expressway, walking aimlessly into busy traffic, or accidental deaths in places far from home, there is a possibility that dementia could be an underlying cause. It is extremely important to diagnose dementia in autopsy diagnoses, not only as direct or underlying causes of death, but also for determining relevant and important background information. These diagnoses could also help in the prevention of the untimely death of elderly people currently suffering from dementia.

We previously attempted to quantify neuropathological findings using image analysis software for the diagnosis of dementia in deceased individuals who underwent forensic autopsies [1]. Among autopsies performed within 48 h of death and excluding those with head injuries, thermal injuries, heat stroke, and intracranial lesions, 8 autopsy cases were clinically diagnosed with dementia, and, thus, were included in the dementia group (group D). The non-dementia group (group non-D) consisted of 6 deceased cases without dementia. In order to compare groups D and non-D, 6 regions and 7 types of pathological findings were examined semi-quantitatively using 4 conventional stains. A semi-quantitative analysis of senile plaques (SP) and neurofibrillary tangles (NFT) was performed with Bielschowsky-Hirano's (BH) silver staining, which showed differences between groups D and non-D. A quantitative analysis of the SP and NFT image data collected was performed using image analysis software. An easy, simple, and effective quantification method

* Corresponding author at: Department of Forensic Medicine, Faculty of Medicine, Fukuoka University, 7-45-1 Nanakuma, Jonan-ku, Fukuoka 814-0180, Japan.
E-mail address: kuboshin@fukuoka-u.ac.jp (S.-i. Kubo).

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of the pathological findings obtained was achieved. However, no significant differences were observed between the two groups, and a diagnosis of dementia based on the quantification of pathological findings was not successful. A diagnosis of dementia using image data may be possible in future studies with a larger number of autopsies and by utilizing staining techniques with higher specificity and sensitivity, such as immunohistochemical staining.

Therefore, the aim of the present study is to develop of a convenient, prompt retrieval method for dementia that uses immunohistochemical staining instead of silver staining. Moreover, the quantification of pathological findings, such as SP and NFT using image analysis software was attempted in order to diagnose dementia.

2. Materials and methods

2.1. Cases, group D, and group non-D

Of the autopsy cases at Fukuoka University between 2008 and 2015, 512 cases were selected according to the following requirements: 1) 65 years old or older; 2) no head injury, thermal injury, or heat stroke; 3) no intracranial lesions; and 4) within 48 h of death.

Among approximately 48 cases that met all of the 4 requirements, 10 had been clinically diagnosed with dementia in a police investigation prior to their death. These cases were included in group D. Group non-D consisted of the 38 other cases that died without any record of a dementia episode.

The 10 cases in group D had been clinically diagnosed with dementia before autopsy. In the present study, we investigated the clinical diagnosis of dementia in these cases in more detail. Two cases were diagnosed with Alzheimer's disease (AD), while 2 other cases were diagnosed with moderate or severe dementia; however, the type was not diagnosed. One case was diagnosed with mild cognitive impairment approximately one year before death. It was not possible to contact the family members of the remaining 5 cases.

In Japan, of the illnesses causing dementia, Alzheimer's disease (AD) was the most frequent (about 68%), followed by vascular dementia (VaD) (about 19%), dementia with Lewy body disease and Parkinson's disease with dementia (DLB/PDD) (about 6%), and mixed dementia and other illnesses. AD, VaD, and DLB/PDD were the cause of more than 90% of dementia cases [2–4].

In our study, to compare groups D and non-D, 6 regions and 7 types of pathological findings were examined semi-quantitatively using 4 conventional stains. The following 6 regions were collected from formalin-fixed forensic autopsy brains: 1) the middle frontal gyrus (MFG), 2) superior and middle temporal gyri (SMTG), 3) Ammon's horn (AH), 4) parahippocampal gyrus (PHG), 5) inferior parietal lobule (IPL), and 6) midbrain (MB) These regions were selected based on previous findings by H. Braak et al. [5] and S.S. Mirra et al. [6]. The following pathological findings were observed in our previous studies: 1) SP, 2) NFT, 3) amyloid bodies (AB), 4) amyloid angiopathy (AA), 5) granulovacuolar degeneration (GVD), 6) lipofuscin deposits (LD), and 7) neuronal inclusion bodies (NIB). Stainings used in the present study were: 1) Hematoxylin-eosin (HE); 2) Luxol fast blue (LFB); 3) Congo red (CR); and 4) BH silver staining [7].

Lacuna necrosis and AA found in VaD were scarcely observed in either group. NIB found in DLB were also scarcely found, and neuronal degeneration in substantia nigra observed in PDD were not obvious in MD. Therefore, our presented group D was considered as suspected AD cases. So, based on those neuropathological findings, we compared groups D and non-D.

The cases examined were summarized in Table 1. No significant differences were observed, except age ($p < 0.01$), between the two groups.

2.2. Neuropathological examinations

In order to compare groups D and non-D, 5 regions and 2 pathological findings were examined using immunohistochemical staining.

2.2.1. Regions

The following 5 regions, within the 6 regions described before, were collected from formalin-fixed forensic autopsy brains; 1) the middle frontal gyrus (MFG), 2) superior and middle temporal gyri (SMTG), 3) Ammon's horn (AH), 4) parahippocampal gyrus (PHG), and 5) inferior parietal lobule (IPL) (Fig. 1). These regions were selected based on previous findings by H. Braak et al. [5] and S.S. Mirra et al. [6]. Based on our previous findings [1], the midbrain was excluded.

2.2.2. Pathological findings

The following pathological findings were observed in our previous studies: 1) SP, 2) NFT, 3) AB, 4) AA, 5) GVD, 6) LD, and 7) NIB [8,9]. These finding were observed by using 4 conventional stains: 1) HE; 2) LFB; 3) CR; and 4) BH silver staining [7].

Based on our previous study [1], SP and NFT were herein adopted from these findings.

2.2.3. Immunohistochemical staining

Tau protein [10,11], beta-amyloid protein [12,13], gephyrin [14], and IL-33 [15] were observed immunohistochemically because they are AD-related proteins. Immunostaining was performed using antibodies against tau (1: 300, Dako, Japan), beta-amyloid (1: 50, Dako, Japan), gephyrin (1: 250, Abcam plc, UK), and IL-33 (1: 100, Abcam plc, UK) with the EnVision™ Detection System/HRP (Dako, Japan) according to the manufacturer's instructions.

The 4 kinds of conventional stains, as described before, were also applied.

2.3. Quantitative analysis of SP and NFT

A quantitative analysis was performed by collecting image data using ProScan™ Stage (Nikon, Japan), which were analyzed by the image analysis software WinRoof™ (Mitani Co., Japan) [16,17]. Within each region, images were taken of 5 different locations (6 locations in AH). Four images taken by a ×20 objective lens were integrated into one image, with a 15% overlap, using the image integration software NIC-Elements™ (Nikon, Japan). One integrated

Table 1
Summary of groups D and non-D.

	Group D	Group non-D
Number of cases	10	38
Gender (male:female)	2:8	14:24
Age (mean ± SD)	85.0 ± 6.5	75.4 ± 7.5
Brain weight (g) (mean ± SD)	1171.4 ± 138.0	1255.5 ± 143.9
Natural cause of death		
Cardiac disease	3	7
Infection	1	4
Others		
Rectal cancer	0	1
Unknown natural death	0	1
Other causes of death		
Bleeding	0	7
Traumatic shock	0	4
Asphyxia	3	3
Drowning	2	9
Others		
Anaphylaxes	0	1
Hypothermia	1	1