ORIGINAL

Quantification of neuropathological findings by image data for the diagnosis of dementia in forensic autopsy cases

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Abstract : The aim of the present study was to quantify neuropathological findings using image analysis software for the diagnosis of dementia in deceased who underwent forensic autopsy. Of the autopsies performed within 48 hours of death and excluding those of patients with head injury, thermal injury, heat stroke, or intracranial lesions, 8 were of autopsy cases clinically diagnosed with dementia and thus included in the dementia group (D). The non-dementia group (non-D) consisted of 6 deceased without dementia. To compare the D and non-D groups, 6 regions and 7 types of pathological findings were observed semi-quantitatively using 4 conventional stainings. Quantitative analysis of collected image data was performed using image analysis software. Semiquantitative analysis of senile plaques and neurofibrillary tangles was performed with Bielschowsky-Hirano's silver staining image data. An easy, simple, and effective quantification method of the pathological findings was achieved. However, no significant differences were observed between the two groups, and diagnosis of dementia by the quantification of pathological findings was not successful. Diagnosis of dementia using image data may be possible in future studies with an increased number of autopsies, and by utilizing staining techniques with higher specificity and sensitivity, such as immunohistochemical staining. J. Med. Invest. 63 : 114-118, February, 2016

Keywords **:** *dementia, senile plaque, neurofibrillary tangle, pathological diagnosis, quantification of neuropathological findings, forensic autopsy*

1. INTRODUCTION

The number of forensic autopsies of the deceased elderly is rapidly increasing as the Japanese society ages. Presence of dementia is often suspected when evaluating the death of elderly people. Therefore, diagnosis of dementia has become increasingly important in forensic autopsies. Previously, we reported that the number or ratio of occupied area of senile plaques in the hippocampus and occipital cortex in a group of dementia subjects was significantly higher than that of the non-dementia group (1). Recently, Alzheimer's disease has become the most common cause of dementia in Japan, while lacunar cerebral infarction has decreased (2-5).

The aim of the present study was to quantify pathological findings for the diagnosis of dementia using image analysis software.

2. Materials and Methods :

2.1. Dementia and non-dementia groups

Autopsies of patients performed at Fukuoka University fulfilling the following requirements were included in the present study : 1) no head injury, thermal injury, or heat stroke ; 2) no intracranial lesions ; and 3) within 48 hours of death.

Of the deceased who met all 3 requirements, 8 had a clinical diagnosis of dementia before death (group D). The non-dementia group (group non-D), whose age and sex were matched to group D, consisted of 6 patients who died without record of having an episode of dementia. There were no significant differences between the two groups, including factors such as brain weight (Table 1).

Table 1 Summary of groups D and non-D

	Group D	Group non-D
Number of patients	8	6
Gender (male : female)	2 : 6	2 : 4
Age (mean±SD)	85.5±6.0	84.5±3.1
Brain weight (mean±SD)	1156.8±147.8	1269.5±104.5
Cause of death		
Cardiac disease	2	1
Infection	1	0
Hypothermia	0	1
Bleeding	1	1
Asphyxia	2	1
Drowning	2	2

2.2. Neuropathological examinations

To compare groups D and non-D, 6 brain regions and 7 types of pathological findings were observed using 4 conventional stainings in formalin-fixed brains. The 6 regions were as follows : 1) 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) (Fig. 1). These regions were chosen based on reports by Braak *et al.* and Mirra *et al.* (6, 7). Pathological findings included 1) senile plaques (SP), 2) neurofibrillary tangles (NFT), 3) amyloid bodies (AB), 4) amyloid angiopathy (AA), 5) granulovacuolar degeneration (GVD), 6) lipofuscin deposition (LD), and 7) neuronal inclusion bodies (NIB) (Fig. 2) (8, 9).

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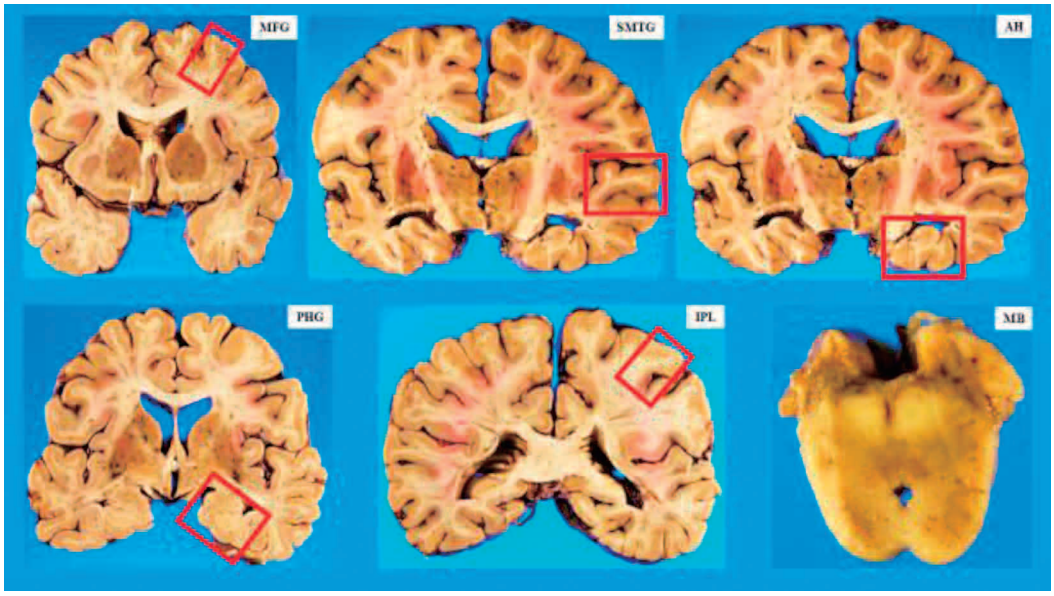

Fig. 1 Regions observed for pathological investigation

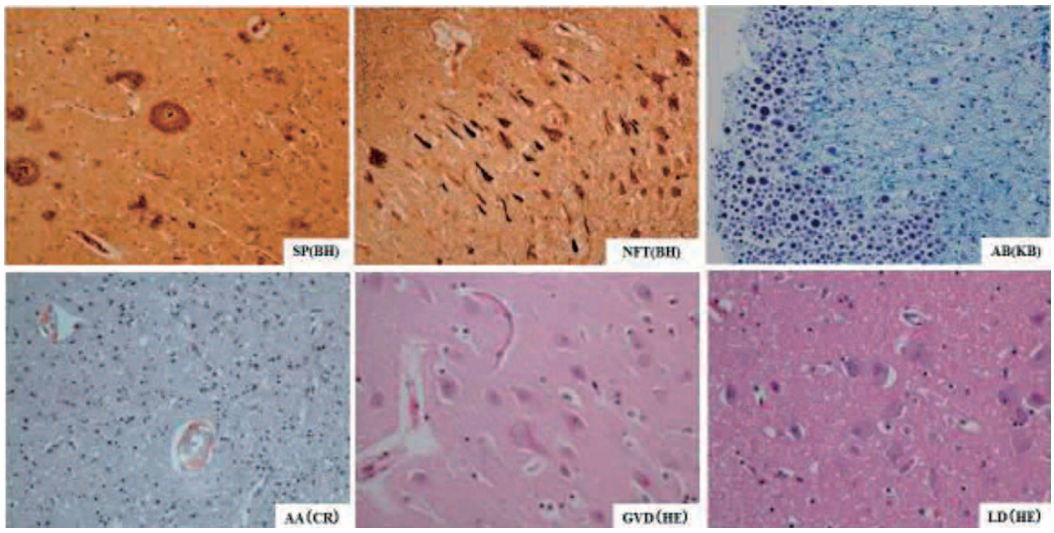

Fig. 2 Pathological findings

Stainings used in the present study were : 1) Hematoxylin-eosin (HE) ; 2) Luxol fast blue (LFB) ; 3) Congo red (CR) ; and 4) Bielschowsky-Hirano's silver staining (BH silver) (10).

2.3. Evaluation of pathological findings

2.3.1. Semi-quantitative analysis

To compare groups D and non-D, the pathological findings were observed separately by two pathologists, and were scored one a scale of four grades :-, +, 2+, or 3+.

2.3.2. Quantitative analysis

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) (11, 12). 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 image was 1.16 mm×0.87 mm in size. All obtained images were processed using the same analytical parameters, such as size and degree of pathological change. The number, gross area, and individual area of each pathological change were quantitatively measured.

2.4. Comparison of groups D and non-D

The distribution of pathological changes observed in groups D and non-D were illustrated as box plots. Analysis of correlation between groups D and non-D was performed using Student's *t*-test. Statistical analyses were performed with the JMP 11.2 software program (SAS Institute, USA). P-values of less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

The 8 cases in group D had been clinically diagnosed as dementia by police investigation before autopsy. In this study, we further investigated the clinical diagnosis of dementia of these cases.

One case was diagnosed as Alzheimer's disease (AD). Two other cases were diagnosed as moderate or severe dementia, but the type was not diagnosed. One case was diagnosed as mild cognitive impairment, about one year before death. The family members of the remaining 4 cases could not be contacted. All 8 cases showed many SP and NFT, and from other pathological findings in the brain they were diagnosed as AD instead of dementia with Lewy bodies and vascular dementia.

3.1. Semi-quantitative analysis

The pathological findings observed in each group are summarized in Table 2 ; due to no observation in either group, GVD and NIB were excluded. Furthermore, due to low frequency of observation, AA, GVD, and NIB could not be compared between groups D and non-D. Although the frequency of observation was high for AB and LD, there were no significant differences between the two groups, suggesting the low specificity of the findings from AB and LD in differentiating the D and non-D groups.

SP and NFT are often used for the pathological diagnosis of Alzheimer's disease. Because occurrence of NFT in areas other than AH and PHG was low in the present study, only AH and PHG were concluded suitable for investigation of NFT presence. On the other hand, other than in MB, SP was frequently observed in all regions ; thus all regions except for MB were considered suitable for investigation of SP.

3.2. Quantitative analysis by image data

From the above-mentioned results, we quantitatively analyzed NFT in AH and PHG, and SP in MFG, SMTG, AH, PHG, and IPL. The total number of NFT in each image was measured, as well as the total number, gross area, and individual area (total gross area/total number) of SP.

NFT and SP data obtained from BH stain images in groups D and non-D are summarized in Table 3 and Fig. 3.

The total numbers of NFT observed in group D, in both AH and PHG, were greater than those of group non-D ; however, these differences were not significant. The p-value of the total number of NFT in AH, $p=0.1633$, was smaller than that in PHG, $p=0.8357$.

Except for individual area, SP parameters were greater in each region of group D compared with those of group non-D. However, there were no significant differences in SP total number, gross area, or individual in each region between the groups. The smallest p-value was observed in the total number of SP in MGF ($p=0.0772$).

In the presented study, we quantified NFT and SP pathological findings using BH staining. One advantage of using image analysis software is that it was relatively easy to use for quantification due to the high contrast of the BH silver staining. However, limitations of such software include the possibility of non-specific staining introducing artifacts that may affect the identification of NFT and SP pathological findings. During image data analysis, the software automatically included non-specific staining ; thus we had to manually remove false-positive findings. However, the image data analysis did not identify any significant differences in findings between groups D and non-D, and we were unable to diagnose dementia by quantification of the pathological findings. Because the presence of false-positives may have affected the results, future studies

Table 2 Semi-quantitative analysis of pathological findings

		SP	(%)	NFT	(%)	AB	(%)	AA	(%)	LD	(%)
Region	Grade	D	non-D	D	non-D	D	non-D	D	non-D	D	non-D
MFG	3+	62.5	33.3	0	0	12.5	0	12.5	0	37.5	33.3
	2+	12.5	33.3	0	16.7	12.5	0	0	0	37.5	50
	+	12.5	0	87.5	0	62.5	100	12.5	16.7	25	16.7
	-	12.5	33.3	12.5	83.3	12.5	0	75	83.3	0	0
SMTG	3+	50	50	0	0	12.5	0	0	0	37.5	16.7
	2+	25	16.7	37.5	0	25	50	12.5	0	25	66.6
	+	0	0	37.5	33.3	62.5	50	25	0	25	16.7
	-	25	33.3	25	66.6	0	0	62.5	100	12.5	0
AH	3+	0	33.3	75	0	37.5	33.3	0	0	0	83.3
	2+	50	33.3	25	83.3	37.5	66.6	0	0	50	16.7
	+	37.5	0	0	16.7	25	0	0	0	12.5	0
	-	12.5	33.3	0	0	0	0	100	100	37.5	0
PHG	3+	75	66.6	75	16.7	25	66.6	0	0	25	50
	2+	25	0	0	66.6	50	16.7	0	0	37.5	16.7
	+	0	0	25	0	12.5	16.7	25	0	25	33.3
	-	0	33.3	0	16.7	12.5	0	75	100	12.5	0
IPL	3+	75	50	0	0	12.5	0	12.5	0	37.5	33.3
	2+	12.5	33.3	0	0	0	50	12.5	0	25	50
	+	0	0	62.5	50	87.5	50	12.5	0	25	16.7
	-	12.5	16.7	37.5	50	0	0	62.5	100	12.5	0
MB	3+	0	0	0	0	12.5	0	0	0	0	50
	2+	12.5	0	0	0	25	50	0	0	37.5	50
	+	0	0	0	0	50	50	0	0	50	0
	-	87.5	100	100	100	12.5	0	100	100	12.5	0

Table 3 Comparison of NFT and SP image data using BH stain

Findings	Regions	Parameters	Group D	Group non-D	p-value
NFT	AH	Total number	75.9±43.5	46.7±23.1	0.1633
	PHG	Total number	84.5±73.8	77.2±47.2	0.8357
SP	MFG	Total number	148.3±111.2	52.7±53.1	0.0772
		Gross area	72308.8±75266.6	26146.7±23883.8	0.1765
		Individual area	365.7±346.7	370.6±329.0	0.9789
	SMTG	Total number	103.8±92.8	47.3±63.0	0.2254
		Gross area	70592.7±101845.7	30330.9±32414.6	0.3729
		Individual area	412.8±373.2	533.5±204.7	0.6138
	AH	Total number	25.0±28.7	12.8±16.7	0.3739
		Gross area	19490.5±22923.9	22523.1±37707.7	0.8546
		Individual area	620.7±465.8	808.8±890.1	0.6157
	PHG	Total number	111.3±78.9	50.7±48.6	0.1244
		Gross area	65929.5±50892.9	43092.7±44688.6	0.3995
		Individual area	567.4±320.4	561.7±503.8	0.9798
	IPL	Total number	139.4±97.6	84.0±131.8	0.3825
		Gross area	53271.8±36971.8	39765.2±41554.4	0.5329
		Individual area	299.0±204.7	484.9±460.3	0.3255

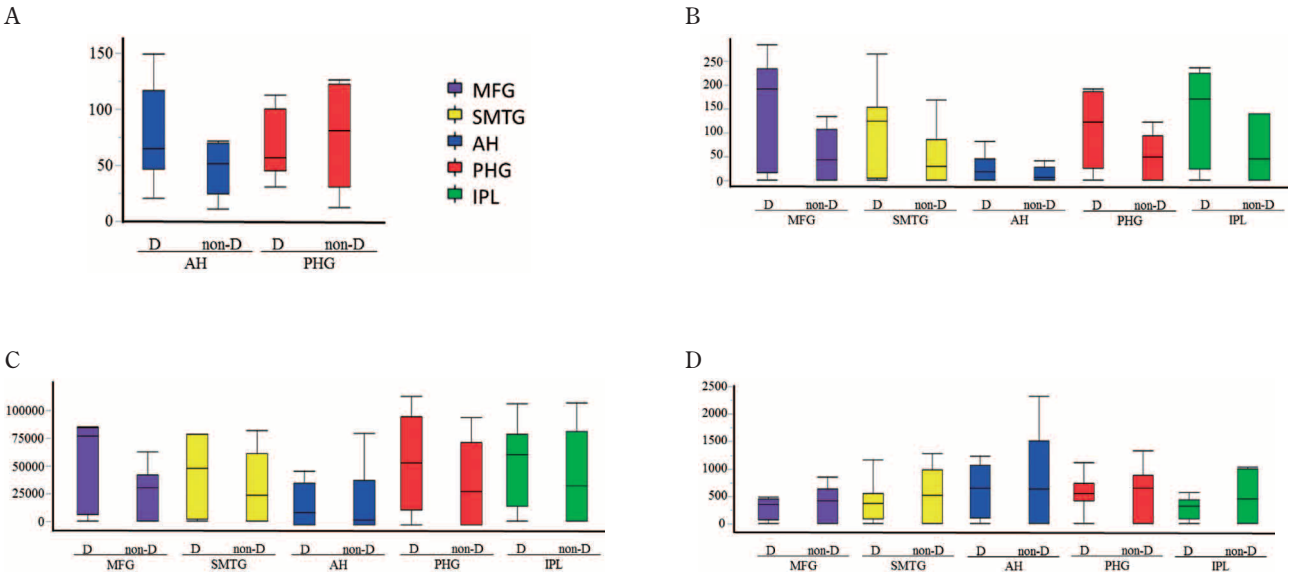

Fig. 3 Comparison of the numbers of NFT or SP in each region between groups D and non-D
A : Number of NFT, B : Number of SP, C : Gross area of SP, D : Individual area of SP

should select more specific staining methods for each pathological finding, with better contrast between stained and unstained regions. For example, sufficient quantification may be possible by immunohistochemical detection of antigens specific for certain pathological findings (13-17). Furthermore, diagnosis of dementia by image data may be possible by increasing the number of autopsies investigated, and/or improving the staining technique.

CONFLICT OF INTEREST

We have no conflict of interest for this case report.

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