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Detection of Senile Plaque and Neurofibrillary Tangle using Bielschowsky-Hirano's Silver Method

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ABSTRACT : Conventional light microscopic morphometrical investigations were performed on senile plaques and neurofibrillary tangles in eighteen senile brains. Specimens from the hippocampal region were investigated statistically whether or not there is any difference in detection of senile plaques and neurofibrillary tangles among the conventional staining methods, that is, Hematoxylin-Eosin stain, Congo red stain, Periodic acid Schiff reaction, Bodian stain and Bielschowsky-Hirano's silver stain. It was clarified that, even in the laboratory without specific antibody to senile plaque and neurofibrillary tangle, Bielschowsky-Hirano's stain is the most useful and convenient staining method for detection of these ageing-related changes.

INTRODUCTION

Morphology using recently developed immunohistochemical methods or molecular biology techniques has become prominent presence even in the field of neuropathology. Senile plaque (SP) can now be accurately detected by the use of antibody to beta-protein (WONG *et al.*[4]), and neurofibrillary tangle (NFT) by the use of antibody to ubiquitin (MORI *et al.*[3]) or tau-protein (IHARA *et al.*[1]). However, it is also true that pathologists involved in a busy schedule of anatomical pathology and diagnostic surgical pathology are compelled to make examinations and diagnoses using conventional staining methods rather than the most recent technology. The most practical approach to the neuropathological investigation of senile changes of the brain is to obtain accurate finding and diagnoses inexpensively by combining classic techniques.

We regularly use Bielschowsky-Hirano's silver stain (Biel) (YAMAMOTO and HIRANO[5]; refined by M. YOSHIMURA) in addition to conventional Congo red stain and Bodian stain for examination of SP and NFT. In the present study, we investigated morphometrically and statistically whether or not there is any difference in detection of SP and NFT among these staining methods, and we demonstrated that Biel is the most useful staing method for detection of these two changes on a routine basis.

MATERIALS AND METHODS

A total of 18 male autopsy cases were used in this study (Table 1). Autopsied during the period from 1972 to 1980 and ranging in age from 70 to 79, they were selected from among the autopsy cases preserved in the Department of Pathology, the Scientific Data Center for the Atomic Bomb Disaster, Nagasaki University School of Medicine. Each of the paraffin-embedded specimens was made into 15 serial sections (8 μm in thickness) and three sections each were stained with Hematoxylin-Eosin stain (H & E ; section #1, 6, 11), Congo red stain (modified by Highman ; CRed ; #2, 7, 12), Periodic acid Schiff reaction (PAS ; #3, 8, 13), Bodian stain (Bod ; #4, 9, 14) and Bielschowsky-Hirano's silver stain (Biel ; #5, 10, 15). The number of SP and NFT found in the sections by various staining methods was counted by two pathologists (MK and MI) independently and the mean value of the two calculations was used. The margin of error was no more than 3 for every three sections counted by the two pathologists. Thus, the mean value can be considered highly reliable.

1) SP : After dividing SP into typical senile plaque with core (SPC) and senile plaque without core (SPP), the total number in five microscopic fields at magnification 200x was counted and the mean value was calculated from the three sections of each stain.

2) NFT : The total number of neurons with NFT in five microscopic fields at magnification 400x was counted and the mean value was calculated from the three sections of each stain.

Statistical calculation for the differences among staining methods were processed using the BMDP statistical software of the University of California, Los Angeles (UCLA).

RESULTS

1) *Senile Plaque (SP)*

Typical senile plaque with core composed of amyloid fibrills (SPC) was observed in 5 of the 18 cases by H&E and CRed, in 6 cases by PAS and Bod, and in 7 cases by Biel (Table 2). The number of SPC was high in examinations by PAS, Bod and Biel. Statistical analysis of the detection ability of the staining methods in terms of the number of SPC stained disclosed a significant difference for PAS ($p<0.01$), Bod ($p<0.05$) and Biel ($p<0.01$) compared to H&E. It was also disclosed that PAS and Biel were superior when compared to CRed ($p<0.05$). There was no significant difference in detection ability among PAS, Bod

Table 1. Materials investigated

Case	Age	Brain (g)	Principal Anatomical Cause of Death
1	70	1360	Hepatoma
2	70	1210	Myocardial infarction
3	70	1430	Bronchopneumonia
4	71	1200	Bronchopneumonia
5	71	1410	Squamous cell carcinoma, larynx
6	71	1260	Adenocarcinoma, stomach
7	72	1300	Myocardial infarction
8	73	1190	Myocardial infarction
9	73	1160	Adenocarcinoma, lung
10	74	1300	Bronchopneumonia
11	76	1350	Cerebral hemorrhage
12	76	1140	Squamous cell carcinoma, larynx
13	77	1300	Adenocarcinoma, stomach
14	77	1240	Myocardial infarction
15	77	1350	Bronchopneumonia
16	78	1250	Bronchopneumonia
17	78	1260	Pulmonary edema
18	79	1210	Hodgkin's disease

Table 2. Detection of senile plaque (cored)

Case	H & E	CRed	PAS	Bod	Biel
1	0	0	0	0	0
2	0	0	0	0	0
3	0	0	5.0	7.3	5.3
4	0.7	0.7	0.7	3.3	8.7
5	0	0	0	0	0
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	2.7	0.7	6.7	7.3	9.0
10	0	0	0	0	0
11	5.3	9.3	20.0	9.0	6.3
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0.7	3.0	2.3	3.0	5.3
16	0	0	0	0	0
17	0	0	0	0	0.3
18	0.3	2.7	6.0	2.7	4.7

H & E : Hematoxylin and Eosin stain
CRed : Congo red stain, Bod : Bodian's stain
PAS : Periodic acid Shiff reaction
Biel : Bielschowsky-Hirano's silver stain

Table 3. Probabilities for the *t*-value : Detection of SPC, between staining methods

	H & E	CRed	PAS	Bod	Biel
CRed	1.0000				
CRed	0.5533	1.0000			
PAS	0.0064	0.0320	1.0000		
Bod	0.0250	0.0978	0.6213	1.0000	
Biel	0.0081	0.0388	0.9370	0.6781	1.0000

Table 4. Detection of senile plaque (primitive)

Case	H & E	CRed	PAS	Bod	Biel
1	0	0	0	0	0
2	0	0	0	0	0
3	1.0	0	13.7	7.3	116.3
4	0.3	0	1.0	2.0	13.7
5	0	0	0	0	0.7
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	10.3	0.7	12.0	21.3	120.3
10	0	0	0	0	0
11	10.7	4.0	17.0	15.7	43.7
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	1.7
15	0	0.7	0.7	2.3	9.7
16	0	0	0	0	0
17	0	0	0	0	0
18	7.0	3.3	7.0	9.7	82.7

H & E : Hematoxylin and Eosin stain
CRed : Congo red stain, Bod : Bodian's stain
PAS : Periodic acid Shiff reaction
Biel : Bielschowsky-Hirano's silver stain

Table 5. Probabilities for the *t*-value : Detection of SPP, between staining methods

	H & E	CRed	PAS	Bod	Biel
H & E	1.0000				
CRed	0.7471	1.0000			
PAS	0.7392	0.5123	1.0000		
Bod	0.5886	0.3882	0.8352	1.0000	
Biel	0.0000	0.0000	0.0000	0.0000	1.0000

and Biel (Table 3).

Senile plaque without core (SPP) was observed in 5 of the 18 cases by H&E, in 4 cases by CRed, in 6 cases each by PAS and Bod, and in 8 cases by Biel. The number of SPP detected by Biel was markedly large while that by CRed was the smallest (Table 4). Statistically, Biel was more effective in the detection of SPP than any of the other staining methods (Table 5). In terms of the number of SP detected as a whole, Bod and Biel were effective (Table 6), but the latter far surpassed the former (Table 7).

2) *Neurofibrillary tangle (NFT)* :

In the hippocampus and gyrus parahippocampalis, NFT stained with H&E was basophilic, and the tissue sections stained with CRed were weakly birefringent when observed through a polarizing plate. However, the number of NFT detected by these staining methods was very small. NFT was not detectable by PAS. Only Bod and Biel were capable of detecting a number of NFT sufficient for statistical analysis, but Biel showed a much higher detection ability than Bod. NFT, though few in number, could be detected by Biel even in cases (#4, #8) of no detection by Bod (Table 8). Statistically there was a significant difference between two staining methods (Table 9).

Table 6. Detection of senile plaque in total

Case	H & E	CRed	PAS	Bod	Biel
1	0	0	0	0	0
2	0	0	0	0	0
3	1.0	0	18.7	15.0	121.7
4	1.0	0.7	1.7	5.3	22.3
5	0	0	0	0	0.7
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	13.0	1.3	18.7	28.7	129.3
10	0	0	0	0	0
11	16.0	13.3	37.3	24.7	50.0
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	1.7
15	0.7	3.7	3.0	5.3	15.0
16	0	0	0	0	0
17	0	0	0	0	0
18	7.0	3.3	7.0	9.7	87.3

H & E : Hematoxylin and Eosin stain
CRed : Congo red stain, Bod : Bodian's stain
PAS : Periodic acid Shiff reaction
Biel : Bielschowsky-Hirano's silver stain

Table 7. Probabilities for the *t*-value : Detection of SP, between staining methods

	H & E	CRed	PAS	Bod	Biel
H & E	1.0000				
CRed	0.8419	1.0000			
PAS	0.4571	0.3459	1.0000		
Bod	0.0000	0.0000	0.0000	1.0000	
Biel	0.0000	0.0000	0.0000	0.0000	1.0000

DISCUSSION

Since the introduction of immunohistochemical techniques in the field of morphology, histological finding provide immediate and invaluable information on etiology and clues to disease components. The techniques have also been applied to studies of senile dementia and have disclosed that one of the components of amyloid deposited on SP is beta-protein (WONG *et al.*[4]), and that NFT is composed of ubiquitin (MORI *et al.*[3]) and tau-protein (IHARA *et al.*[1]) among other proteins. These immunohistochemical techniques can probably also be applied to routine diagnostic procedures. At present, however, the above antibodies are not commercially available and their use is restrcted to certain research institutions. In routine pathological diagnosis, it is important to use simple and inexpensive staining methods which are available to any pathology institution and yet are highly effective in identifying specific findings. We believe that Biel is a staining method which meets these demands. The purpose of this paper is to demonstrate statistically the truth of this assertion.

Generally, there is a tendency to assume that CRed is the most useful stain for the detection of SP because of its association with amyloid. This may be true in the sense that the identification of SPC requires demonstration of amyloid in the core portion. However, since an accurate estimate of the number of SPC is essential in the diagnosis of senile dementia, PAS, Bod and Biel with an excellent ability to detect SPC are obviously superior (Table 2, Table 3). In the brain showing ordinary senile changes in addition to senile dementia, SPP appears more numerously than SPC (Table 2, Table 4). For detection of SPP, CRed seems to be inferior to H&E, but there was no significant difference statistically. With regard to detection of SPP, PAS and Bod differed little from H&E and CRed ; only Biel demonstrated an outstanding achievement (Table 4. Table 5). It should be emphasized, therefore, that Biel is the best method for detection of SP (Table 6, Table 7). Although not mentioned in the statistical analysis, diffuse SP was not detectable by the four other staining methods. To the best of our knowledge, therefore, Biel is the only viable method other than immunohistochemical techniques on beta-protein.

Silver impregnation staining has been used preferrably for detection of NFT. In the present study, Bod and Biel were much more effective for the detection of NFT than H&E and CRed. The fact that NFT, which was not detectable by Bod in two cases (#4, #8), was detected by Biel indicates the outstanding usefulness of Biel (Table 8, Table 9).

In a comparison of SP and NFT detected by Bod and Biel, only one case (#14) showed no NFT and only SP, whereas six cases (#1, #6, #7, #8, #10, #16) showed no SP and only NFT. In other words, NFT seems to be more fre-

quent than SP in general senile changes.

Autopsy criteria for the diagnosis of Alzheimer's disease, are mentioned in a conference report by KHACHATURIAN[2]. Says the author with regard to the number of SP in the neocortex at 200x magnification field : "For any patient between 66 and 75 years of age, the number of senile plaques must be greater than ten per field. In any patient greater than 75 years old, the number of senile plaques should exceed 15 per microscopic field." It is not stated in his report whether SP refers only to SPC or includes SPP as well. In the present study, the counting area included the archicortex (hippocampus) and neocortex (parahippocampal gyrus). The number of SP including SPP in case # 3 and case # 9 calculated for one microscopic field in the Biel specimens was 24 and 25 respectively. The number was more than 10 in the neocortex area only, thereby exceeding the criteria of KHACHATURIAN[2]. However, clinical symptoms of dementia were not observed in case # 3, who died of bronchopneumonia due to fistula of the esophagus, nor in case # 9, who died of primary pulmonary adenocarcinoma. The number of SPC in these two cases was 1 or 2 per field. Case # 18, a case of Hodgkin's disease having no clinical symptoms of dementia, showed more than 17 senile plaques per field, which exceeded the criteria of KHACHATURIAN[2] for a patient over 75 years old. In any case, morphometrical studies of SP and NFT as well as staining techniques are undoubtedly necessary in various regions of the brain in patients without dementia. It may also prove significant to review the size of SPC, SPP and diffuse plaque in addition to their number.

Table 8. Detection of NFT

Case	Bod	Biel
1	14.7	50.7
2	0	0
3	13.7	36.0
4	0	1.3
5	1.0	9.7
6	3.0	6.3
7	4.0	23.7
8	0	4.0
9	25.0	49.7
10	20.0	29.7
11	9.3	27.0
12	0	0
13	0	0
14	0	0
15	2.0	6.3
16	40.3	144.7
17	4.7	7.0
18	6.3	11.3

NFT : Neurofibrillary tangle
Bod : Bodian's stain
Biel : Bielschowsky-Hirano's silver stain

Table 9. Probabilities for the *t*-value : Detection of NFT, among Bod and Biel

	Bod	Biel
Bod	1.0000	
Biel	0.0050	1.0000

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