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Histopathological Differentiation of Endometrial Adenomatous Hyperplasia from a Well Differentiated Type of Endometrial Adenocarcinoma by Statistical Methods

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Summary. **This histopathological analysis was statistically performed between specimens of endometrial adenomatous hyperplasia (aH) regarded as a benign proliferative lesion and those of a well-differentiated type of endometrial adenocarcinoma (wC) with low-grade atypiam. Among the examined statistical indices of this histological atypism, the index of glandular area per unit area (IGA), the index of cellular area per unit area (ICA), and the index of gland-in-gland configuration per unit area (IGG) were statistically significant at 0.001, 0.001, and 0.02 levels (t-test), respectively. The multivariate statistical analysis with these reliable indices led the following quadratic discriminant functions: $Q(x)=0.01227x_1^2-0.07634x_2^2-0.45338x_3^2-0.11596x_1x_2+0.42307x_2x_3+0.11860x_3x_1+2.08872x_1+8.90431x_2-16.82170x_3-160.49600$ (corrective discriminating rate of the aH and the wC: 0.857 and 1.000, respectively), $Q'(x)=0.03324x_1^2-0.07129x_2^2-0.02868x_4^2-0.11888x_1x_2+0.06813x_2x_4+0.03027x_4x_1-1.20861x_1+6.62168x_2-0.98273x_4-35.40290$ (corrective discrimination of the aH and the wC: 1.000 and 1.000, respectively) (x_1=IGA, x_2=ICA, x_3=IGG, x_4=age). The multivariate statistical analysis of histopathology is valuable for the differentiation of the wC from the aH.**

INTRODUCTION

The endometrial hyperplasia is classified into cystic glandular hyperplasia (cH), adenomatous hyperplasia (aH), and atypical hyperplasia (AH).[1-5] In the hyperplasia-carcinoma morphological continuum, the AH is regarded as a borderline malignancy between the aH and the well differentiated type of endometrial adenocarcinoma (wC). The histological patterns of stromal invasion, regarded as the only histological clue to making a diagnosis of carcinoma, were frequently too difficult to confirm, especially in the wC. Furthermore, a pseudo-invasion was histologically difficult to exclude from a true invasion in a small biopsy specimen. Hence any evidence of stromal invasion could not always indicate non-carcinoma, and vice versa; the AH, diagnosed histologically because of a lack of invasion, always had the possibility of being confused with the wC.

However the histochemical differentiation of wC may be useful, the judgement of stainabilities may look different according to each pathologist's subjective judgement.[3,4] On the other hand, the histopathological differentiation was also digitally quantified with severel indices of atypism; furthermore, the discriminant function was drawn for the histopathological criteria of malignancies.[4,6,7]

As for an accuracy and reproducibility in this study, the examined specimens were restricted to histologically authentic cases of the aH and the wC.[6,7] The aH was confirmed by examination of the repeated full-curettage of the specimens after hormonal curettage or extirpated uteri and the wC were diagnosed by a frank invasion in extirpated uteri. Furthermore, the examined statistical indices were limited to a structural atypism because the analysis of the complicated cellular atypism took time. Four indices of structural atypism were statistically analyzed, and the discriminant functions between the aH and the wC were analyzed.[4,6,7]

MATERIALS AND METHODS

The examined cases consisted of five normal proliferative phase endometria (pN), eleven cH, seven aH, and eight wC. The difference in the average age of cases was statistically significant (Table 1). Sections from the formalin-fixed and paraffin-embedded blocks were stained with hematoxylin and eosin. The

Table 1. Classification of cases

	number of cases	age (years) (m±σ)	※1	※2	※3	※4
pN	5	40±2.9			※3	※4
cH	11	52±7.3	※1	※2		
aH	7	45±5.8			※3	
wC	8	62±9.6				

pN: normal proliferative phase, cH: cystic hyperplasia, aH: adenomatous hyperplasia, wC: well-differentiated type endometrial adenocarcinoma, t-test: ※1: 0.05, ※2: 0.02, ※3: 0.01, ※4: 0.001.

specimens of pN were obtained from extirpated uteri, which were resected because of myoma uteri. In the cH and the aH, each examined area was selected from the field of the severest atypism in each series of repeated biopsied specimens or the extirpated uterus. The specimens used as the wC were selected from the regions of the least atypism in frankly invasive lesions. Hence, in this discriminating study between the aH and the wC, the definitive wC of the least atypism was compared with the reliable aH of the severest atypism.

First, each specimen was microscopically photographed under the x10 objective lens. Second, this photo, printed twenty-five times enlarged, was traced on a sheet of graph-paper by a diagraph, which magnified the photo five times. Lastly this copy on a sheet of graph-paper with three thousand units to count (5 mm-square/unit) was calculated for indices.

The statistical indices consisted of (1) index of glandular area per unit area (IGA), (2) index of cellular area per unit area (ICA), (3) index of nuclear stratification per unit area (INS), and (4) index of gland-in-gland configuration per unit area (IGG). Each index was calculated as follows:

(1) IGA=sum of square-units of whole glandular area x100/ 3000 (total square-units of a graph paper)

(2) ICA=sum of square-units of whole glandular cellular area x100/3000

(3) INS=total number of nuclei of glandular cells/total number of the lining nuclei seated just on the besement membrane

(4) IGG=total number of glandular lumens/total number of glands

The multivariate analysis was performed by a digital computer (personal computer: PC-9801 VX, NEC, TOKYO, Soft disk: PC-9801/E/F/M/VF/VM/U/UV/VX/XL, 5″2HD, Kyouritsu Press Co., Tokyo).[7]

RESULTS

The value of morphometric indices corresponded with the development of lesions (Table 2). In the IGA, the differences between the pN and the cH, the aH, or the wC were statistically valuable ($p=0.05$, 0.001 and 0.001 in t-test, respectively). The significant difference was similarly confirmed between the aH and the wC ($p=0.001$). In the ICA, the differences between the pN and the aH or the wC were statistically identified ($p=0.001$), and there was also a great difference between the aH and the wC ($p=0.001$). As for the INS, the pN was statistically distinguished from the wC ($p=0.001$). Regarding the IGG, the wC was statistically diffenentiated from the pN and aH ($p=0.05$ and 0.02, respectively). Hence the above findings suggested that the IGA, the ICA, and the IGG were valuable indices for the differetiation of the wC from the aH.

With the use of the IGA and ICA, being the most reliable indices for differentiating the wC from the aH, the reliant ellipse of the groups of aH and wC were drawn (reliant rate: 95%, Figure 1). The functions of ellipse were as follows: (1) the group of the wC: $x^2/339+y^2/14.1=1$, O(60.9, 50.9) and $\tan 2a=-3.30$, (2) the group of the aH: $x^2/120+y^2/19=1$, O(45.7, 28.9) and $\tan 2a=-0.99$ (x-axis: IGA, y-axis: ICA). There was one case of wC in the reliant ellipse of the group of aH.

The valuable parameters, including the IGA, the ICA, the IGG, and age, were applied to the multivariate statistical analysis, and the quadratic discriminant functions were drawn as follows: (1) with the utilization of the IGA (x_1), the ICA (x_2), and the IGG (x_3); $Q(x)=0.01227x_1^2-0.07634x_2^2-0.45338x_3^2-0.11596x_1x_2+0.42307x_2x_3+0.11860x_3x_1+2.08872x_1+8.90431x_2-16.82170x_3-160.49600$,(2) with the utilization of the IGA (x_1), the ICA (x_2) and age (x_4); $Q'(x)=0.03324x_1^2-0.07129x_2^2-0.02868x_4^2-0.11888x_1x_2+0.06813x_2x_4+0.03027x_4x_1-1.20861x_1+6.62168x_2-0.$

Table 2. Classification of morphometric indices

	IGA	ICA	INS	IGG
pN	16.5±7.01	12.7±3.40	1.52±0.110	0.970±0.080
	※1 ※3 ※3	※3 ※3	※3	※1
cH	37.5±14.2	21.4±7.57	1.91±0.360	1.15±0.170
aH	45.7±7.27	28.9±4.11	2.20±0.750	1.60±1.04
	※3	※3		※2
wC	60.9±12.2	50.9±16.2	2.51±0.350	2.19±1.09

pM:normal proliferative phase, cH: cystic hyperplasia, aH:adenomatous hyperplasia, wC: well-differentiated type endometrial adenocarcinoma, IGA: index of glandular area per unit area, ICA: index of cellular area per unit area, INS: index of nuclear stratification per unit area, IGG: index of gland-in-gland configuration, t-test: ※1: 0.05, ※2: 0.02, ※3: 0.001.

$98273x_4 - 35.40290$. As to the discriminant function of the Q(x), the reliability of aH, showing a positive value in the Q(x), and the wC, showing a negative value in the Q(x), were 0.857 and 1.000, respectively, so that 14.3% of the aH cases showed a false negative value in the Q(x) and were diagnosed as the wC. Concerning the function Q'(x), on the other hand, the aH was completely differentiated from the wC (both corrective discriminating rate : 1.000).

DISCSSION

In the endometrial hyperplasia-carcinoma consequenece, the endometrial hyperplasia was regarded as a foregoing lesion of carcinoma and was clinically found earlier than a carcinoma.[1-3,5] The age of cases in this study was statistically significant between the hyperplasia and the wC. The re-biopsy after hormonal curettage for a differential diagnosis was especially added to an early premenopausal patient in her thirties or forties. The age was clinically an important factor for preventing an overdiagnosis of carcinoma.

In histologically differentiating the wC from the AH, it was a criterion that the wC showed a stromal invasion, including an irregular glandular budding with surrounding stromal reactions, cribriform patterns, and prominent papillary growths.[1-3,5] However, the wC could not always reveal these typical signs in a small preoperative biopsy specimen; the

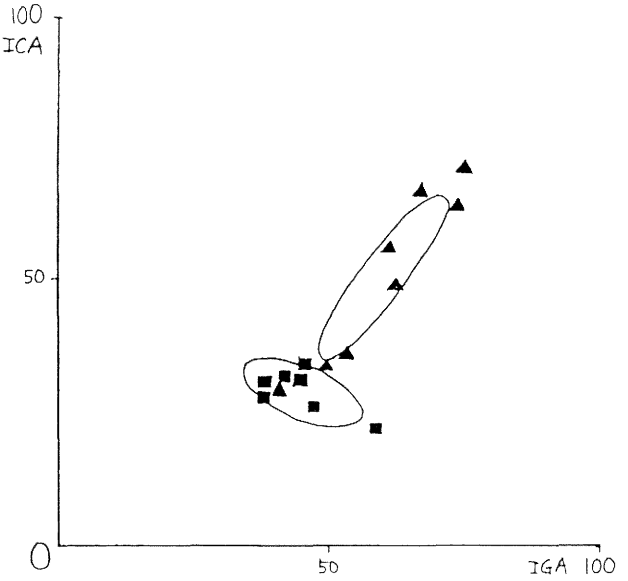

Fig. 1. Reliant ellipse of adenomatous hyperplasia (■) and well differentiated type endometrial adenocarcinoma (▲) with the use of the IGA (x-axis) and the ICA (y-axis) (reliance : 95%).

AH, therefore, could be confused with the wC.[2,3] Hence, in reliant research into histopathological differentiation of the wC from borderline malignancies, it was inevitable that the examined cases had been confirmed to be histologically authentic as the wC and the aH, and not the AH.

It was also reasonable and unbiased that the pathological diagnosis could be done from a mathematico-statistical analysis of atypism.[4,6,7] Both structural and cytological atypisms were required for unequivocal diagnosis of the wC.[2] However, only a structural atypism was calculated in this study because of its instantaneous counting, which was clinically needed especially in the preoperative pathological diagnosis. Working criteria of a structural atypism were required for a diagnosis of the wC. Excluding a stromal invasion, we chose the following three criteria based on our examined indices: (1) confluent glands of a high gland-to-stroma ratio, corresponding to the IGA, (2) gland-in-gland pattern, corresponding to the IGG, (3) marked cellular stratification, corresponding to the ICA, the INS, and the IGG. The morphometric indices of the IGA, the ICA, and the IGG were statistically significant to differentiate the wC from the aH.

Important as each index was, each could not completely separate these two lesions by itself. Furthermore, the differential trial by reliant ellipse with the combination of the IGA and ICA failed in differentiating the wC from the aH. From these results, it was necessary to combine various indices of atypism into a multivariate discrminant.[6,7] The discriminant with the combination of the IGA, the ICA, and the IGG, regarded as more reliable structural indices, was insufficient for differentiating the wC from the aH. The quadratic discriminant with a combination of the IGA, the ICA, and age was the most reliable criterion for differentiating between these two lesions. Consequently, it was suggested that both architectural and clinical indices should be considered in making the diagnosis of the wC with a multivariate analysis.

In the process of drawing a discriminant quadratic, it was necessary to determine the degree of importance between a structural atypism and a cellular atypism. And this degree of discrimination was influenced by the examined numbers of the wC and the aH.[6,7] Hence a discriminant unsusceptible to influences of the examined numbers was required. Further studies with more cases into these automatic discriminating analyses by multivariate analysis are important in reducing both the number of borderline malignancies like the AH and to clear the histogenesis of the wC.

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