STEREOLOGICAL ESTIMATION OF THE NUMBER OF GLANDS PER AREA AND GLANDS TO STROMA RATIO IN ENDOMETRIAL HYPERPLASIA

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SUMMARY :

Purpose: *We aimed to find more quantitative and objective criteria for defining and classifying endometrial hyperplasias characterized by glandular crowding and increased glands to stroma ratio.* ***Methods :*** *The number of glands per area (mm^2) and glands to stroma ratio were measured using a stereological method in endometrial curettings from 129 patients containing secretory endometrium and six groups of endometrial proliferations including proliferative endometrium, simple hyperplasia without atypia, simple hyperplasia with atypia, complex hyperplasia without atypia, complex hyperplasia with atypia, or well differentiated carcinoma. Mann-Whitney U Test was used for statistical analysis.* ***Results :*** *The number of glands per area showed a significant increase in proliferative endometrium, complex hyperplasia with atypia, and well differentiated carcinoma. Moreover, a significant increase of glands to stroma ratio was found in well differentiated carcinoma and complex hyperplasia with atypia.* ***Conclusion:*** *Our results suggested that the number of glands more than 20 per mm^2 and glands to stroma ratio greater than 1.5 are more quantitative criteria in distinguishing well differentiated carcinoma from complex hyperplasia with atypia.*

Key Words: *Endometrial Hyperplasia.*

INTRODUCTION

Published criteria for defining and classifying endometrial hyperplasia are not quantative and unbiased. This problem causes misdiagnosis of endometrial hyperplasia and well differentiated carcinoma. In distinguishing atypical endometrial hyperplasia from well-differentiated carcinoma, pathologists have not agreed with each other in more than 85 % cases (1). Attempts have been made to find more objective criteria for endometrial hyperplasias using aspects of quantative cytomorphometry (2, 3). These techniques partially succeeded in discriminating various histopathologic entities within the continium of endometrial proliferations.

In this study, we used a stereological method to measure the number of glands per mm^2 and glands to stroma ratio (G/S) to characterize specimens from secretory endometrium (SE) and six groups within the proliferative spectrum including proliferative endometrium (PE), simple hyperplasia without atypia (SH), simple hyperplasia with atypia (SHA), complex hyperplasia without atypia (CH), complex

hyperplasia with atypia (CHA), and well differentiated carcinoma (WDC). We then examined the correlation within the groups using a statistical method to find out quantative and objective criteria.

MATERIAL AND METHODS

Paraffin blocks from routine archival cases of endometrial curettings were retrieved from the Dokuz Eylül University Medical School, Department of Pathology files. One-hundred-twenty-nine cases, involving seven different groups were studied. Of these, 14 cases were SEs, 16 were PEs, 31 were SHs, 2 were SHAs, 19 were CHs, 10 were CHAs, and 27 were WDCs.

The criteria for classifying endometrial hyperplasia were those described by Kurman et al (4, 5). Simple hyperplasia is characterized by an increased glands to stroma ratio without glandular crowding. Complex hyperplasia has crowded glands with less intervening stroma as compared with simple hyperplasia. Atypical hyperplasia refers to cytologic atypia and is diveded into simple and complex, depending on the corresponding glandular architecture as described (Fig. 1). WDC was distinguished from atypical hyperplasia by features of invasion as described by Kurman and Norris (6). These features were the replacement of stroma by cribriform formations, papillary processes, and accompanying desmoplastic stromal response displacing the normal endometrial stroma (Fig. 2).

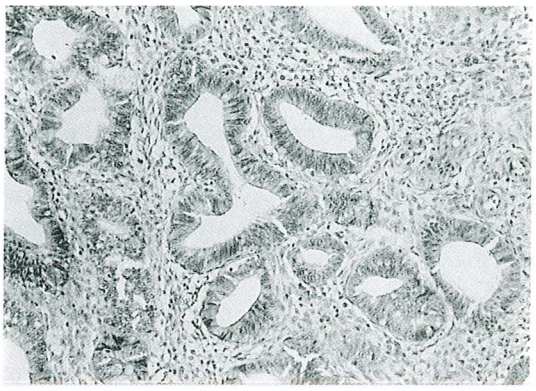

Fig - 1 : Complex hyperplasia with atypia. Crowded glands showing epithelial atypia with less intervening stroma (Hematoxylene-Eosin x100).

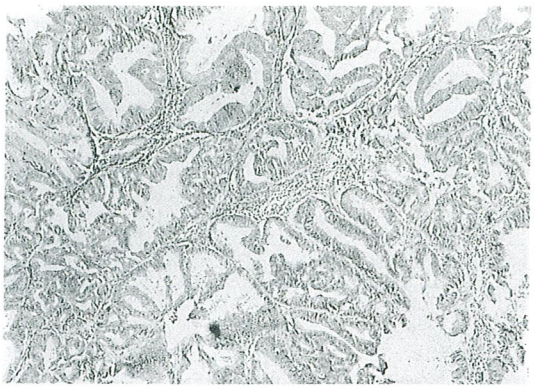

Fig - 2 : Well differentiated carcinoma. Replacement of stroma by crowded glands showing cribriform formation, papillary processes, and cytologic atypia (Hematoxylene-Eosin x100).

All tissues were originally fixed in formalin. The blocks were sectioned at 6 μm and stained with hematoxylene-eosin. A single representative area of the lesion was selected from the section and marked for the stereological analysis.

The microscopic image obtained at x 4 objective was projected by a CCD camera (Sony) to a monitor (Sony, Trinitron) attaching to the microscope (Nikon, Optiphot). The representative field on the monitor was superposed with a transparancy containing the counting frame with a set of regularly spaced points. The final magnification was calculated as x 2100.

The stereological measurements of the number of glands and G/S were done using the method described by Gundersen et al (7). The coefficient error and % Nug of the method were kept 5 % and % 25, respectively as described in reference 7.

Statistical analysis of the association between the groups was done with the use of Mann-Whitney U test by Excell 5.0. A probability level of 0.05 or less was choosen to represent the statistical significance.

RESULTS

Table 1 and 2 provide an overall summary of the results. An increased G/S was seen in CHA and WDC, in addition to increased number of glands per mm^2 in PE, CHA, and WDC.

	PE	SE	SH	SHA	CH	CHA	WDC
minumum	0.12	0.13	0.12	0.39	0.59	0.66	0.50
maximum	0.53	1.05	0.88	0.42	1.07	2.02	9.02
mean	0.28	0.43	0.38	0.40	0.45	1.14	2.85
SD	0.12	0.24	0.21	0.02	0.27	0.47	1.91

Abbreviations: SE: secretory endometrium, PE: proliferative endometrium, SH: simple hyperplasia without atypia, SHA: simple hyperplasia with atypia, CH: complex hyperplasia without atypia, CHA: complex hyperplasia with atypia, WDC: well differentiated carcinoma, SD : standart deviation.

Table 1 : Endometrial glands to stroma ratio in 129 patients.

	PE	SE	SH	SHA	CH	CHA	WDC
minumum	6.40	8.80	2.80	13.60	10.00	7.10	5.60
maximum	44.20	32.80	32.60	14.40	26.40	38.40	52.00
mean	18.84	16.90	15.59	14.00	16.46	18.64	27.76
SD	10.93	6.81	6.92	0.56	5.44	8.89	13.80

Abbreviations: SE: secretory endometrium, PE: proliferative endometrium, SH: simple hyperplasia without atypia, SHA: simple hyperplasia with atypia, CH: complex hyperplasia without atypia, CHA: complex hyperplasia with atypia, WDC: well differentiated carcinoma, SD: standart deviation.

Table 2 : Number of glands per area (mm^2) in 129 patients.

The statistical analysis showed a significant increase in the number of glands per mm^2 in WDC when compared with CHA (p=0.037). However, the difference for this parameter was not found to be significant between PE and CHA or WDC (p>0.05). G/S showed significant increase in WDC when compared with CHA (p=0.004), in CHA when compared with SHA (p=0.009), and in CHA when compared with PE (p=0.0073).

DISCUSSION

Quantative analysis of histologic images aims to enhance the specificity and sensitivity of diagnostic pathology by isolating the application of individual criteria and increasing the precision in the evaluation of quantative criteria. The expense of a quantative technique must be balanced against the utility of the information. However, visual examination of a slide requires less time and effort than does image analysis (1, 8, 9).

Stereology is a set of simple and efficient method for quantation of three-dimensional microscopic structures which is specifically turned to provide reliable data from sections. Two concepts are refered to stereology: "unbiased" and "efficient" and used in a statistical mean "without systematic deviation from the true value" and "with a low variability after spending moderate amount of time", respectively. Moreover, the necessary equipment and the calculations used in these methods are invariably simple and easy (7).

In the development or modification of a classification system, the main factors to be taken into consideration are reproducibility of histopathologic criteria and clinical relevence. For the past several years, there existed numerous classifacation schemes for endometrial hyperplasia, many of which were poorly defined, resulted in poor reproducibility, and failed to provide a rational approach to patient care. In an attempt to provide more precise definitions for endometrial hyperplasia, the International Society of Gynecological Pathologists has proposed a new terminology considering both architectural and cytologic features described by Kurman and Norris (4, 5, 10-12).

Architecturally, hyperplasias are either simple or complex with the major differing features being the complexity and crowding of the glandular elements. Simple hyperplasia is characterized by

an increased glands to stroma ratio and distinguished from the complex hyperplasia by lacking the glandular crowding (4, 10, 11). Hyperplasias must be differentiated from normal proliferative and secretory endometrium.

Atypical endometrial hyperplasia may involve both simple and complex hyperplasia, and refers to cytological atypia including large nuclei of variable size and shape that have lost polarity, increased nuclear to cytoplasmic ratios, prominent nuclei, and irregularly clumped chromatin (11, 12). Cytologic atypia seems to be the best indicator for higher risk of progression to carcinoma. Therefore, atypical hyperplasias must be differentiated from well differentiated carcinoma characterized by the architectural features described by Kurman and Norris, such as the replacement of stroma by cribriform formations, papillary processes, and accompanying desmoplastic stromal response displacing normal endometrial stroma (6, 13, 14).

Published criteria for classifying endometrial hyperplasias and their differential diagnosis are lacking quantative features. In this study, we used the stereological method to increase precision in the evoluation of quantitative criteria. Our results suggested a significant increase in the number of glands per area in PE, CHA, and WDC, and in G/S in CHA and WDC. We did not find any quantitative criteria for distinguishing endometrial hyperplasia from PE. However, the diagnosis of WDC should be suggested when the number of the glands exceeds 20 and G/S is greater than 1.5.

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