ACTA INFORM MED. 2012 Jun; 20(2): 94-98

doi: 10.5455/aim.2012.20.94-98
Recieved: 05 February 2012
Accepted: 30 March 2012

ENDOMETRIAL BIOPSY: A KARYOMETRIC AND STEREOLOGICAL STUDY

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Original paper
SUMMARY
Background: Diagnosis of premalignant endometrial lesions is a major challenge to pathologists. The D-score system is more reproducible than the WHO scheme, however it is not widely available, this study attest a simple custom-made workstation for estimation of the "D-score". **Material and methods**: Fifteen endometriam with low grade hyperplasia, fifteen proliferative and eight secretory phase endometria were analyzed by light microscopy and stereology. We determined the volume and surface density glands & stroma. karyometric parameters of the endometrial glandular epithelial cells were estimated. D-score was calculated from some architectural & karyometric parameters. **Results:** The volume of stroma (VPS) was significantly higher in proliferative than secretory and hyperplastic endometria. The glandular surface density were higher in secretory endometria than proliferative and hyperplastic endometria, however, the differences was statistically insignificant. The ratio of longest nuclear axis to shortest nuclear axis was significantly less in secretory than proliferative and hyperplastic endometria. Secretory endometria also have significantly lower mean values of shape factor & significantly higher mean values of contour index than proliferative and secretory endometria. 67% of hyperplastic endometria show D-score values < 0. **Conclusion:** it seems feasible to estimate D-score using a semi-automated workstation based on the simple stereologic and morphometric principles.
Keywords: premalignant endometrial lesions, stereological study, D-score.

1. INTRODUCTION

Endometrial carcinoma is the most common malignancy of the female genital tract (1), and it is often preceded by proliferative precursor lesions which fall under the diagnostic umbrella of endometrial hyperplasia". Thus, early accurate diagnosis and proper treatment of endometrial hyperplastic lesions are essential to prevent endometrial cancer development (2).

For many years, endometrial hyperplasia has been a diagnostic problem for pathologists (3). This is understandable because the condition comprises a spectrum of histological changes from simple exaggeration of the normal proliferative state at one extreme to changes that are difficult to distinguish from carcinoma at the other end of the spectrum (4).

Many studies on different organs have shown that in such continuous spectral lesions, ordinary qualitative subjective microscopical evaluations are not completely reproducible, even among experts, and that the resulting differences may be associated with important prognostic variations. To further complicate the issue, the presence of several classification systems and the use of descriptive diagnostic terms have resulted in low interobserver and intra-observer diagnostic reproducibility for endometrial hyperplasia (2, 3, 4).

The diagnosis of hyperplasia in the past has often led to hysterectomy, even though only a relatively small proportion of cases with endometrial hyperplasia is associated with cancer in the follow up.

Therefore, it is important to characterize high or low risk groups before initiation of therapy, because about 1–28% of hyperplasias progress to carcinoma, depending on the degree of severity (5). The WHO 94 endometrial hyperplasia classification system is still adopted by many pathologists but is plagued by poor diagnostic reproducibility, mandating a more reliable substitute (2).

Earlier morphometrical studies have shown that the measurement of nuclear features can predict progression to cancer with better results when quantitative architectural and nuclear criteria were combined into a single index "D-score" (6).

Identification of endometrial precancers by morphometric D-score has proven to be both diagnostically reproducible and predictive of clinical outcome (5). Because the automated D-score measurement systems (e.g., QProdit, Leica Cambridge, UK) is not widely available, it is fortunate to find a simple alternative to diagnose a lesion as endometrial intraepithelial neoplasia (EIN) or not.

This study is an attempt to design a simple custom-made workstation for estimation of the "D-score" index by evaluating architectural and karyometric parameters.

2. MATERIALS AND METHODS

Case selection

Cases were selected by review-

ing the pathology reports of all cases of abnormal vaginal bleeding accessioned between January 2010 and December 2011, at Al-Hilal & Al-Rahma medical laboratories, Tikrit, Iraq. Out of total of (85) cases, (38) were eligible to be enrolled in this study. Cases of abnormal vaginal bleeding due to abortion, retained piece of placenta, cervical pathology, or endometrial carcinoma were excluded.

The cases studied were divided into the following groups: proliferative endometrium (n = 15), secretory endometrium (n = 8), and endometrial hyperplasia (n = 15).

The specimens were routinely processed fixed in buffered formaldehyde, embedded in paraffin wax, and standard histological sections were made.

The age of the patients ranged from 20-70 years old. Morphometric evaluation was performed at the Department of Pathology, Tikrit college of Medicine, Tikrit.

Enrolled H&E stained sections were reviewed to identify field(s) most representative of the lesion and three images of that field were captured using a Benq® digital camera (image acquired at 4x, 10x & 100x magnification).

The architectural measurements were performed using the Cavalieri estimator. Briefly, the point-counting method consists of overlying selected section with a regular grid of test points, which is randomly positioned; the number of test points hitting structures of interest on the sections is tallied[7].In this study, a digital test system (JAVA applet) with 256 points was used (the 2 points length were calibrated in terms of micrometer, using a calibrated eyepiece reticule before each measurement

1. Architectural parameters: For each histopathological section two architectural features were assessed

(i) Volume density of stroma (VPS), which assesses the percentage of endometrial tissue composed of stroma (i.e., the inverse of glandular percentage, a measure of crowding). VPS was obtained using the following formula:

$VPS = P_{stroma}/P_{total}$

	VPS (Mean ± SD)		outSD (Mean ± SD)	
Secretory	38.6 ± 12.4	p-value <0.001	18.2 ± 14.5	p-value: NS
Proliferative	67.7 ± 16.1		9.7 ± 10.8	
Hyperplasia	45.5 ± 17.1	p-value 0.001=	12.3 ± 18.4	p-value: NS
p-value # (secretory vs hyperplasia)	0.001<		NS	

Table 1. Mean values of architectural parameterts. VPS: volume percentage of endometrial stroma. outSD: outer surface density of endometrial glands. # Unpaired Student's t-test

	Hyperplasia (Mean ± SD)	p-value*	Proliferative (Mean ± SD)	p-value*	Secretory (Mean ± SD)	p-value* secretory vs hyperplasia
D (longest nuclear axis) μm	7.8 ± 2	NS	7.1 ± 1.8	NS	6.8 ± 2.6	NS
d (shortest nuclear axis) μm	4.3 ± 0.9	NS	3.8 ± 0.5	NS	4.7 ± 1.7	NS
D/d ratio	1.8 ± 0.5	NS	1.9 ± 0.5	0.001	1.4 ± 0.1	0.004
M Mean axis (μm)	5.8 ± 1.2	NS	5.2 ± 0.8	NS	5.7 ± 1.97	NS
Perimeter (μm)	19.5 ± 4.3	NS	17.6 ± 3.6	NS	18.29 ± 6.4	NS
Area (μm^2)	27.2 ± ` 10.8	NS	21.6 ± 6.9	NS	28.2 ± 17.1	NS
Volume (μm^3)	0.0036 ± 0.003	NS	0.0043 ± 0.002	NS	0.014 ± 0.032	NS
Shape factor	0.87 ± 0.1	NS	0.86 ± 0.1	0.005	0.95 ± 0.022	0.007
Contour index	3.8 ± 0.2	NS	3.8 ± 0.3	0.002	3.6 ± 0.04	0.002

Table 2. karyometric parameters of endometrial glandular epithelial cell * Unpaired student's t-test

where P_{stroma} denotes the number of points hitting stroma and P_{total} is the total number of test points.

(ii) Gland outer surface density (out SD), which is a measurement of basement membrane length about the endometrial glands (measurement of gland complexity). Intersections of gland outer surfaces with calibrated horizontal lines of the test grid were tallied and the outer surface density was calculated by underlying formula

$outSD = 2I/dL \times \Sigma Pi$

where I is the number of intercepts between line and surface of interest, dL is the length of a test line , and Pi is the number of profiles in a counting frame. (Figure 1).

2. Karyometric Parameters. The following karyometric parameters of the endometrial glandular epithelial cells were estimated according to *Picoli et al*: the longest axis (D), the shortest axis (d), geometric mean axis (M), ratio of the longest to the shortest axis (D/d ratio), perimeter (P), area (A), volume (V), shape factor, and contour index.[8]

3. D-score. The D-score was calculated, for each case, as described previously using the following

Formula:

$D\text{-score} = 0.6229 + (0.0439 \times VPS) - [3.9934 \times Ln\ (SDSNA)] - (0.1592 \times outSD)$

Where VPS refers to volume percentage of stroma, Ln (SDSNA) for natural logarithm of standard deviation shortest nuclear axis, and outSD for outer surface density

Statistical analysis

Data were statistically analyzed by Medcalc® version 11.6.1 software and SSP (Smith statistical package) version 2.8, briefly

a) To assess the significance of our observations, the mean of morphometric measurements were compared using the unpaired Student's t-test

b) The overlap index, a nonparametric, mathematically derived index useful for quantifying the degree of overlap between two sets of data and, in the case of NHL, as a method for evaluating which nuclear feature best distinguishes be-

	Proliferative vs Hyperplasia	Proliferative vs Secretory	Secretory vs Hyperplasia
D (longest nuclear axis) μm	0.67	0.98	0.76
d (shortest nuclear axis) μm	0.74	0.59	0.76
D/d ratio	0.86	0.32	0.35
M Mean axis (μm)	0.71	0.76	0.97
Perimeter (μm)	0.73	0.87	0.97
Area (μm^2)	0.71	0.77	0.96
Volume (μm^3)	0.72	0.77	0.97
Shape factor	0.95	0.30	0.37
Contour index	0.95	0.32	0.37

Table 3. Indices of Overlap across diagnostic groups

tween the various subtypes. The value for the overlap index will be zero if there is no overlap between two sets of observations and one if both samples have the same median.[9]

3. RESULTS

1- Architectural parameters. Results of stereological evaluation are summarized in Table I. the volume density of stroma (VPS) was significantly higher in proliferative (67.7±16.1) than secretory and hyperplastic endometria (38.6± 12.4 and 45.5±17.1) respectively. Among the three diagnostic groups, secretory phase endometria have -significantly- the least VPS

The mean values of glandular surface density (outSD) were higher in secretory endometria (18.2±14.5) as compared to proliferative (9.7±10.8) and hyperplastic endometria (12.3 ± 18.4), however, the differences was statistically insignificant

2. Karyometric parameters. Table II summarizes the mean values of nuclear morphometry. All of evaluated parameters are insignificantly differ between hyperplastic and proliferative endometria. The ratio of longest nuclear axis to shortest nuclear axis (D/d) was significantly less in secretory (14.1±0.1) than proliferative (1.9±0.5 and hyperplastic (1.8±0.5) endometria

Secretory endometria also have significantly lower mean values of shape factor and significantly higher mean values of contour index than proliferative and secretory endometria

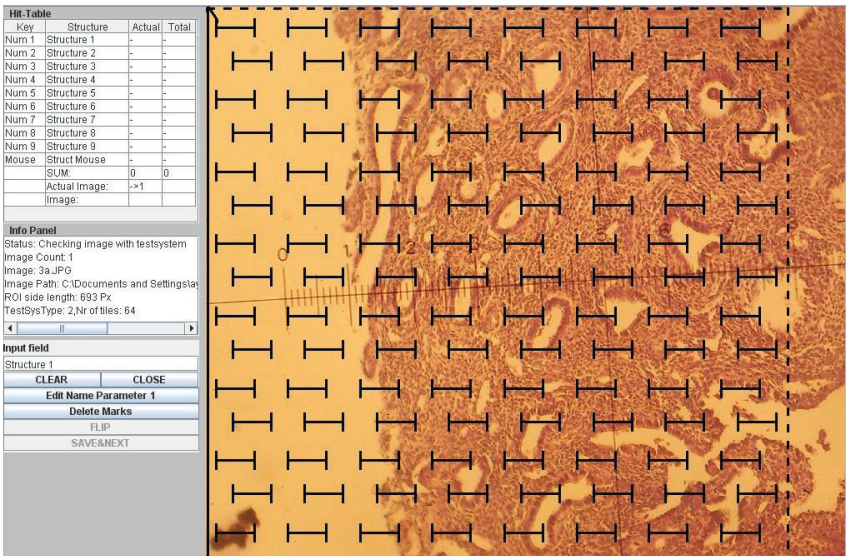

Figure 1. Test grid system applied to a digitized image of an endometrial section. H&E x10

3. Overlap index. Table 3 portrays a great overlap in all nuclear parameters when proliferative and hyperplastic endometria are contrasted against each other, overlap index range from 0.67-0.95).

Relatively low overlap is found when secretory endometria are compared to proliferative and hyperplastic endometria. Overlap indices of shape factor, contour index and D/d ratio range from 0.32-0.37.

4. D-score: The majority of hyperplastic endometria (67%) show D-score values < 0, Figure 2.

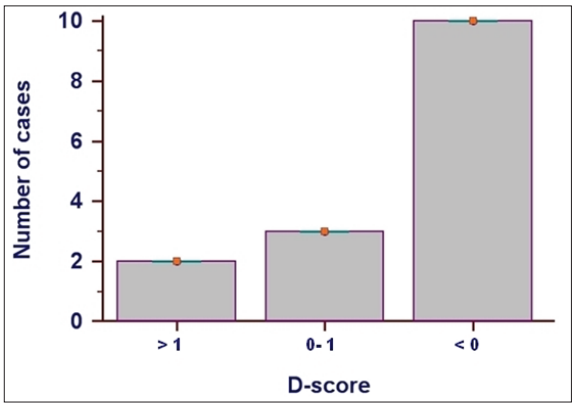

Figure 2. D-score in patients with endometrial hyperplasis

4. DISCUSSION

Architectural parameters

A tissue sample can be divided into stromal and glandular compartments, and their relative proportions used to assess glandular crowding. Focusing on the stroma provides a homogenous and more distinctive field amenable to visual assessment than the relatively complex, variably shaped glandular component. EIN lesions have a stromal volume less than that of the glands.

Results of stereological evaluation are summarized in Table 1. The volume density of stroma (VPS) was significantly higher in proliferative (67.7±16.1) than secretory and hyperplastic endometria (38.6± 12.4 and 45.5±17.1) respectively. Among the three diagnostic groups, secretory phase endometria have significantly the least VPS. This is in agree with Avvad-Portari et al. (10).

Simple hyperplasia had smaller VPS than proliferative endometrium; this may be due, probably, to the relative decrease of the stroma caused by the increase in number and size of the hyperplastic glands. In simple hyperplasia the glands usually have a tendency to be crowded and with great diameters (luminal dilatation).

The mean values of glandular surface density (outSD) were higher in secretory endometria (18.2±14.5) as compared to proliferative (9.7±10.8) and hyperplastic endometria (12.3±18.4), however, the differences was statistically insignificant.

Baak *et al*. (11) found similar results concerning the glandular Sv[outer] in well differentiated and in the moderately differentiated carcinomas. This result could be explained by the fact that, in our study, cystic dilatation of some of the glands in the simple hyperplasia when compared with proliferative endometrium and then, part of the outer surface of the cystically dilated glands may disappear outside the frame. Therefore, the outer surface per mm3 decreases, although of course, in the total tissue volume the total glandular surface may still increase.

Secretory endometrium poses a special problem in diagnosis of EIN. Normal secretory endometrium is nonuniform throughout the endometrial thickness. Basal areas without significant stromal predecidual change have much more gland crowding than near the surface where expanding stromal cells push the glands apart. Combined with cytologic differences in secretory activity between the basal and superficial gland elements, it is very easy to misinterpret an isolated fragment of basal secretory endometrium as a localizing EIN lesion (12).

Karyometric parameters

Morphometric measurements of the cytoplasm characteristics were not performed because of the unsharp cell margins and overlapping, as also reported elsewhere (13).

In this study, all of evaluated nuclear parameters are insignificantly differ between hyperplastic and proliferative endometria (Tables 2) and 3 with great overlap in all nuclear parameters when proliferative and hyperplastic endometria are contrasted against each other, overlap index range from 0.67-0.95), i.e., none of nuclear parameters can be used to differentiated between proliferative and hyperplastic cases.

This is in agreement with Skaarland et al. (14), who found that the range of the mean nuclear area of the normal cases included 70% of the malignant values. Furthermore, individual cell groups in a normal cell population often gave values well within the malignant range. In another study (15), Skaarland et al. observed that the scatter in values in the different conditions overlapped to such a degree as to make nuclear size of little importance as a diagnostic criterion. There were no differences in nuclear shape between normal, hyperplastic, and malignant conditions.

In a recent study (13), Mahovlic et al. compared karymetric parameters via Kruskal-Wallis test according to nucleus area, convexity and breadth produced no statistically significant difference ($p>0.05$) between moderately differentiated adenocarcinoma and atypical hyperplasia, or between simple and complex hyperplasia of the endometrium. The parameters of nucleus perimeter, maximum radius and length showed no statistically significant difference ($p>0.05$) between simple and complex hyperplasia. On comparison of the minimal radius yielded no statistically significant differentiation between simple and complex hyperplasia, atypical hyperplasia and moderately differentiated adenocarcinoma.

The ratio of longest nuclear axis to shortest nuclear axis (D/d) was significantly less in secretory (1.4±0.1) than proliferative (1.9±0.5) and hyperplastic (1.8±0.5) endometria.

Secretory endometria also have significantly lower mean values of shape factor and significantly higher mean values of contour index than proliferative and secretory endometria. Thus, the shape factor, contour index, and the D/d ratio are useful parameters to distinguish secretory from proliferative and hyperplastic endometria

Relatively low overlap is found when secretory endometria are compared to proliferative and hyperplastic endometria. Overlap indices of shape factor, contour index and D/d ratio range from 0.32-0.37.

Like other authors (13, 14, 15, 16), we also observed the values of some morphometric parameters to overlap between particular endometrial categories, thus a combination of several qualitative 2–4 and quantitative parameters, including clinical ones should be used for their reliable differentiation (4).

4. D-score

The majority of hyperplastic endometria (67%) show D-score values < 0, this value puts the corresponding patients at high risk level for developing subsequent endometrial carcinoma, however, this study is conducted to test the feasibility of estimating D-score using a costume-made workstation, unfortunately without a "gold standard" method for comparison such as the QProdit system of Leica, Cambridge, UK.

In conclusion, it seems feasible to estimate D-score for suspected EIN lesions using a semi-automated workstation based on the simple stereologic and morphometric principles described in this study, preferably, if fortified by a future comparative study in which a reference method such as the QProdit system is used.

Conflict of interest: none declared.

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