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Fractal Analysis: An Objective Method for Identifying Atypical Nuclei in Dysplastic Lesions of the Cervix Uteri[1]

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Objectives. **Fractal geometry is a tool used to characterize irregularly shaped and complex figures. It can be used not only to generate biological structures (e.g., the human renal artery tree), but also to derive parameters such as the fractal dimension in order to quantify the shapes of structures. As such, it allows user-independent evaluation and does not rely on the experience level of the examiner.**

Methods. **We applied a box-counting algorithm to determine the fractal dimension of atypical nuclei in dysplastic cervical epithelium. An automatic algorithm was used to determine the fractal dimension of nuclei in order to prevent errors from manual segmentation. Four groups of patients (CIN 1–3 and control) with 10 subjects each were examined. In total, the fractal dimensions of 1200 nuclei were calculated.**

Results. **We found that the fractal dimensions of the nuclei increased as the degree of dysplasia increased. There were significant differences between control and atypical nuclei found by an analysis of variance. Atypical nuclei associated with CIN 1, CIN 2, and CIN 3 also differed significantly among these groups.**

Conclusion. **We conclude that the fractal dimension is a valuable tool for detecting irregularities in atypical nuclei of the cervix uteri and thus allows objective nuclear grading.** © 1999 Academic Press

Key Words: **fractal analysis; dysplasia; cervical intraepithelial neoplasia.**

INTRODUCTION

Usually, the structure of an object can be described utilizing tools of common geometry. A square, for example, can be described by the measure of its sides. However, "complicated" objects, particularly naturally occurring objects such as clouds, mountains, and coastlines, do not apparently appear as a sum of triangles and lines. Such objects are better described using fractal geometry. Fractal geometry has been known as a mathematical concept for many years and was introduced by B. Mandelbrot [1]. Its tools were applied successfully to characterize irregularly shaped and complex figures by a mathematical value wherever Euclidean geometry fails. One of the advantages of fractal analysis is the ability to quantify the irregularity and complexity of objects with a measurable value, which is called the fractal dimension. The fractal dimension can be determined using the box-counting method [2]. Fractal analysis techniques are common tools in physics and image processing. In the past few years they have gained increasing attention in medical sciences, particularly in cardiology, pathology, and radiology [3–8], and have been applied in healthy as well as in certain pathological conditions [9–19]. During the past years fractal analysis has been applied also in tumor pathology to characterize irregular boundaries of tumors and their nuclei [20–26]. Whereas dysplastic lesions of the oral cavity were investigated by Landini and Rippin [21], the different stages of dysplasia of the uterine cervix remained to be analyzed by fractal geometry. All these studies emphasize the usefulness of fractal parameters in tumor pathology. Such parameters are especially important for improving the assessment of tumor grading, which still remains largely a subjective judgment. We, therefore, applied this technique to 1200 nuclei of regular and dysplastic epithelia of the uterine cervix in order to investigate the practicality of this method.

MATERIALS AND METHODS

A total of 1200 nuclei (30 nuclei/patient) were selected from 40 cone biopsies of the uterine cervix from patients with mild to severe dysplasia (cervical intraepithelial neoplasia (CIN) grades 1–3) or without any dysplastic lesion, as a control. From each patient three epithelial areas (in total 0.12 mm^2/patient) in three different sections of hematoxylin–eosin-stained slides were used. The digitized image of the microscopic picture was captured by a digital camera (CCD; ProgRes3012; Jenoptik, Germany) fixed on an Axioplan 2 microscope (Zeiss, Germany). The magnification and the resolution used were $\times 600$ and 2312×1740 pixels (Fig. 1) in all cases, resulting in a final pixel size of 0.2 μm.

An algorithm based on color (RGB) thresholding implemented in the KS300 software package (Zeiss Vision, Germany) was applied to the digitized images to determine the outlines of the nuclei. In this way, user-introduced errors due to manual segmentation were minimized. The resulting binary

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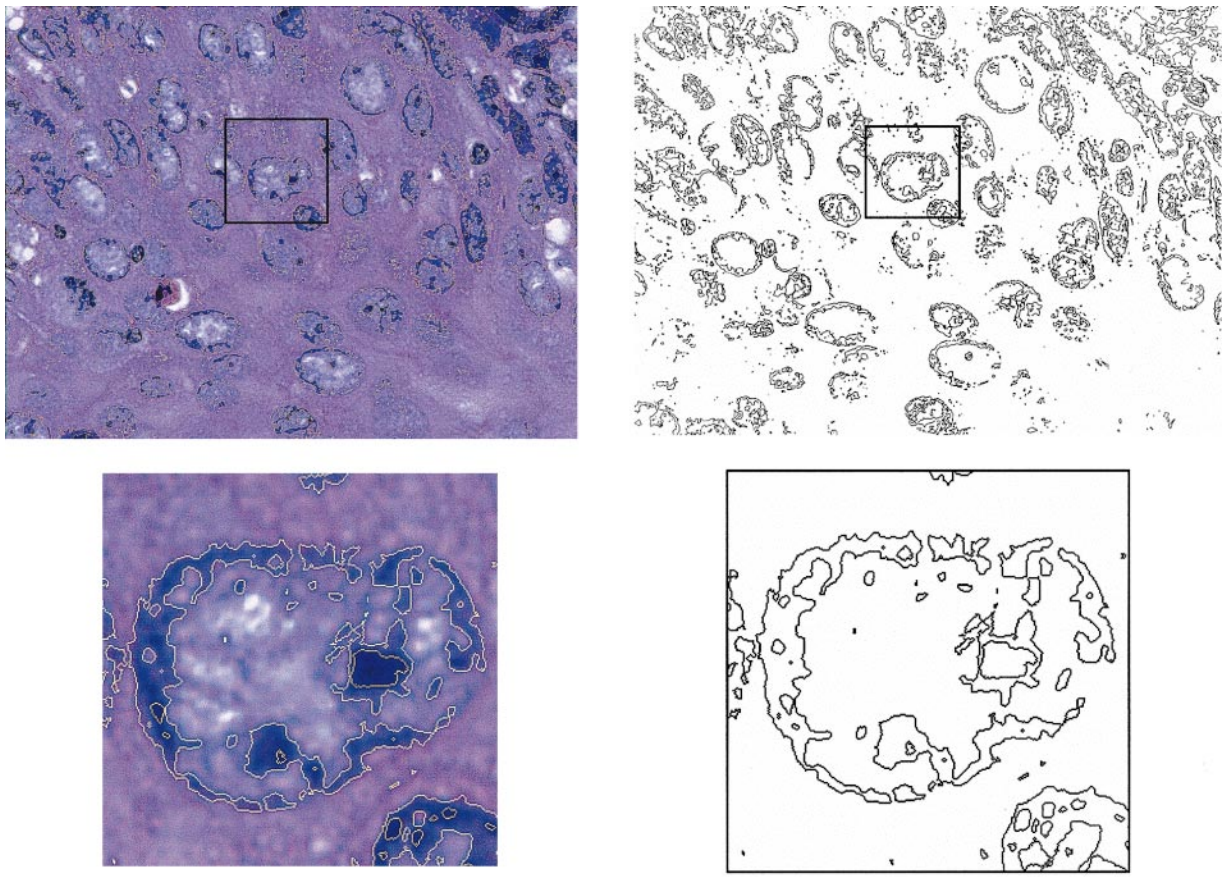

FIG. 1. Digitized images of the cervical epithelium are shown (left top) with segmentation of nuclei (left bottom). The corresponding binary images are given on the right.

(black and white) images were cleared of nonnuclear structures using a scrap filter. Square images of 300 × 300 pixels in length and containing a single nucleus were extracted and underwent fractal analysis. Only complete and nonoverlapping nuclei were taken into account.

The box-counting algorithm was used for fractal dimension calculation: the binary images were covered with different grids (box length ϵ), and the number of boxes $N(\epsilon)$ required to cover the structures of the nuclei was recorded. If an object is an ideal fractal, $N(\epsilon)$ increases according to the relation

$$N(\epsilon) = C\epsilon^{D_F}, \tag{1}$$

where D_F is the fractal dimension and C is constant. For a straight line, $N(\epsilon)$ doubles with every halving of the box length, corresponding to a dimension of 1. From Eq. (1) the fractal dimension D_F can be derived as

$$D_F = \lim_{(\epsilon \to 0)} [-\log(N(\epsilon))/\log(\epsilon)]. \tag{2}$$

Therefore, the fractal dimension can be determined as the slope of a double-logarithmic plot of $N(\epsilon)$ over ϵ.

In our study 300 pixel-sized binary images of the nuclei were analyzed for their fractal dimension by covering them with 150 different grids (box length ranged from 0.2 to 30 μm). For each grid the number of nucleus-containing squares $N(\epsilon)$ was determined, i.e., 150 values per nucleus (Fig. 2). For each nucleus, $N(\epsilon)$ was plotted over the reciprocal of ϵ in a double-logarithmic way. The slope of the graph was derived by a linear regression analysis and yielded the fractal dimension. The method was checked against structures with known Euclidean dimension (line, circle = 1) and known fractal dimension (Koch's curve) with a mean error of 0.001 (Fig. 3). The data were expressed as means ± standard error.

Differences between the control and the groups CIN 1–3 as well as between subject classes were examined by an analysis of variance using the statistical package SAS. All probability values are two-tailed and $P < 0.05$ was considered statistically significant.

RESULTS

The mean fractal dimension (mean ± standard error) of control nuclei was $D_F = 1.02 \pm 0.004$ and of atypical nuclei CIN 1, $D_F = 1.32 \pm 0.01$; CIN 2, $D_F = 1.37 \pm 0.009$; and CIN

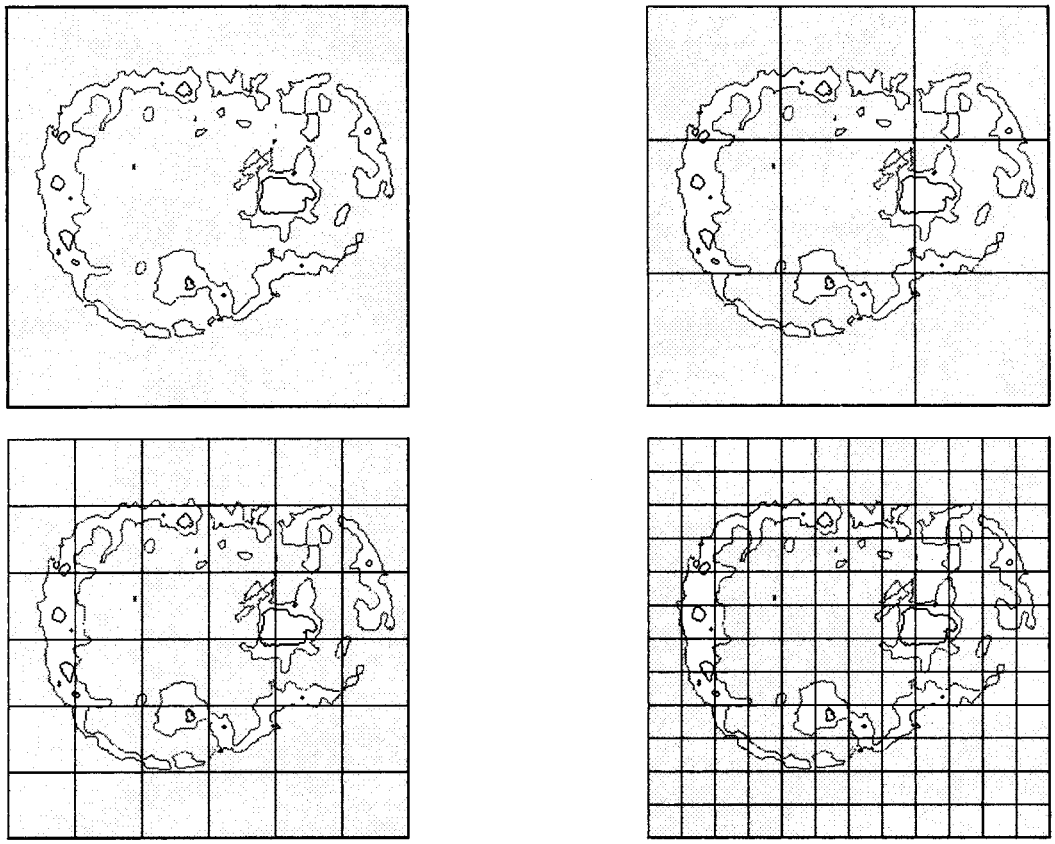

FIG. 2. Edited image used for fractal analysis with variation of $N(\epsilon)$ of the box sizes (in total 150 different grids were used).

3, $D_F = 1.40 \pm 0.008$. The fractal dimension of atypical nuclei was significantly greater than that of nuclei of the control group (control/CIN 1–3 $P = 0$; Figs. 4 and 5). In addition, the fractal dimension of atypical nuclei increased as the degree of dysplasia increased (CIN 1/CIN 2 $P = 0.003$, CIN 1/CIN 3 $P < 0.0002$, CIN 2/CIN 3 $P = 0.0024$; Fig. 5).

DISCUSSION

In this study fractal analysis was applied in order to quantify structural irregularities of atypical nuclei associated with dysplastic lesions of the uterine cervix (CIN 1–3). We found that the fractal dimension is a reliable measure of atypical nuclei, since it is able to distinguish between control and atypical nuclei of dysplastic epithelia. In addition, we found a small but statistically significant difference in atypical nuclei among the CIN groups. The fractal dimension (a measure of irregularity) of atypical nuclei was increased as the degree of dysplasia increased. Thus, fractal analysis yields an objective measure of the irregularity in shape of cervical nuclei. At present, the assessment of nuclear irregularities remains largely a subjective judgment, with high observer dependence and variation. Therefore, such mathematical discrimination which provides additional and more objective information in overall classification of dysplastic lesions would be of great benefit and may help to identify objectively the pathological status of the cervical epithelium. The fractal dimension of atypical nuclei in CIN lesions alone, however, is not enough for a histopatho-

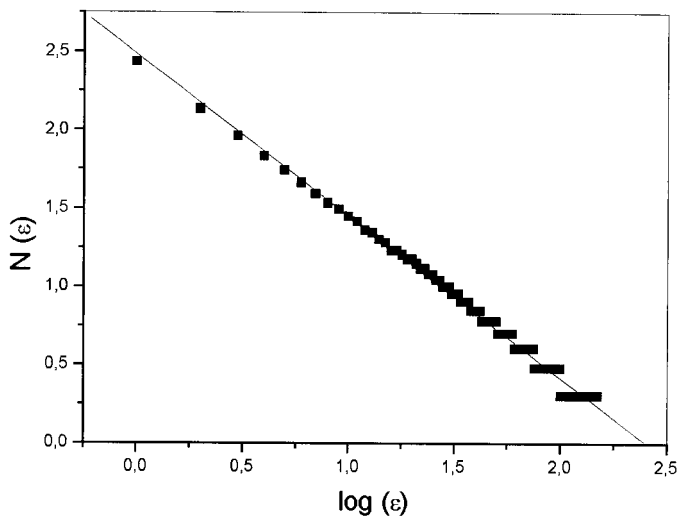

FIG. 3. Fractal dimension of a line with Euclidean dimension 1. The revealed fractal dimension was $D_F = 1.001$.

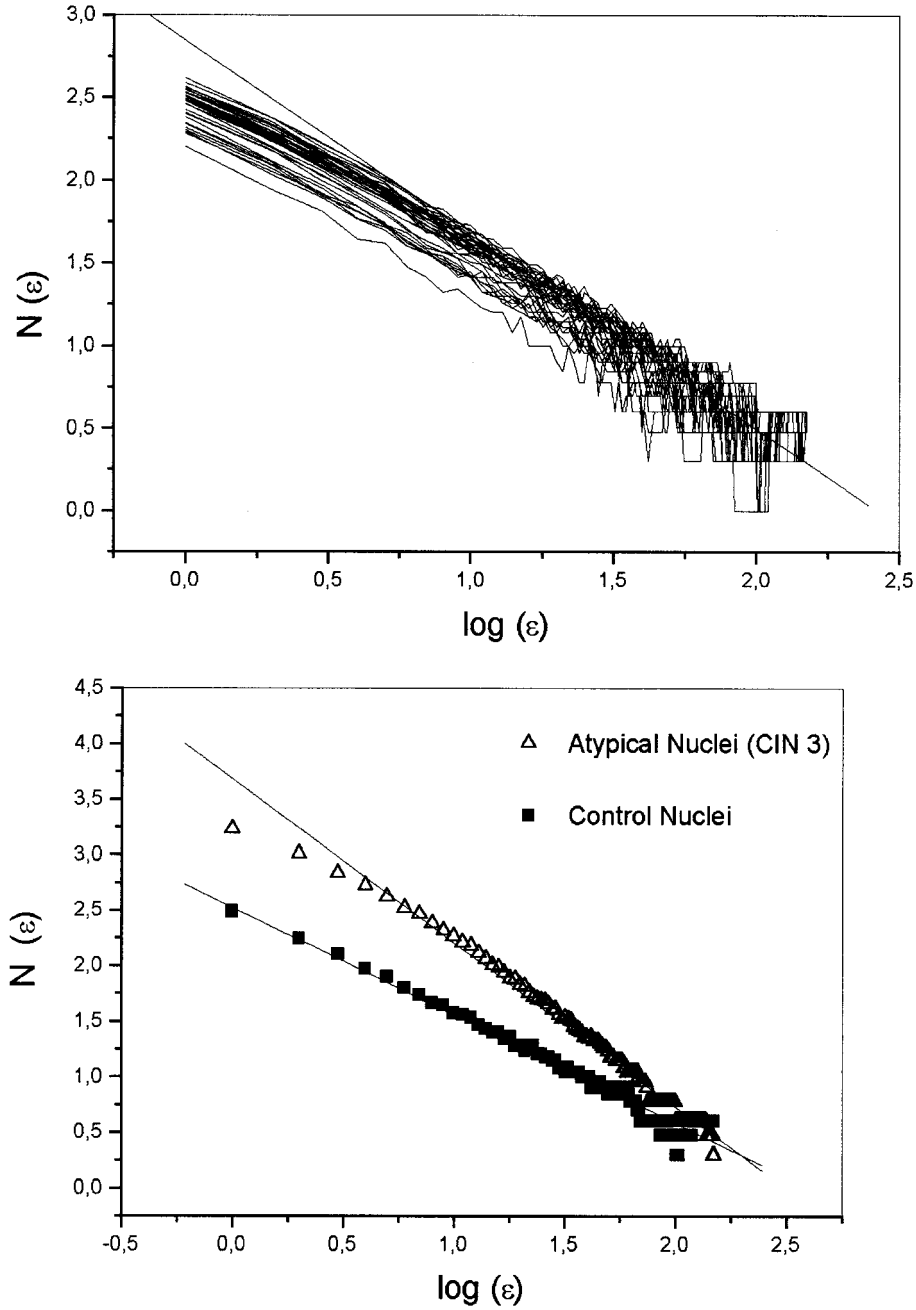

FIG. 4. Fractal dimension of nuclei from the uterine cervix. Top: Individual fractal dimension of the control nuclei. Bottom: Mean fractal dimension of the control nuclei ($D_F = 0.97$; squares) and the atypical nuclei of severe dysplastic epithelium (CIN 3; $D_F = 1.47$; triangles).

logical diagnosis. In daily histological practice the assessment of the grade of CIN lesions involves several criteria such as the size of the nuclei, the pleomorphism, the staining intensity, the pattern of the chromatin, the appearance of nucleoli and/or vacuoles, mitotic figures, and the extent of these changes in relation to the thickness of the mucosa. Two of these criteria, the chromatin clumping and the structure of nucleoli, have been assessed by measuring the lacunarity of chromatin texture and by using the spectral dimension and the Minkowski dimension in fine-needle aspirations of breast tissue [27]. It was noted that, even if objects have the same fractal dimension, they may differ in their texture as evaluated by a parameter such as lacunarity. Another valuable parameter is the determination of the fractal dimension of the epithelial–connective tissue border, as it was shown for dysplastic lesions of the oral mucosa [20]. A combination of these measurable parameters would yield an objective index which supports the individual assessment of epithelial irregularities by the pathologist.

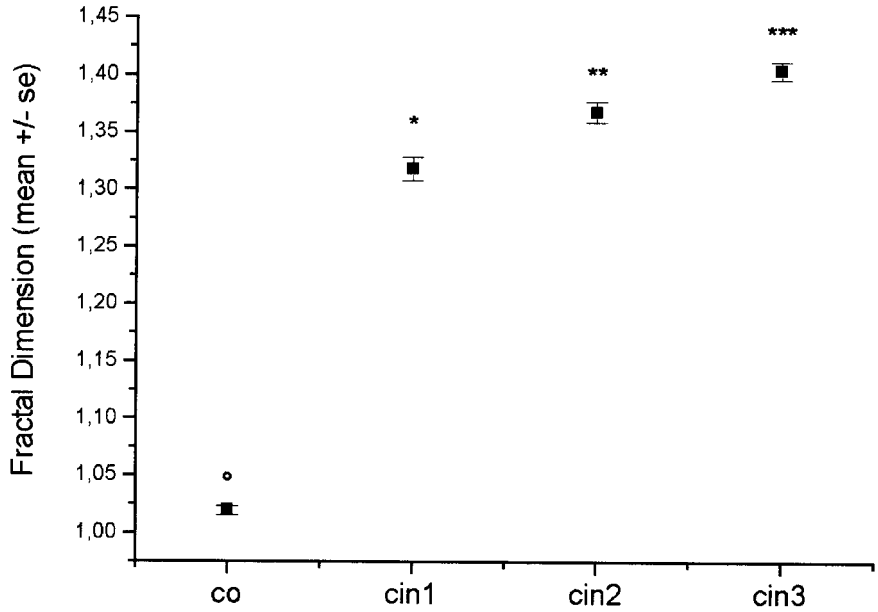

FIG. 5. Differences in categories. Significantly larger fractal dimension of atypical nuclei than of control nuclei: control/CIN 1–3 (°/*/**/*** $P = 0$). Significantly larger fractal dimension CIN 1/CIN 2 (*/** $P = 0.003$), CIN 1/CIN 3 (*/*** $P < 0.0002$), CIN 2/CIN 3 (**/*** $P = 0.0024$).

A further advantage of fractal analysis is its scale independence. A previous study has shown that usual shape descriptors (e.g., form factor, shape factor) vary significantly with magnification in dysplastic lesions of the oral mucosa [23]. Therefore, fractal dimension could be used at different levels of magnification, which still provides a discriminant and comparable parameter. Recently, it was shown that a changed fractal dimension was associated with functional changes of breast cancer cells [28]. Thus, fractal analysis not only has potential for providing a measure of irregularity in shape of nuclei but also reflects the corresponding functional status of the cell. Fractal analysis could, therefore, be used to investigate hormonal influences that may change the structure of nuclei of the cervical epithelium.

In summary, this study has demonstrated a reliable method for distinguishing between control nuclei and atypical nuclei of cervical dysplasia. This finding presents the opportunity to set a cutoff point of fractal dimension above which a nucleus can be identified safely and objectively as atypical. The application of such a cutoff point would contribute to automated screening techniques in cervical smears. Fractal analysis provides a new objective assessment important for the screening of cervical smears in which the computer can discriminate accurately healthy from atypical samples. We also found that it may be possible to distinguish between dysplastic categories using this observer-independent method. In addition, we can see a further potential of the method by adding other parameters, such as lacunarity, which may also be helpful in the assessment of cervical smears.

ACKNOWLEDGMENT

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