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Fractal dimension of hepatocytes' nuclei in normal liver vs hepatocellular carcinoma (HCC) in human subjects - preliminary results

ABSTRACT

Background: *With the aim of determining the fractal dimension, as an expression of quantitative measure of nuclear shape, in normal and malignant hepatocytes, the box-counting method was performed, followed by linear approximation of necessary numeric values by the first degree polynoma.*

Materials and methods: *A total of 308 human hepatocytes' nuclei on digitized microscopic images of histological sections (119 normal and 189 hepatocellular carcinoma nuclei) were submitted to fractal analysis, using haematoxylin-eosin stained liver biopsy specimen.*

Results: *The obtained mean fractal dimension for nuclei of normal hepatocytes was 1.0521±0.16 versus nuclei of malignant hepatocytes, where it was 1.15299±0.126.*

Conclusion: *The average values of the nuclear fractal dimensions presented, among normal and malignant hepatocytes are statistically significantly different(p<0.0039).*

Key words: *Hepatocellular carcinoma; Nucleus; Fractal analysis; Mathematical model; Humans*

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INTRODUCTION

Although the concept and rules of fractal geometry (1) have been used in basic research for several years recently, the transition between aesthetic contemplation of fractal images and the application of fractal geometry to the analysis of naturally-occurring images has been relatively slow (2). The basic concepts of fractal geometry hold that natural objects (including microscopic images) are not regular sets of points (lines, circles, rectangles, cubes, etc.) and cannot be described well enough using ideal constructions of Euclidean geometry. As far as an important characteristic of fractal geometry is the property of "self-similarity", in a statistical sense, they should have a constant amount of detail as the image is viewed at different (higher and higher) levels of magnification, i. e. at different range of scale (3,4). These facts have been used for developing methods for the ˝complexity measuring˝ of different natural shapes (cells, particles, geographical objects, etc) (5-7,2,9-14). In other words, shapes can be measured and expressed as comprehensible digits that represent their measure of regularity or irregularity, thereby becoming comparable to the other ones.

The intent of this paper is to report evidence that hepatocytes from both, normal and malignant liver biopsy sources have a fractal nature, and that the fractal dimension, as a quantitative measure of cell's shape complexity, could serve as a valid tool for the determination of their characteristics and distinction to different groups.

The expected results, concerning cell shape complexity, were that the fractal dimensions of the same cell type differ in their characteristics, depending whether they belong to normal or malignant cells, which is to be a numeric evidence of cell shape complexity and cytoarchitectonic characteristics. Opposite to this assumption, we learned that some authors found no statistically significant morphometric differences among nuclear size of cirrhotic liver, borderline lesions and HCC or normotrabecular HCC and cirrhotic or normal nuclei (15,16).

New facts offered by the fractal dimension itself, as quantitative feature of cell morphology, could add and offer more relevant parameters and better insight into the mode of nuclear behavior in specific situations, too.

MATERIALS AND METHODS

Histological method

Patients who had undergone liver biopsy were selected either as suspects for hepatocellular carcinoma - HCC (23 patients) or patients who had undergone liver biopsy because of sarcoidosis or lymphomas, and whose pathohistological findings proved to be completely normal (17 patients). The material was obtained by fine needle liver biopsy or ultrasound guided liver biopsy. All biopsy samples were fixed in alcohol routinely processed, cut at 6 mm and stained with haematoxylin eosin.

Analytical method

For further cell image analysis, the following hardware line was used: light microscope - CCD video camera - analog black-and-white monitor - digitizer - personal computer (PC) connected to a printer.

Image data acquisition and digitalization

Isolated nuclear shapes of normal and malignant liver cells were found by a "Polyvar" Reichert-Jung light microscope with standard magnification (objective:10x20). Images were captured by a CCD video camera ("Sanyo" VDC 3824 F, #95750810) with the standard lens

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mounted on a photographic stand. Images captured via the cine port of the microscope were switched over to the black-and-white monitor in the form of analog signals. The monitor displays a real-time analog image, which permits focusing and positioning of the sample and also allows adjustment of light conditions such as illumination intensity, iris opening of the lens, and brightness/contrast controls of the digitizer. The BIOMED (*Advanced American Biotechnology & Biomed Instruments, Inc, Fullerton, California, USA*; user #021708607) digitizer was used (512x512 pixels, 8 bits, optical density (OD) range 0-255), which was inserted into the personal computer. The input port of the digitizer was connected directly to the black-and-white monitor. After the adjustment of optimal conditions, two referent spots of the microscopic image on the analog black-and white screen were determined: spots of the highest and the lowest OD using manual modification of this automated function. After digitization of analog microscopic image, further analysis was performed on the digitized image (Figure 1 and 2). The OD determination of all the points of interest (points that present nuclear

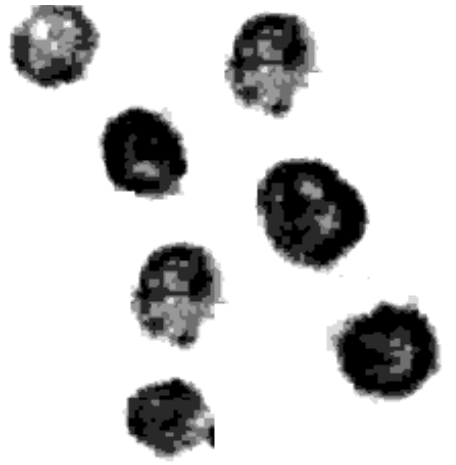

Figure 1. Isolated nuclear shapes of normal liver cells after digitization of analog microscopic image and contour subtraction of the 'noisy' background

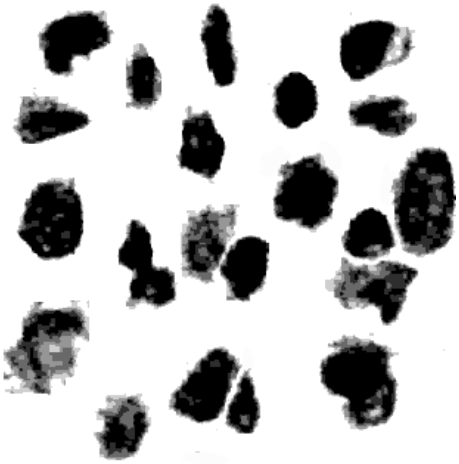

Figure 2. Isolated nuclear shapes of HCC liver cells after digitization of analog microscopic image and contour subtraction of the 'noisy' background

contour- silhouette) was performed followed by the contour subtraction of the ″noisy″ background cytoplasm). No further modifications were made with respect to any normal variability of individual cell morphology, in order to avoid any bias resulting from geometrical transformations of the original image data. In this way, the nuclear contour was saved on the PC's hard drive.

The digitized images were changed using Marr-Hildreth convolution algorithm (17, 18.) from gray scale to binary silhouettes. Silhouettes that became the objects of further quantitative studies were used to obtain a one-pixel-wide border of the cell image.

Applied fractal analysis

The fractal dimensions of normal and malignant nuclei were determined and studied by box-counting (square-covering) method (1, 19-22). The registered binary border-image of each nucleus, was expressed with an ensemble of 512 x 512 pixels, and transformed from the gray level of 0-255. The binary border to be analyzed was superimposed on a succession of square grids, containing a successive ascending number of squares (with a decreasing edge length for each of them). The number of grid squares (boxes, tiles) that the cell border-image contacts (no matter how many pixels of the border it contains) was counted. The log number of squares encountered was plotted against the log of the box edge length, as given by:

$$D_B = \lim_{\varepsilon \to 0} \frac{\log N(\varepsilon)}{\log(1/\varepsilon)}$$

where D_B is the box-counting fractal dimension of the nuclear image is in binary form, ε so as the side length of the box, and N(ε) is the smallest number of boxes with side length ε required to completely cover the outline of the object being measured (20, 22). In a log-log plot of the $(1/\varepsilon)$ (X-axis) versus N(ε) (Y-axis) the linear approximation of the first-degree polynoma (Y=A+BX) was performed, where the slope (B) of the least square fitted line presents the box-counting fractal dimension (Figure 3 and 4). To evaluate the worth of the fit of regression lines, the coefficient of determination was used (higher values of which indicate better fit). The range of data points which provided the value of the coefficient of determination of 0.99 was assumed to be the range in which present materials show self-similarity). All these processes were performed on a personal computer system (Intel, PC-80486), using Molecular Dynamics Image Quant (3.3) and MicroCal Origin (3.0) (MicroSoft Windows key No 422-0465974).

RESULTS

The obtained mean fractal dimension for nuclei of normal and malignant hepatocytes are shown in Table 1.

Our results were statistically evaluated by a

Table 1. Basic statistical parametars on fractal dimensions of normal malignant nuclei

Summary table of means (index.sta) N=308 (No missing data in dep. var. list)			
Nuclei	N°	Mean	sd
Normal	119	1.10521	0.160
Malignant	189	1.15299	0.126

one-way analysis of variance (ANOVA) regarding the fractal dimension of nuclear shape in normal and malignant nuclei as the variable category (Table 2).

The results showed that, at a significance

Table 2. One-way analysis of varience (ANOVA) regarding the fractal dimensions of nuclear shape in normal and malignant nuclei as the variable category

Analysis of Variance (index.sta)

Marked effects are significant at p<0.050

SS Effect	df Effect	MS Effect	SS Error	df Error	MS Error	F	p
0.16666	1	0.16666	6.01772	306	0.019666	8.474789	0.003866

level of 1% (p=0.000368 0.01), there was a significant difference among both groups containing analyzed nuclei in (normal and malignant) bioptic specimens. Using the test of parallelisms of regression lines, statistically significant difference between two analysed regression lines (Figure 3 and 4) was found (t=2.22 ($t_{26,0,0.5}$=2.056), at a significance level of 5%. The

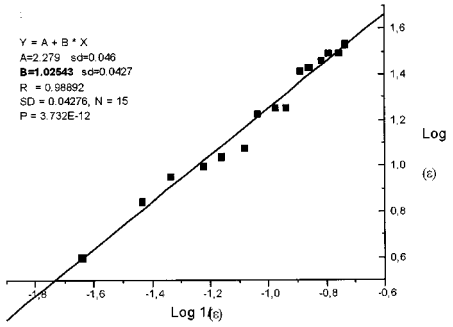

Figure 3. Linear regression on the data obtained by successive block counting (ascending number of frames) of a nucleus of normal hepatocyte.

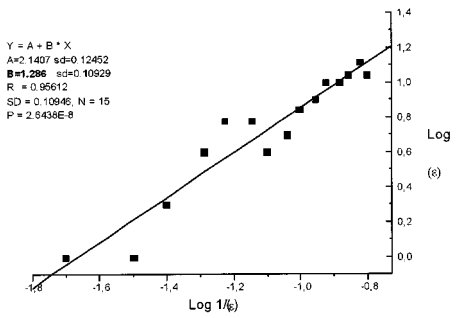

Figure 4. Linear regression on the data obtained by successive block counting (ascending number of frames) of a nucleus of HCC.

frequency distribution of fractal dimensions of malignant and normal cells shows normal distribution in both groups (Figure 5 and 6).

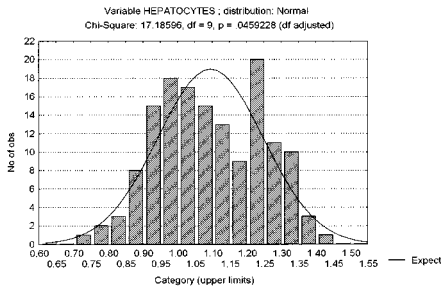

Figure 5. The frequency distribution of fractal dimensions of normal hepatocytes.

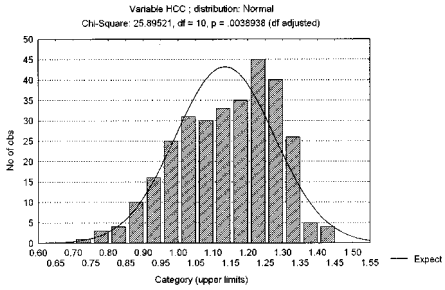

Figure 6. The frequnecy distribution of fractal dimensions of malignant cells.

DISCUSSION

Method applied

The method for digitizing the microscopic image and its immediate use as a medium for the application of the fractal analysis of natural shapes is innovative. This method was created on the basis of available data from the references and by modifying the existing equipment. Thus, it was necessary to check the validity of the method applied in two specific ways:

a) In terms of testing the reliability of the digitalization of the microscopic image as a method for presenting the two-dimensional natural shape, and

b) In terms of confirming the modified method of fractal analysis.

The necessary tests were done using similar methods:

a) By utilizing the method described above, a fractal analysis was performed on an undigitized microphotograph of a rat's cerebellar Purkinje cell (23). Using the applied method in this case, the fractal dimension value obtained for the observed cell was D_B=1.56559. According to the reference data (19), where the fractal dimension of a similar cell from an European hamster was D_B=1.550, the estimated error was 1.00%.

b) In order to show the accuracy of the applied modified system for the evaluation of fractal dimension, the fractal analysis of some typical geometrical figures and mathematically described fractal patterns was performed using the same analytical procedure. The values of the determined fractal dimensions were compared to theoretically expected values. The determined fractal dimensions and observed errors are presented in Table 3.

At present we did not classify our patients with HCC into the groups according to histological grading. It would be of interest to see whether further sub classification into such groups would lead to further differences. This will request more patients and measurements, which we intend to complete in future.

Tabela 3. Calculated fractal dimensions of some geometrical figures versus fractal dimensions determined by present system.

Subject	Expected D_B	D_B	R^2	Error (%)
Line	1.0000	0.98930 ± 0.02300	0.998	1.07
Circle	1.0000	1.04050 ± 0.11467	0.998	4.05
Koch snowflake	1.2618	1.22183 ± 0.02475	0.999	3.17
Sierpinski carpet	1.8928	1.81823 ± 0.07034	0.998	3.94

In the measurements of this kind, one would expect comparison of the nuclear fractal dimensions of normal and malignant hepatocytes from the same source i.e. bioptic material from both HCC and normal tissue obtained by ultra sound guided needle biopsy from the same patient. However, the high incidence of cirrhotic alteration of such lesions (as HCC, which is mostly found on cirrhotic ground) could affect and mislead the conclusions based on such measurements. Hence, the selection of patients whose pathohistological findings proved to be completely normal seems to be a reasonable approach. Nevertheless, this group of cirrhotic patients, (regarded as a transitional group from normal towards HCC) will be the subject of our further investigation.

CONCLUSION

The fractal dimension determined for a two-dimensional digitized image of human hepatic nuclei is an objective measure of the complexity of their shape, which undoubtedly indicates the fractal nature of this cell type. Our measurements clearly show that there is a significant statistic difference between nuclei of normal hepatocytes vs nuclei of HCC. These measurements could be used for distinction of shape regularity and/or unregularity since unusual and bizzare nuclear shapes are one among other attributes of malignancy. The fractal analysis applied on digitized cell shapes is a reliable method for cell complexity measurment that can be used alone or as an additional parameter along with morphometrical measurements both in routine work and research.

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