Trakia Journal of Sciences, Vol. .5, No. 1, pp 65-68, 2007

Available online at:
http://www.uni-sz.bg

ISSN 1312-1723

Original Contribution

COMPUTER-ASSISTED FRACTAL ANALYSIS OF SPONTANEOUS CANINE MAMMARY GLAND TUMOURS ON CYTOLOGIC SMEARS

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ABSTRACT

Fifty-two spontaneous canine mammary gland tumours (fibroadenomas, tubulopapillary carcinomas, solid carcinomas, anaplastic carcinomas, fibrosarcomas, liposarcomas and osteosarcomas) were selected and analysed by computer-assisted nuclear fractal analysis on Hemacolor® stained cytologic specimens. Computerized cytomorphometry was performed and the fractal dimension of the studied nuclear surfaces was assessed. A minimum of hundred nuclei per lesion was examined. The statistical analysis revealed significant differences between benign and malignant neoplasms, but not between different types of malignant canine mammary gland neoplasms. The results indicated that computer-assisted fractal analysis could be used as an additional method for differentiating between benign and malignant canine mammary gland tumours on cytologic specimens.

Key words: cytology, computer-assisted fractal analysis, canine mammary gland tumours

INTRODUCTION

Usually, the structure of an object can be described utilizing tools of Euclidian 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. The fractal geometry is the branch of mathematics, which studies the properties and behaviours of fractals. The word "fractal" was introduced for the first time by Mandelbrot from the Latin *fractus* meaning broken (1, 2, 3). Fractal objects are mainly characterized by four properties: a) the irregularity of their shape; b) the self-similarity of their structure; c) their non-integer or fractal dimension, and d) scaling, which means that the measured properties depend on the scale at which they are measured (4 , 5, 6). 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 (7).

Fractal analysis techniques are common tools in physics and image processing. Recently, the interest in this analysis in medical science, especially in oncology has been progressively increasing (2, 6, 8, 9, 10, 11). The latest investigations in medicine have shown the usefulness of fractal geometry in diagnosis of tumours of the reproductive system (12, 13), colonic cancer (14), bone marrow tumours (15), skin disorders (10, 11, 16) and breast lesions (17, 18, 19, 20, 21, 22, 23). The goal of the present study was to investigate whether computer-determined fractal dimension could be used as an additional tool for distinguishing between benign and malignant canine mammary gland tumours on cytologic specimens.

MATERIAL AND METHODS

The study was performed on fifty-two spontaneous canine mammary gland tumours. The tumours were selected from the Department of Surgery, Faculty of Veterinary Medicine, Trakia University, Bulgaria. A total of 52 tumours was included in this study and

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tumours were distributed as follows: fibroadenomas (n=8), tubulopapillary carcinomas (n=9), solid carcinomas (n=6), anaplastic carcinomas (n=7), fibrosarcomas (n=9), liposarcomas (n=9) and osteosarcomas (n=4). Tumour cells were preoperatively obtained by fine-needle aspiration biopsy, immediately fixed with Merckofix® spray *(Merck®, Darmstadt, Germany)* and stained with Hemacolor® *(Merck®, Darmstadt, Germany)*. The fine-needle aspiration biopsy was performed by sampling cells from four different areas of tumour formations. Subsequently all tumours were histopathologically confirmed according to WHO International Histological Classification of Tumours of Domestic Animals (24). The digitised images of the cytologic findings were captured by Motic Professional B3 digital microscope *(Motic®, China Group Co, Ltd*). The computer used was equipped with 2.00 GHz Celeron® Intel® processor with 256 Megabits of RAM and 17-inch monitor (*Samsung Electronics, Slovakia Ltd, Galanta, Slovakia*). The images created by the computer system were formatted as jpeg files. The magnification and the resolution used were 1000x and 1024/768 pixels in all cases. Only intact, non-overlapping nuclei and those with easily detected boundaries were analysed. Computer-assisted morphometry of randomly selected nuclei was automatically performed by image analysis program Image Pro Plus® (*Image Pro Plus 4.5.0.29. for Windows 98/NT/2000, Media Cybernetics, USA*). A minimum of hundred nuclei per lesion was examined. The investigated morphometric parameter was fractal dimension. Computer-assisted fractal analysis of selected nuclei was performed automatically by Image Pro Plus. When the quality of digitised images was not optimal, we used the segmentation function. Segmentation is a process by which certain colours (and subsequently objects of interest, such as nuclei) in an image can be visually identified, and then isolated from the image as whole. The Image Pro Plus program uses a modification of the "hand and divider" method ("perimeter-stepping" method) for the calculation of fractal dimension. The fractal dimension is defined as the slope of the linear part of the function that relates the log of the outline length to the log of the "stride" length, that is, the steps taken in marking the perimeter of the object. For closed curves in the plane, this dimension is always >1 (a perfect circle) and is always <2 (values >1 indicate irregular shapes). In our study we did not threshold the images to produce a binary image before calculation because the program computed fractal dimension without the need for "binarisation" of digital images. The data from computerized cytomorphometry were analysed using the ANOVA/LSD test *(Statistica 6.0, StatSoft, USA)* at a level of significance $p < 0.05$.

RESULTS

The mean fractal dimension (mean ± standard deviation) of fibroadenomas was 1.049 ± 0.009, tubulopapillary carcinomas - 1.057 ± 0.004, solid carcinomas - 1.061 ± 0.005, anaplastic carcinomas-1.061 ± 0.004, fibrosarcomas- 1.056 ± 0.006, liposarcomas - 1.059 ± 0.051 and osteosarcomas – 1.057 ± 0.004. The data from statistical analysis are presented on **Table 1**.

The statistical analysis revealed significant differences between malignant and benign tumours, but not between different types of malignant canine mammary gland neoplasms. Analysis of our results showed that the fractal dimension had significance between malignant and benign tumours, but not between different types of malignant canine mammary neoplasms. The most significant differences were found between fibroadenomas and solid carcinomas, and fibroadenomas and anaplastic carcinomas ($p<0.001$). The comparison of fractal dimension between fibroadenomas and liposarcomas showed less significance ($p<0.01$). Among the groups of fibroadenomas, tubulopapillary carcinomas, fibroadenomas, fibrosarcomas, fibroadenomas and osteosarcomas, significance was the lowest ($p<0.05$).

DISCUSSION

Because of the absence of information on morphometric parameter of fractal dimension in canine mammary gland tumours, we compared our results with selected studies in human medicine. Sedivy and Windischberger (1998) applied fractal analysis to mammography as well as the histologic sections of breast carcinomas. Their results showed a reliable difference between benign and malignant breast tumours. Grizzi et al. (2001) studied fractal dimension and coefficient of roundness, two mathematical descriptors of irregularly shaped objects, in order to discriminate between 12 benign and 11 malignant mammographic lesions. Morphometrical and fractal analysis of the breast lesions were automatically performed

by a computer-assisted image analysis system. Their results showed that fractal geometry allowed quantitative measurements of the complex morphology of benign and malignant mammographic lesions. Ohri et al. (2004) examined fractal dimension in randomly selected fine needle aspiration cytological smears of 42 infiltrating duct carcinomas and 38 fibroadenomas of the breast. The Mann-Whitney U test showed a significant difference in fractal dimension between these two groups. The authors concluded that fractal dimension might be a helpful discriminatory tool to distinguish benign and malignant cells. The results from research of Dey and Mohanty (2003) were similar. They studied fractal dimension of fine-needle aspiration cytological smears of 14 cases of histopathologically proven infiltrating duct carcinomas and 7 cases of fibroadenoma of the breast. Computer-assisted image analysis indicated a significant difference in the investigated parameter of malignant versus benign cells; Mann-Whitney's nonparametric test was used again. The authors confirmed that measurements of fractal dimension might be helpful in differentiating between malignant and benign cells.

Table 1. *Values of fractal dimension in canine mammary gland fibroadenomas, tubulopapillary carcinomas, solid carcinomas, anaplastic carcinomas, fibrosarcomas, liposarcomas and osteosarcomas*

Groups	***Value***		***Significance of differences (P)***						
	N	*Mean ± SD*	*FA*	*TC*	*SC*	*AC*	*FS*	*L*	*O*
Fibroadenoma (FA)	8	1.049±0.009	-	*	***	***	*	**	*
Tubulopapillary carcinoma (TC)	9	1.057± 0.004	*	-	-	-	-	-	-
Solid carcinoma (SC)	6	1.061 ±0.005	***	-	-	-	-	-	-
Anaplastic carcinoma (AC)	7	1.061 ±0.004	***	-	-	-	-	-	-
Fibrosarcoma (FS)	9	1.056 ± 0.006	*	-	-	-	-	-	-
Liposarcoma (L)	9	1.059 ± 0.051	**	-	-	-	-	-	-
Osteosarcoma (O)	4	1.057 ±0.004	*	-	-	-	-	-	-

*Level of significance (P) of differences among groups using ANOVA/LSD test (*p<0.05, **p<0.01, ***p<0.001)*

In conclusion, this study demonstrated that the parameter of fractal dimension could be used as an additional method for distinguishing between benign and malignant canine mammary gland tumours on cytologic specimens. The objectivity of fractal analysis depends on the cell processing and on the use of the analysis software (23). Therefore, it is necessary to standardize the fixation and staining methods before performing each morphometric analysis and also to standardize the computer-assisted techniques and the estimated fractal dimension in the biologic objects (6, 22). In the present study, standardisation of the cell fixation was performed by immediate fixing of the cytologic material after obtaining the cells with Merckofix spray (Merck). These fixatives protect cells with polyglycol film for several weeks. Larger studies are needed to set a threshold of fractal dimension value above which the nucleus can be safely and objectively identified as malignant. This application could contribute to the introduction of automated techniques in veterinary oncology.

REFERENCES

1. Coffey, D. Self-organization, complexity, and chaos: the new biology for medicine. *Nat Med*, 4: 882-885, 1995
2. Gross, S. Fractals in pathology. *J Pathol*, 182: 1-8, 1997.
3. Mandelbrot, B. *The fractal geometry of nature.* Freeman, New York, USA, 1983.
4. Losa, G. Fractals in pathology: are they really useful? *Pathol*, 87: 310-317, 1995[a].
5. Losa, G. Fractal morphometry of cell complexity. *Riv Biol*, 95: 239-258, 1995[b].

6. Losa, G., Nonnemacher, T. Self-similarity and fractal irregularity in pathologic tissues. *Mod Pathol*, 9: 174-182, 1996.
7. James, W., Rajesh, K. Fractals and cancer. *Cancer Res*, 60: 3683-3688, 2000.
8. Guarini, G., Onofri, E. New horizons in medicine. Fractals. *Rec Prog Med*, 84: 438-442, 1993.
9. Landini, G. *Fractal horizons*. C.A. Pickover, St.Martin's Press, New York, 1996.
10. Landini, G., Rippin, J. How important is tumor shape? Quantification of the epithelial-connective tissue interface in oral lesions using local connected fractal dimension analysis. *J Pathol*, 179: 210-217, 1996[a].
11. Landini, G., Rippin, J. Quantification of nuclear pleomorphism using an asymptotic fractal model. *Anal Quant Cytol Histol*, 18: 167-176, 1996[b].
12. Sedivy, R., Windischberger, C., Svozil, K. Fractal analysis: An objective method for identifying atypical nuclei in displastic lesions of the cervix uteri. *Gynec Oncol,* 75, 78-83, 1999.
13. Dey, P., Rajesh, L. Fractal dimension in endometrial carcinoma. *Anal Quant Cytol Histol*, 26: 113-116, 2004.
14. Esgiar, A., Naguib, R., Sharif, B. Fractal analysis in the detection of colonic cancer images. *IEEE Trans Inform Technol Biom*, 6: 54-58, 2001.
15. Moatamed, F., Sahimi, M., Naeim, F. Fractal dimension of the bone marrow in metastatic lesions. *Hum Pathol*, 29: 1299-1303, 1998.
16. Landini, G., Rippin, J. Fractal dimension of the epithelial-connective tissue interfaces in premalignant and malignant epithelial lesions of the floor of the mouth. *Anal Quant Cytol Histol*, 15: 144-149, 1993.
17. Lefebvre, F., Benali, H. A fractal approach to the segmentation of microcalcification in digital mammograms. *Med Phys*, 22: 381-390, 1995.
18. Pohlman, S., Powel, K., Obuchowski, N. Quantitative classification of breast tumor in digitalized mammograms. *Med Phys*, 23: 1337-1345, 1996.
19. Velanovich, V. Fractal analysis of mammographic lesions: a feasibility study quantifying the difference between benign and malignant masses. *Am J Med Sci*, 311: 211-214, 1996.
20. Sedivy, R., Windischberger, C. Fractals analysis of a breast carcinoma-presentation of a modern morphometric method. *Wien Med Wochen*, 148: 335-337, 1998.
21. Grizzi, F., Muzzio, P., Maggio, A. Geometrical analysis of benign and malignant breast lesions. *Radiol Med*, 101: 432-435, 2001.
22. Dey, P., Mohanty, S. Fractal dimension of breast lesions on cytology smears. *Diag Cytopathol*, 29: 85-86, 2003.
23. Ohri, S., Dey, P., Nijhawan, R. Fractal dimension in aspiration cytology smears of breast and cervical lesions. *Anal Quant Cytol Histol*, 26: 109-112, 2004.
24. Misdorp, B., Else, R., Hellmen, E. Histological classification of mammary tumors of the dog and cat. WHO International Histological Classification Of Tumors of Domestic Animals, 2 nd series, Vol VII, Washington DC: Armed Forces Institute of Pathology, American Registry of Pathology, 2001.