Fractal Dimension of Dog Kidney Proximal Convoluted Tubuli Sections by Mean Box-Counting Algorithm

Dimensión Fractal de Secciones de los Túbulos Contorneados Proximales del Riñón Mediante un Algoritmo de Recuento de Cajas

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SUMMARY: The possibility of characterizing the proximal convoluted tubuli of the dog kidney by means of a unique and objective value, is an attractive idea in order to automate its recognition in anatomy and patology.

For this, we obtained a fractal dimension of the proximal convoluted tubuli in the dog kidney by mean box-counting method. The fractal value we obtained is a flat dimension, and it is different than the line fractal dimension of the dog kidney arterial pattern, which was previously calculated by us (Gil *et al.*, 2006). Thus, from optical microscopy images, we are able to obtain a single quantitative measure to discriminate these dog kidney components. This measures can use to automate its recognition.

Fractal geometry provides many advantages when examining the complex microscopical images of natural objects.

KEY WORDS: Fractal; Kidney; Proximal convoluted tubuli.

INTRODUCTION

In the cortex of each kidney is located the pars convoluta of the nephron, which consists of renal corpuscles and numerous proximal and distal convoluted tubules (Fig.1). The possibility of being able to characterize a morphological structure, like the proximal convoluted tubuli, by means of a single unique and objective value, for example a number. It could be an attractive idea, for instance to automate its recognition by mean a computer. In the kidney, calculation of the fractal dimension may open this way (Gil *et al*., 1992, Gil *et al.*, 2006).

However, application of fractal geometry to the analysis of naturally-occurring images has received relatively poor attention. This analysis can only be useful in natural structures if they hold fractal properties (Cross, 1994; Canals *et al.*, 1998).

We know the proximal convoluted tubule has a fractal lineal geometry (Gil *et al.*, 2006), but is it the only one possible?

MATERIAL AND METHOD

For the purpose of this study we have used five crossbred male adult dogs weighing 20 kg each, provided by the local Service of Animal Protection. The animals was euthanatized by mean the protocol approved by Zaragoza University Animal Care Committee.

Afterwards, each abdominal cavity was opened and the left kidney was extracted and fixed. The fixation was carried out at room temperature for seven days in a standard fixative solution of buffered formol 10%. We obtained four pieces from each kidney cortex. Later, pieces of renal tissues were handed following conventional procedures for optical microscopy. Sections 5 µm thick were stained with Hematoxilin and Eosine (Merck).

The twenty X400 microscopy pictures showing proximal convoluted tubuli like in Fig. 1, were digitalized by means of a Hewlett Packard ScanJet IIc. Each picture was obtained from different places within the kidneys cortex. We chose only convoluted tubuli to feel queer

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perpendicularly. Slant or folding convoluted tubuli were refused.

Coordinates of the outline image of each proximal convoluted tube were obtained with AtlasMapMaker 4.03 program (Strategic Mapping Inc).

We have calculated the fractal dimension of the proximal convoluted tubule with a computer program designed by us, using the box-counting method. Our program was tested on the Koch curve. The program was written in Pascal (Symantec v. 4.0.2).

With box-counting method, the outline image of each proximal convoluted tubule was utilized. These plane images areas were covering with grids of different mesh sizes. Then we compared the grid sizes and the number of squares containing at least part of the image (Fig. 2).

After that, a log-log graph of box-counting measurements is made. The X axis is the box size. Fractal dimension is the negative slope of the regression line calculated from de plot of log-log graph (Fig. 2).
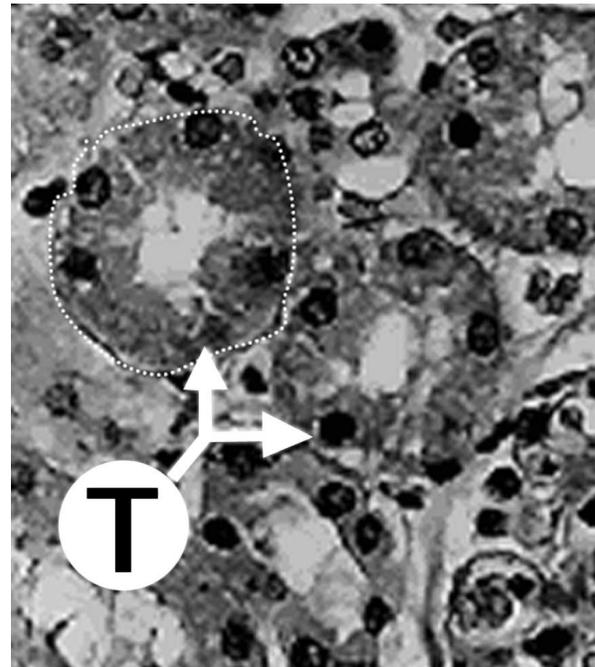

Fig. 1. Cortex of kidney dog. T. Proximal convoluted tubuli, left one has its outline surrounded. H-E. X400.
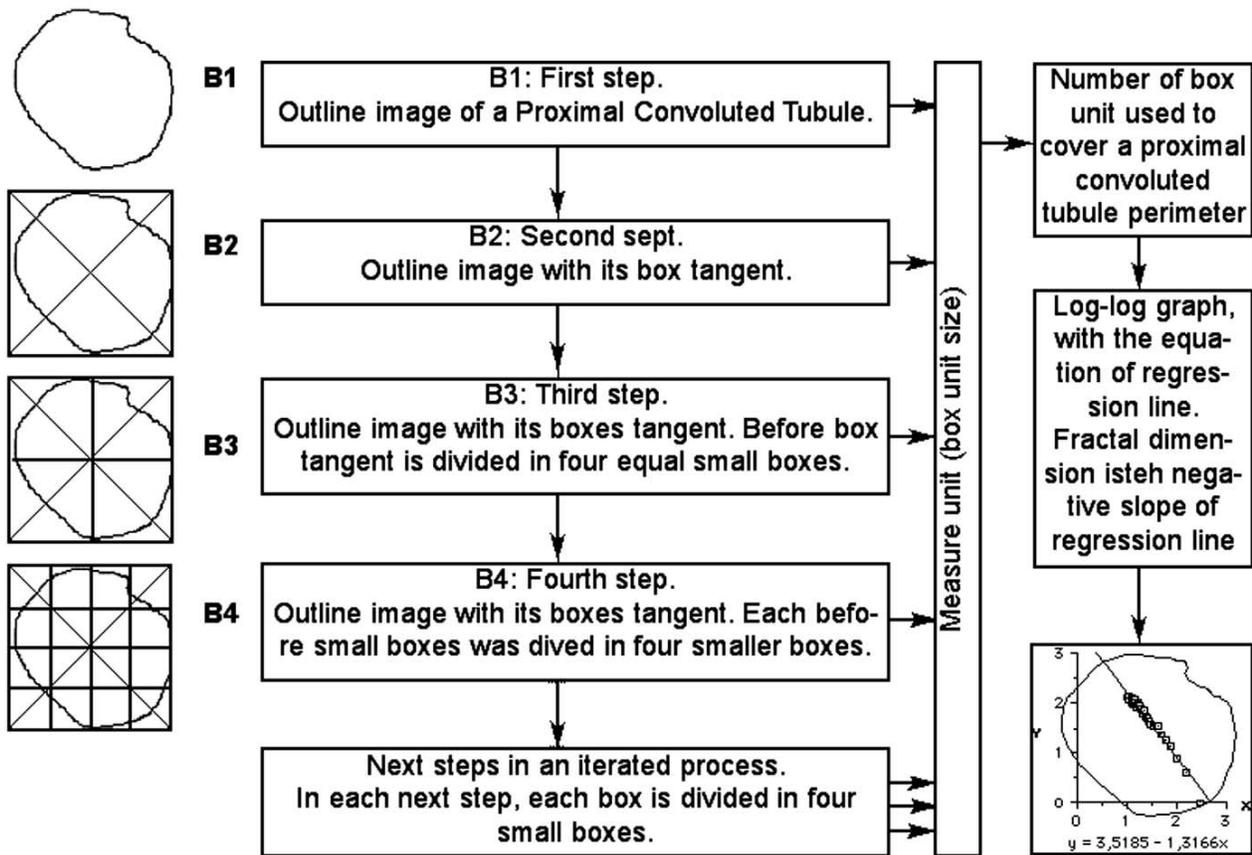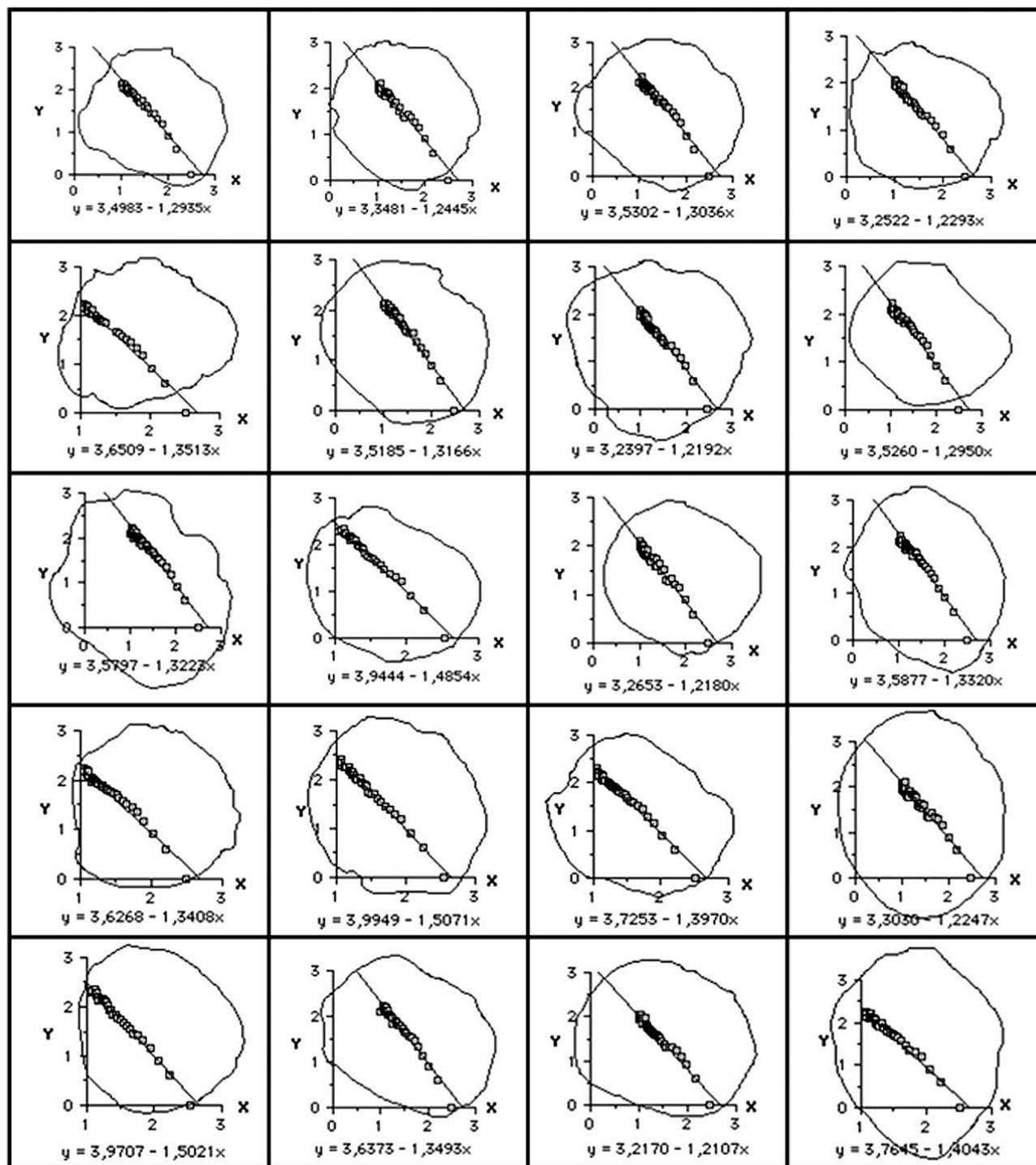

Fig. 2. Box-counting method.

Fig. 3. The twenty one outline images of dog proximal convoluted tubuli and its log-log graphs, with the equation of each regression line. X axis is the log box size and Y axis the log number of outline-containing squares.

Furthermore, our program gave us the perimeter and the area of each proximal convoluted tubule outline image. Graphics and statistical calculations were made with StatView SE+ (Abacus) and Cricket Graph III (Computer Associates International Inc), programs. All computer tasks were made with a Macintosh IIfx computer.

RESULTS

All the dog kidneys used in this study showed a normal, healthy appearance. The twenty proximal convoluted tubuli analyzed and the calculations of their fractal dimensions, perimeters and areas, are shown in Fig. 3 and Table I. All calculations were made in pixel unit. A pixel is the smallest dot in screen computers. In our computer we have 72 pixels per inch.

The fractal dimension of the proximal convoluted tubule of the dog kidney that we have obtained is 1.33 ± 0.18 with 95% security coefficient (Table II). Calculations were made with a precision of 0.04.

The concept of fractal dimension encloses a certain complexity because of its nature is far of our usual experience in perception of the space. With the aim of finding some possible relationships with other measures which we are usually more accustomed with, we have calculated a statistical index on convoluted tubuli areas and perimeters (Table II).

And we have calculate correlation coefficients between fractal dimensions, areas and perimeters (Table III). We have obtained very high correlation coefficients 0.8 and 0.9.

Table I. Set of measurements obtained from twenty one proximal convoluted tubuli outline images. (A pixel is the smallest dot in screen computers. In our computer we have 72 pixels per inch).

LEFT KIDNEY	FRACTAL DIMENSION	PERIMETER (pixels)	AREA (pixels^2)
	1.2935	597	32605
1	1.2445	604	34620
	1.3036	566	32290
	1.2293	605	32032
	1.3513	646	35167
2	1.3166	618	36216
	1.2192	583	33393
	1.2950	604	34569
	1.3223	614	35232
3	1.4854	634	41007
	1.2180	614	34880
	1.3320	645	38688
	1.3408	599	33548
4	1.5071	669	44009
	1.3970	649	39484
	1.2247	540	30375
	1.5021	702	43586
5	1.3493	625	36299
	1.2107	562	31341
	1.4043	629	38267

Table II. Statistic calculations on fractal dimensions

Statistic calculation	Fractal dimension	Perimeter	Area
Count	20	20	20
Mean	1.33	615.25	35880.4
Maximum	1.51	702	44009
Minimum	1.21	540	30375
Range	0.3	162	13634
Std. Dev.	0.09	37.66	3871.59
Std. Error	0.02	8.42	865.71
Variance	0.01	1418.41	149849235.31
Coef. Var.	7.06	6.12	10.79

Table III. Correlation Coefficient.

Correlation coefficient between	Value
Dimension fractal and perimeter	0.8
Dimension fractal and area	0.9
Perimeter and area	0.9

DISCUSSION

Functionally, the renal tubule is made up of highly metabolically active cells, and functional disorders are more often. Proximal convoluted tubules are distinguished by the brush borders of their epithelial cells, and somewhat scalloped appearance of the apical surface of their cells when the latter are seen in profile (Bacha & Wood,1990). Any pathological lesion affecting one nephron component, will usually have an impact on the other. Tubular epithelial cells have considerable powers of recovery and regeneration, and acute renal failure may be reversible under such circumstances.

The tubular components of the nephron may be primarily damaged as a result of hypovolaemic shock, by inorganic and organic toxins, or as the result of infection. (Wheather, 1985).

Nephron components are utterly dependent for their normal function on adequate perfusion by circulating blood, and if this is disrupted there are serious consequences to both nephron components.

As we showed in a previous paper, the fractal dimension of the arterial tree of the dog kidney can be measured (Gil *et al.*, 1992). In this paper, we have analyzed the flat fractal geometry of the proximal convoluted tubule and we have also calculated its fractal dimension (Mandelbrot 1985), which value is greater than Euclidian dimension (De Guzman *et al.*, 1993), suggesting that proximal convoluted tubuli are fractal objects.

The fractal dimension of the proximal convoluted tubule of the dog kidney that we have obtained is 1.33 ± 0.18 with 95% security coefficient (Table II). This value is different from the arterial fractal dimension of the dog kidney, That is 1.94 (Gil *et al.,* 1992). Consequently, we can have a single quantitative measure to discriminate each of these two dog kidney components by mean automatic tools. Also this dimension value is different from the linear fractal dimension we obtained (Gil *et al.*, 2006), logic because we used different measure unit. We think this work method is easy to use and more sure to obtain results.

Using pictures with magnification X400 for this work has a further advantage of the procedure is that, in almost all circumstances, the fractal component of dimension is retained when a fractal object is projected to a lower order dimension (Falconer, 1993)

The correlation found between fractal dimension and perimeter or area of the proximal convoluted tubule of the dog kidney, supports the idea of some characteristic natural background that may be expressing the fractal geometry of this organ in order to automatizing its identification. Many models have been developed to analyze morphological changes within organs (Caruthers & Harris, 1994). Fractal analysis based on microcomputer studies of image systems may provide many advantages to the understanding of the complex microscopical arrangement of natural objects (Cross & Cotton, 1992).

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RESUMEN: La posibilidad de caracterizar los túbulos contorneados proximales del riñón de perro, mediante un valor único y objetivo, es una idea interesante para automatizar su reconocimiento en anatomía y en patología.

Para ello, hemos obtenido la dimensión fractal de los túbulos contorneados proximales del riñón de perro, mediante la técnica de recuento de cajas. El valor fractal obtenido es una dimensión de superficie, diferente de la dimensión fractal lineal que calculamos previamente (Gil *et al.*, 2006). De esta forma, desde imágenes microscópicas, nos es posible obtener una medida simple y cuantitativa para diferenciar estos compontes del riñón de perro.

Esta medida puede usarse para automatizar su reconocimiento.

PALABRAS CLAVE:Fractal; Rinón; Túbulo contorneado proximal.

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