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• Clinical Research •

Analysis of normal human retinal vascular network architecture using multifractal geometry

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Abstract

• AIM: To apply the multifractal analysis method as a quantitative approach to a comprehensive description of the microvascular network architecture of the normal human retina.

• METHODS: Fifty volunteers were enrolled in this study in the Ophthalmological Clinic of Cluj-Napoca, Romania, between January 2012 and January 2014. A set of 100 segmented and skeletonised human retinal images, corresponding to normal states of the retina were studied. An automatic unsupervised method for retinal vessel segmentation was applied before multifractal analysis. The multifractal analysis of digital retinal images was made with computer algorithms, applying the standard box-counting method. Statistical analyses were performed using the GraphPad InStat software.

• RESULTS: The architecture of normal human retinal microvascular network was able to be described using the multifractal geometry. The average of generalized dimensions (D_q) for q=0, 1, 2, the width of the multifractal spectrum ($\Delta\alpha=\alpha_{max} - \alpha_{min}$) and the spectrum arms' heights difference ($|\Delta f|$) of the normal images were expressed as mean±standard deviation (SD): for segmented versions, D_0=1.7014±0.0057; D_1=1.6507±0.0058; D_2=1.5772±0.0059; $\Delta\alpha$=0.92441±0.0085; $|\Delta f|$ = 0.1453±0.0051; for skeletonised versions, D_0=1.6303±0.0051; D_1=1.6012±0.0059; D_2=1.5531±0.0058; $\Delta\alpha$=0.65032±0.0162; $|\Delta f|$ = 0.0238±0.0161. The average of generalized dimensions (D_q) for q=0, 1, 2, the width of the multifractal spectrum ($\Delta\alpha$) and the spectrum arms' heights difference ($|\Delta f|$) of the segmented versions was slightly greater than the skeletonised versions.

• CONCLUSION: The multifractal analysis of fundus photographs may be used as a quantitative parameter for the evaluation of the complex three-dimensional structure of the retinal microvasculature as a potential marker for early detection of topological changes associated with retinal diseases.

• **KEYWORDS**: generalized dimensions; multifractal; retinal vessel segmentation; retinal image analysis; retinal microvasculature; standard box-counting method

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INTRODUCTION

The human retinal vascular imaging, as a noninvasive research tool, can provide precise quantification of subtle retinal vascular changes associated with retinal vascular diseases[1-3].

The human retinal microvasculature is the only microcirculation with a complex topological architecture that can be observed *in vivo* using a retinal camera and can provide potentially valuable disease biomarkers[3-5].

Over the last few decades, the human retinal microvascular network is especially relevant to modern ophthalmology because it has the potential to reveal important information for detection and quantify the retinal systemic vascular diseases[1,5-7] and non-vascular pathology[8-10]. Previous studies have established that the ocular vascular diseases modify the three-dimensional morphology of retinal blood vessels and the optimal density for capillary networks[2,7,9].

The analysis of the relationship between the changes in retinal blood vessel topological parameters and retinal diseases, using different computerized methods, is used to detect and treatment of the retinal diseases in the early stages[2-3,5].

The natural morphological complexity of the eye is a major factor contributing to the limits of the application of Euclidean geometry to the study of the complex architecture of the human retinal microvasculature[7,11-13].

Digital retinal photographs of the complex patterns structures of the human retinal microvasculature that can not be described and measured properly in a correct manner using Euclidean geometry can be characterized using fractal[8,11-12,14-22] and multifractal geometry[13,23-26].

In ophthalmology, the fractal and multifractal geometries offer a mathematical approach for the quantification of the human retinal microvasculature, over a range of spatial, temporal, and organizational scales[7,14,22-24].

Previous reports indicated that the mechanisms and factors implied in the development of human retinal microvasculature, both normal and pathological, owing to their complex hierarchical organization, are not fully understood and need to be researched further[7-8,14].

The three-dimensional human retinal microvascular network can be analyzed and estimated if it is considered as fractal or multifractal object in a "scaling window", which normally ranges in two to three orders of magnitude[27].

Fractal analysis describes the global measurement of complexity of the human retinal vessel branching, which is expressed by the mean fractal dimension (D) value (a valid estimator with a fractional value)[7,14,19,22].

In several fractal analysis studies of the normal human retinal microvascular network, determined by box-counting method, the mean fractal dimension (D) value was approximately 1.7[15,17-19].

Some reports indicated that human retinal microvascular network has a multifractal geometrical structure[23-26].

Multifractal analysis provides us a deep insight into the complex nature of spatial distribution and geometry (a description over the retinal regions both locally and globally)[23-26].

In many studies, researchers have found different fractal dimensions D[14,17-20] or generalized dimensions D_q (for q=0, 1, 2) values[23-26] associated with normal human retinal microvascular network and their results sometimes reveal discordances because of differences in the experimental and methodological parameters involved.

The fractal and multifractal analyses of retinal vascular network depend on the fractal analysis methods, multifractal methods, including the algorithm and specific calculation used *etc*[7,23,28-31].

MATERIALS AND METHODS

Multifractal Analysis In our study the multiple scalings are analyzed through multifractal spectrum.

The common multifractal measures are: 1) the generalized fractal dimensions function D_q, where q is a real parameter that indicates the order of the moment of the measure; 2) the $f(\alpha)$ singularity spectrum, where α is named Hölder or singularity exponent[23-25]. There are some mathematical methods to compute this spectrum, according references[23-25].

The generalized dimensions D_q for q=0, 1 and 2, are known as the capacity (or box-counting), the information and correlation dimensions, respectively. The capacity dimension D_0 is independent of q and provides global (or average) information about the structure, D_1 quantifies the degree of disorder present in the distribution, and D_2 measures the mean distribution density of the statistical measure[25]. All dimensions are different, satisfying $D_0>D_1>D_2$.

The retinal microvascular network can be associated with a multifractal structure if there is a statistically significant difference between D_0, D_1 and D_2[23-25].

Segmentation Method The segmentation consists of extracting geometric information about the retinal vessels from the eye fundus images. The quality of the segmentation is quite dependent on the parameters used such as image quality, choice of threshold, and choice of structuring elements[31-35]. The color images were converted in a binary format.

In this study, an automatic unsupervised method for the segmentation of retinal vessels[32] was applied before multifractal analysis, according to the segmentation methodology and computer algorithms proposed in reference[33-35].

Materials This study was conducted according to the Declaration of Helsinki and the European Guidelines on Good Clinical Practice for research in human subjects.

All adult volunteers were provided with written informed consent prior to enrollment in the study. The approval of the Ethics Committee of "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania, was obtained.

Normal healthy adult subjects consisted of 50 volunteers (male gender, 52%; age, 49±6.1y) with normal posterior Pole, without ocular or systemic diseases and no current smoking history participated in this study from January 2012 to January 2014. All participants underwent a standardized interview, including a medical history review, and ocular and systemic examination.

After maximal pharmacologic pupil dilation, retinal images centered on the macula and optic disc of each eye, available for retinal vascular analysis, were captured digitally using a fundus camera at 45° field of view (VISUCAM^{Lite} Zeiss; Carl Zeiss Meditec AG 07740 Jena 2008, Germany). Digital retinal photographs were taken of both eyes. Each subject was examined in a room at (24℃±1℃), with standard indoor levels of illumination (300 lx) and humidity (50%±1%), and no air drafts.

A set of 100 segmented and skeletonised human retinal images, corresponding to normal states of the retina has been studied. The results were expressed as mean±standard deviation (SD).

Methods The multifractal analyses were made according to the multifractal methodology proposed in reference[23]. An analysis of the digital retinal files was conducted based on the original algorithm (in MATLAB software R2012b, MathWorks, Inc.)[36]. This is derived directly from the definition of the box-counting fractal dimension[37].

Multifractal analysis was performed, for the entire retinal image, applying the standard box-counting algorithm to the digitized data, in two cases: 1) on the segmented images (seg.); 2) on the skeletonised (skl.) version.

The $f(\alpha)$ spectrum was computed in the range $-10 \le q \le 10$ for successive 1.0 steps.

After adjustments of every digital image, the segmented version of retinal vessel structure contains the vessel silhouettes extracted from the fundus photographs.

The vessel maps (8-bit) were then skeletonised. This procedure was applied for all analyzed structures and all the skeletonised images were obtained with the Image J skeletonising algorithm[38].

Statistical Analysis The statistical processing of the results (Table 1) was done using GraphPad InStat software program, version 5.00[39]. A value of $P<0.05$ was regarded as being statistically significant. The average results were expressed as mean value and SD.

RESULTS

Results of Multifractal Analysis A summary of the obtained results of the generalized dimensions (D_q) for q=0, 1, 2, the width of the multifractal spectrum ($\Delta\alpha=\alpha_{max}-\alpha_{min}$) and the spectrum arms' heights difference ($|\Delta f|$), all with average±SD, of 100 analyzed images, for normal (N) status, in segmented and skeletonised variants, is presented in the Table 1.

Figures 1-4 show the results of multifractal analyses, plots of $\alpha(q, r)$ versus q, $f(q, r)$ versus q and of $f(q, r)$ versus $\alpha(q, r)$, for normal retinal vessel network (Figure 5A), in segmented (seg.) and skeletonised (skl.) variants (where r is box size in the given scale).

$|\Delta f| = f_{max}-f_{min}$, when $f(\alpha)>0$, represents the spectrum arms' heights difference.

DISCUSSION

Improvements in digital retinal imaging have led to quantification of the complex three-dimensional structure of the normal human retinal microvasculature network. Our findings confirm that normal human retinal microvascular network can be described and estimated using the multifractal geometry.

The average of generalized dimensions (D_q) for q=0, 1, 2, the width of the multifractal spectrum ($\Delta\alpha=\alpha_{max}-\alpha_{min}$) and the spectrum arms' heights difference ($|\Delta f|$), of the normal images were expressed as mean±SD: 1) for segmented versions, D_0=1.7014±0.0057; D_1=1.6507±0.0058; D_2=1.5772±0.0059; $\Delta\alpha$= 0.92441±0.0085; $|\Delta f|$ = 0.1453±0.0051; 2) for skeletonised versions, D_0=1.6303±0.0051; D_1=1.6012±0.0059; D_2=1.5531± 0.0058; $\Delta\alpha$=0.65032±0.0162; $|\Delta f|$ = 0.0238± 0.0161.

Table 1 The generalized dimensions (D_q) for q=0, 1, 2, the width of the multifractal spectrum ($\Delta\alpha=\alpha_{max}$ - α_{min}) and the spectrum arms' heights difference ($|\Delta f|$), all with average±SD, of 100 analyzed images, for normal (N) status, in segmented and skeletonised variants

Status (N)	Segmented	Skeletonised	*P*
D_0	1.7014±0.0057	1.6303±0.0051	0.035
D_1	1.6507±0.0058	1.6012±0.0059	0.038
D_2	1.5772±0.0059	1.5531±0.0058	0.032
$\Delta\alpha=(\alpha_{max}-\alpha_{min})$	0.92441±0.0085	0.65032±0.0162	0.029
$\|\Delta f\|=f_{max}-f_{min}$	0.1453±0.0051	0.0238±0.0161	0.033

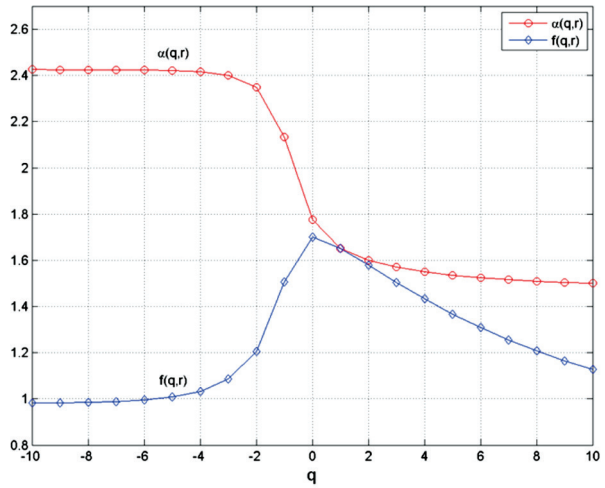

Figure 1 Plot of $\alpha(q, r)$ versus q and of $f(q, r)$ versus q, in segmented variant The $\alpha(q, r)$ curve decreases from 2.4254 (for q= -10) and tends to 1.5009 (for q=10). The $f(q, r)$ curve increases from q=0.9816 (for q=-10) to a maximum value of 1.7015 (for q=0), then decreases and tends to 1.1269 for q=10.

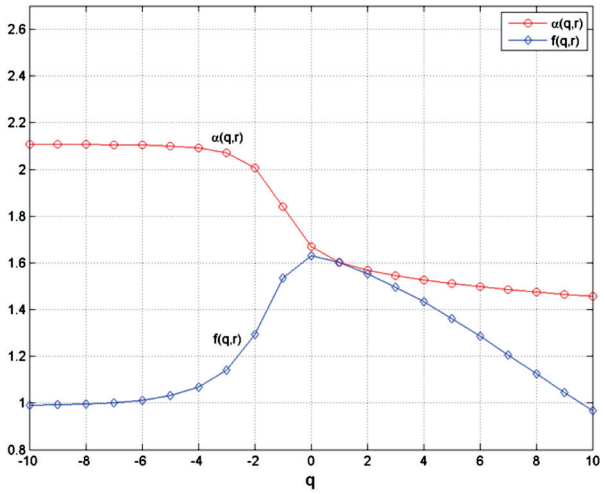

Figure 2 Plot of $\alpha(q, r)$ versus q and of $f(q, r)$ versus q, in skeletonised variant The $\alpha(q, r)$ curve decreases from 2.1072 (for q= -10) and tends to 1.4569 (for q=10). The $f(q, r)$ curve increases from q=0.9910 (for q=-10) to a maximum value of 1.6306 (for q=0), then decreases and tends to 0.9672 for q=10.

The average of generalized dimensions (D_q) for q=0, 1, 2, the width of the multifractal spectrum ($\Delta\alpha$) and the spectrum arms' heights difference ($|\Delta f|$) of the segmented versions are slightly greater than the skeletonised versions. This study confirms the obtained results in previous multifractal studies shown in reference[24].

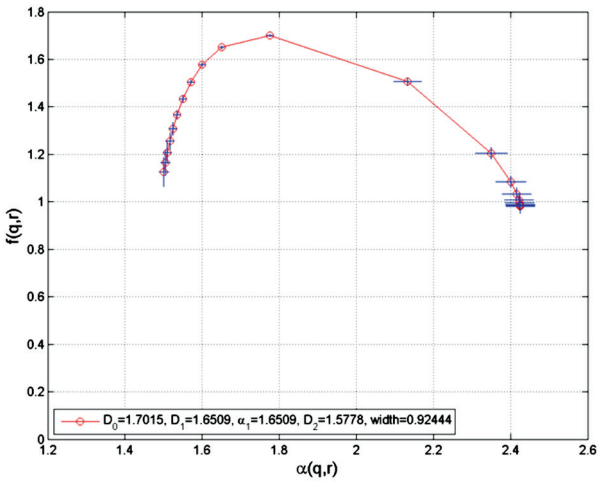

Figure 3 Plot of *f(q, r)* versus *α(q, r)* in segmented variant The left branch of the spectrum corresponds to large *q* values (*q*>0), while the right branch of the spectrum corresponds to small *q* values (*q*<0). The *f(q, r)* curve has a maximum value of 1.7015 corresponding to *q*=0.

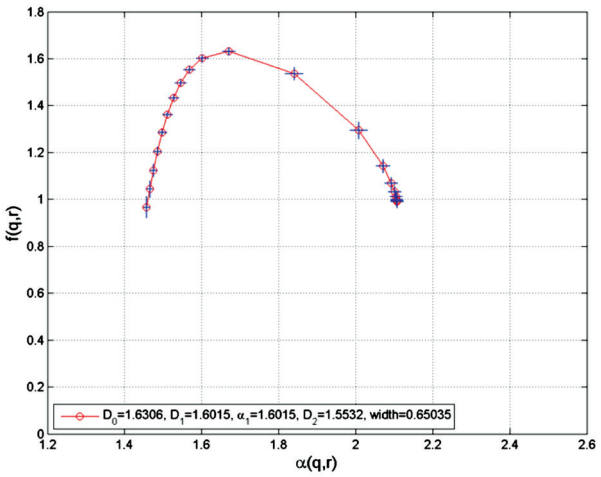

Figure 4 Plot of *f(q, r)* versus *α(q, r)* in skeletonised variant The left branch of the spectrum corresponds to large *q* values (*q*>0), while the right branch of the spectrum corresponds to small *q* values (*q*<0). The *f(q, r)* curve has a maximum value of 1.6306 corresponding to *q*=0.

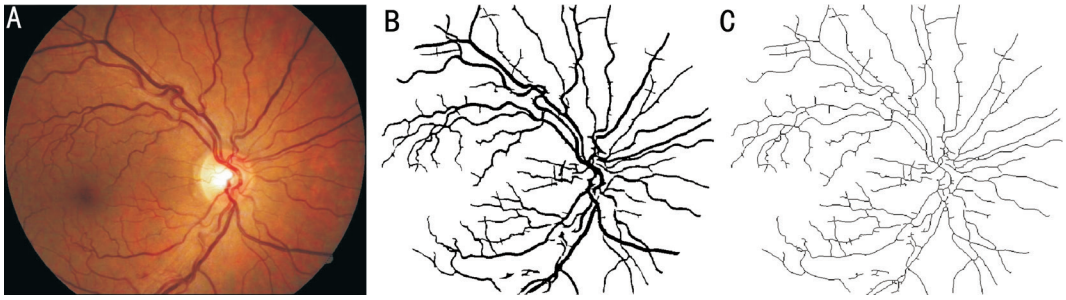

Figure 5 Images of the retina vessel network for a normal subject A: Original color; B: Segmented (seg.); C: Skeletonised (skl.) variants. The optic disc is the brightest region having elliptical shape which appears bright orange pink with a pale center. The retinal vessels coming from the optic disc have a normal branching pattern.

In conclusion, the non-Euclidean three-dimensional nature of human retina microvascular network across the physiopathological spectrum can be analyzed and quantified using the multifractal geometry.

The graphs in Figures 1-4 demonstrate the multifractal geometrical organization of the normal human retina microvascular network, that is not evident in the fractal dimensions. A benefit of the proposed multifractal methodology is the elegance of the algorithm, computational effectiveness and its interpretation.

The multifractal analysis of fundus photographs (with all the intrinsic limitations as a mathematical model) may be used as an objective and quantitative parameter for the evaluation of the human retina microvascular network. It is reasonable to assume that its identification as a potential marker for early detection of topological changes associated with retinal diseases will help investigators to design specific retinal treatment strategies.

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