Fractal analysis of mucosal microvascular patterns in oral lichen planus

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Fractal analysis of mucosal microvascular patterns in Oral Lichen Planus: a preliminary study.

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ABSTRACT

Objectives. The objective of this study was to assess local vascular architecture in atrophic-erosive oral lichen planus (OLP). ***Materials and Methods.*** We investigated the capillary structure of the oral mucosa in 31 OLP patients and 32 healthy controls. Capillaries images were captured in vivo through a capillaroscope. We applied fractal analysis to quantify the microvasculature morphometric changes in the oral mucosa of atrophic-erosive OLP patients in terms of their fractal dimension (D). ***Results.*** The oral vascular networks of atrophic-erosive OLP lesions had a significantly higher D, both in buccal mucosae (D=1.167, p=0.019) and in tongue (D=1.196, p=0.038), when compared to the control population (1.123 for both locations, respectively). ***Conclusion.*** The present study confirms previous literature data on a close relationship between abnormal vascular architecture and atrophic-erosive OLP. Fractal analysis provided a quantitative descriptor of the complexity of the vascular patterns, which increases in the OLP samples. These data may provide new information on the OLP pathogenesis, as well as serve as morphological quantifiers in the monitoring treatment strategies.

Keywords: fractal analysis; fractal dimension; vascular patterns; oral mucosa; oral lichen planus.

Abstract words: 168

INTRODUCTION

Lichen planus (LP) is a chronic idiopathic inflammatory disease of the skin and mucous membranes, characterized by an immune process directed against basal keratinocytes that leads to epidermal damage[1]. It is still unknown, however, how this process is activated in vivo[1].

It is estimated that LP has a prevalence of 1-2% in the general population, 50% of which presents with skin and oral lesions, while in 25% of patients oral lesions are the only feature (oral lichen planus, OLP)[2] commonly involving the buccal mucosa, tongue and gums[3]. Clinically, OLP lesions are often multiple, bilateral, symmetrical and may present as different subtypes: papular, plaque-like, reticular, erosive, atrophic, bullous[4-6].

Although OLP pathogenesis is still unclear, there is growing evidence pointing to cell-mediated immunity mechanisms that involve oral keratinocytes secreting chemokines that attract lymphocytes[7]. The T CD4+ cells stimulated by antigens associated with major histocompatibility complex (MHC) class II (expressed by Langerhans cells) activate T CD8+ cells that appear to lead to basal keratinocyte apoptosis[8].

The production of Th1 cytokines (IFN-γ, IL-2 and TNF-α) by activated T cells stimulates the expression of intercellular adhesion molecule-1 (ICAM-1) on Langerhans cells and macrophages, and somehow induce the expression of class II MHC molecules by keratinocytes, thus contributing to a chronic injurie[9]. In addition, the activity of cytokines seems to be facilitated by an over-expression of their receptors, and possibly up-regulation of several chemokines[10]. Furthermore, lymphocytes seem to influence the development and extent of lesions through the production of chemokines such as RANTES which trigger degranulation of mast cells, releasing TNF-α and chymase, which in turn promote the discharge of further RANTES, attracting in more mast cells and also inducing their degranulation[7].

TNF-α and IFN-γ polymorphisms have also been suggested to play a role the in susceptibility to OLP, while cytokines polymorphisms appear to influence some OLP clinical presentations[11].

Similarly to other chronic inflammatory diseases[12-15], neo-angiogenesis is a common event in OLP[5,8,16,17] and a relationship between abnormal angiogenesis and atrophic-erosive OLP types has been demonstrated[5,18]. Angiogenesis has been suggested to occur as a direct consequence of the dense lympho-histiocytic infiltration (a typical band-like pattern in the lamina propria), which causes hypoxia in the inflamed stromal area[19].

The microvasculature in abnormal angiogenesis is often structurally and functionally altered[20]. The vessels tend to be disorganized, unevenly distributed, dilated and more permeable. Arterioles and venules can be morphologically undefined, with premature and lack of mural cells, high interstitial pressure and sluggish blood flow. These vascular phenotypic changes are also likely to affect the balance of pro- and anti-angiogenic signaling in the tissue[21,22].

Lately, investigation of the quantitative microangioarchitecture has also focused on morphometric descriptors such as those used to characterise fractals[23] which we set to investigate here.

Fractal Analysis

Mandelbrot, in his work aimed to provide mathematical interpretations of real phenomena dominated by randomness and chaos and to devise means of describing shapes endowed of complexity and self-similarity, by developing the concept of fractals[24]. Mathematically, a fractal object has two main features:

a) Fractals often have a fractional, or non-integer, dimension[25]. In terms of Euclidean 'topological' dimensions, points have a zero dimension, straight lines have 1 dimension (length), plane surfaces have 2 and volumes have 3. Fractals however typically have dimensional values that exceed their topological dimension[26]. Fig. 2 shows an approximation of a von Koch type of fractal curve. This object should have (by being a line) a topological dimension of 1, but increasing the scale of observation reveals that it is not a rectifiable curve: the length of the curve between any two points is (in its limit) infinite because increasingly more detail is revealed upon increasing magnification.

b) Fractals exhibit self-similarity independent of scaling[25]. Natural objects, such as coastlines, trees and, in this case, vascular trees are only approximations to mathematical fractals and exhibit some degree of statistical self-similarity (i.e. smaller pieces are 'statistically similar' to the whole, but not necessarily identical, while maintaining a similar level of structural complexity) over some range of scales[27]. In those cases, one way to describe their complexity is by means of the fractal dimension (D). This number defines the space-filling properties of the object[26] by describing the rate of space filling in the embedding space. For an object in 2 dimensional space, D can take values between 0 and 2 — the closer an object's fractal dimension gets to 2 (the embedding space), the more the object appears to fill space and the more geometrically complex it is. Therefore D becomes a convenient quantifier of the complexity and space filling properties of the microvessels within the environment.

It is interesting to note that in addition to global quantifiers of complexity, Fractal geometry can also be used to obtain quantifiers of the local morphological complexity of objects[23,28-30]. In the context of vascular structures, it has been shown that both physiological and pathological vascular trees can be described as fractals[23,30].

We applied fractal analysis to quantifying global microvasculature morphometric changes in the oral mucosa of atrophic-erosive oral lichen planus (OLP) patients.

MATERIALS AND METHODS

Patients

This study - approved by the Ethical committee of the Second University of Naples - enrolled 63 patients referring to the Oral Pathology Unit at the Second University of Naples, Italy, after informed and written consent. Thirty-one patients were affected by atrophic-erosive OLP and 32 healthy patients were used as controls.

Diagnosis of OLP was made evaluating both clinical and histological features according to the criteria established by World Health Organization[31]. Patients diagnosed with OLP were further divided into atrophic-erosive and reticular subgroups according to the clinical classification generally accepted[5,32-34]

Patients with reticular lichen planus were not included because a previous study reported that microvessel density (MVD) of atrophic-erosive OLP was significantly higher than that of the reticular OLP and there was no significant difference of MVD between reticular OLP and a control group[5]. Moreover, Scardina et al.[35,] compared microcirculation among the different clinical OLP forms by video-capillaroscopy and observed a higher density and the presence of a greater number of branched, tortuous loops in the atrophic form compared to the exclusively reticular form.

For all 31 patients with OLP, the lesions were originally atrophic-erosive (first onset) and did not have involvement of the skin, genital mucosa, or other anatomic sites. The characteristics of the subjects are shown in Table I.

Capillaroscopic investigation

Intravital videomicroscopy (capillaroscopy) allows evaluation of in vivo microcirculation[35] (Fig. 1) and permits a simple, repeatable, non-invasive exam of oral mucosa in an orthogonal projection. The videocapillaroscope is a digital microscope that can be connected to a computer/camera allowing the acquisition of images of the mucosal vasculature (in this case with a field size of 1280x1024 pixels). Oral mucosa vascular architecture imaging was performed using computerized videocapillaroscopic techniques (Dino-lite) and related software (DinoCapture2.0- 0.9.5b). White LED lights are built-in in the device. All the captured images were taken at 200x magnification. The capillaroscopy analysis was always performed by the same operator, with the same light source, always in the morning, with the camera was positioned into the oral cavity at a right angle.

Estimation of the Fractal Dimension (D)

D is a global quantifier of the complexity of a set and it is here related to the number of vessels, their variability in shape and magnitude, and distribution pattern[29,30]. In this case D can be considered as a quantitative parameter to characterise the visible vasculature.

The images captured with capillaroscopy were converted to 8bit greyscale and thresholded using Otsu's method implemented in the ImageJ analysis software[36]. Regions smaller than 2 morphological dilations were deleted and finally a median filter of radius 2, was applied to remove any remaining noise. The resulting images were then skeletonized (reduced to single pixel paths) to simplify the geometric pattern while still retaining a reproducible and computable representation of the original vessel distribution (Fig. 1). The box-counting method[25] was used to calculate D of the vascular skeletons (Fig. 3).

Statistical analysis

The differences in D in the images across the OLP and control patients were analysed for significance using F-test (for variability across groups) and Student's t-test for significance of the difference of the means using Microsoft Excel (Microsoft Corp.).

RESULTS

Table II summarizes the average of the D in OLP lesions and in healthy oral mucosae (Fig.4).

The statistical analysis of the D data is shown in Table II. An F-test revealed that variability in D across groups was not statistically significant ($p>0.05$), but the mean D values were statistically different. The oral vascular networks of atrophic-erosive OLP lesions had a significantly higher D, both in buccal mucosae ($D=1.167$, $p=0.019$) and in tongue ($D=1.196$, $p=0.038$), when compared to the control population (1.123 for both locations, respectively). However, the range of values across groups had an important overlap, possibly due to individual variation and this would make it difficult to enable D to be used alone as a unique discriminant of OLP. Nevertheless, it might prove useful for monitoring changes in vascularity as response to treatment or the status of the vascular networks on an individual basis.

DISCUSSION

Fractal-like properties have been described in numerous macro and microscopic anatomical structures[37], suggesting that they might provide functional or developmental benefits. Self-similarity, has been observed in biological trees[38], neural structures[39] and vascular network in developing embryos[40]. Microcirculation in normal and pathological conditions, (e.g. in neoplastic development and growth[36,41]), has also been investigated in terms of fractals[42,43] and it has been hypothesized that a fractal morphology might optimize the supply of nutrients while minimizing the energy required for

blood flow and metabolic exchange[26,43]. It should not be surprising therefore that the geometric organization of vascular networks becomes altered when is influenced by local stimuli, e.g. growth factors released by neoplastic tissues[44,45] or by chronic inflammatory diseases like OLP[5,9].

In this paper we exploited Fractal analysis to quantify the phenotypic changes in the vascular patterns, but the analysis also has the additional advantage to be a multi-scale characterization, i.e. it summarises the rate of change of space occupancy across scales (box size, in the current analysis). Such multi-scale analysis is more robust than single scale descriptors, such as 'density', which for fractal objects is not as meaningful as first thought (unintuitively e.g. a fractal sponge, the bigger it is, the less dense it becomes).

The oral vascular networks of atrophic-erosive OLP lesions had a significantly higher D, when compared to the control population, confirming previous evidence of a relationship between abnormal vascular architecture and morphological changes in atrophic-erosive OLP[5,18]. It furthermore highlights the feasibility to use fractal analysis to assess quantitatively the microvascular patterns in OLP lesions to characterise and monitor progression and response to treatment.

The present analysis deals with a projection of the 3d pattern onto a 2d image plane and while it could be extended to the more realistic case of 3d microvascular networks, this is technically challenging as 3d data is difficult to obtain at these small scales using non-invasive methods. Nevertheless, 2d projection patters still preserve useful microcirculation information, as reported here and in other publications[26,36].

The central role of vascular architecture in autoimmune disorders and chronic inflammation has prompted the use of some therapeutic strategies in the treatment of some chronic inflammatory diseases. For example, anti-angiogenic therapy proved promising in alleviating the severity and preventing progressive chronic inflammation in disorders like rheumatoid arthritis, collagen-induced arthritis and peritoneal fibrosis[46-49]. Considering that in some OLP cases conventional immunosuppressive and/or anti-inflammatory therapy has been found ineffective, and that the vascular architecture is altered, targeting angiogenesis might be a valid therapeutic target to fight such persistent inflammation cases. This idea seems to be supported by results obtained with thalidomide, an inhibitor of angiogenesis, in the treatment of erosive OLP cases where the corticosteroid therapy was contraindicated or had no effects[50-52]. It is also important to note that OLP is a potentially malignant disorder. Despite the numerous factors associated with malignant change, there is a scarcity of definite clinical, histological and molecular predictors of malignant development for OLP[53]. While some authors[54] proposed microvessel density analysis as a parameter in determining potential malignant progression of vulvar lichen sclerosus, this has not been studied extensively in OLP. In this context of malignant

transformation, fractals also have been shown to be indicators of premalignant and malignant epitelial profiles[55] and that could also be integrated in the analysis of OLP lesions.In this preliminary study, we presented an application of fractal geometry to develop an objective, operator-independent and *in vivo* technique capable of quantifying morphological changes in microvascular patterns in atrophic-erosive OLP lesions. Further study is guaranteed to elucidate the value of fractal geometry as a quantitative marker in monitoring the status of the disease, the effectiveness of therapies and the early diagnosis of malignant transformation.

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FIGURE LEGENDS

Table I. Clinical features of subjects in this study.

Table II. The average of the D in OLP lesions and in healthy oral mucosae.

Fig. 1 – A) Capillaroscopy image of healthy mucosa; B) Capillaroscopy image of patient affected by lichen; C) Skeletonized image of image A; D) Skeletonized image of image B.

Fig. 2 - A von Koch curve example. Fractal objects have a non-integer, dimension which exceeds their topological dimension and exhibit self-similarity independent of scaling.

Fig. 3 - The slope of the line represents the Fractal dimension of the microvessels. A) healthy subject; B) OLP patient.

Fig. 4 - The bar graph shows the average fractal dimension in the four groups.

Table I. Clinical features of subjects

Group	Number of subjects	Age		Gender	Site	
		Mean ±SD	*Range*	F/M	*Buccal mucosa*	*Tongue*
Atrophy-erosive OLP	31	64.903 *± 6.705*	54 - 78	16/15	22	9
Control	32	61.593 *± 9.417*	43 - 73	16/16	22	10

OLP: Oral Lichen Planus

Table II. The average of the FD in OLP lesions and in healthy oral mucosae.

	Normal		OLP	
	Tongue	Buccal mucosa	Tongue	Buccal mucosa
N	10	22	9	22
Average	1.123	1.123	1.196	1.167
SD	0.083	0.052	0.053	0.068
Min	0.990	1.045	1.089	1.040
Max	1.225	1.253	1.281	1.281

Statistical differences (FD normal vs. OLP)

	Tongue	Buccal mucosa
F-test p	0.227	0.221
T-test p	0.038	0.019

SD: standard deviation, FD: fractal dimension, OLP: oral lichen planus

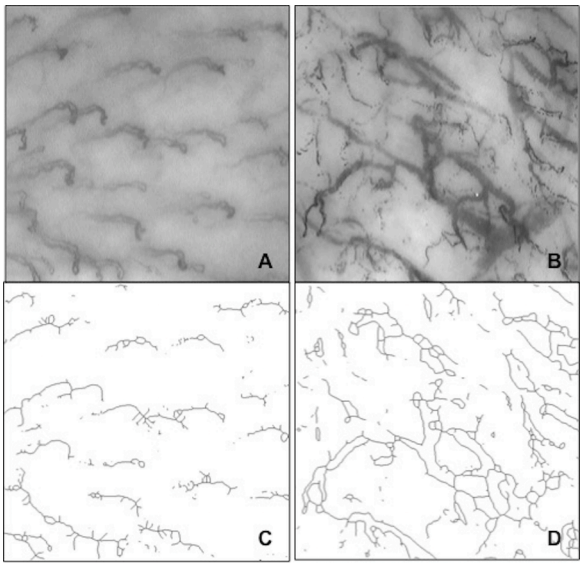

Figure 1

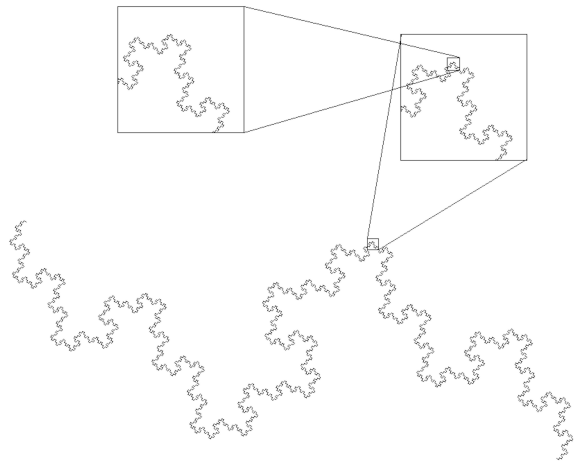

Figure 2

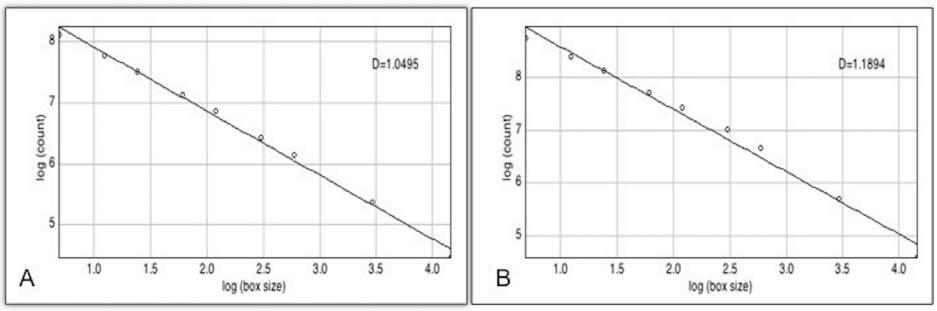

Figure 3

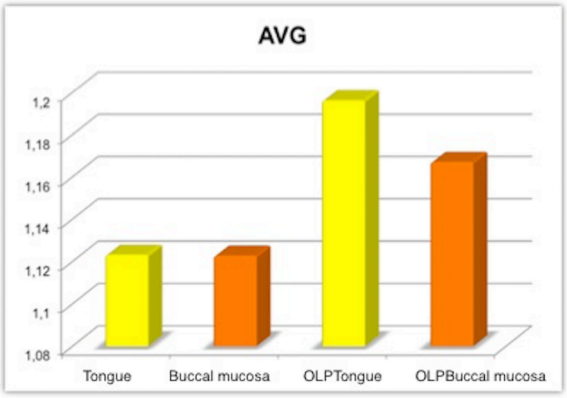
AVG
1,2
1,18
1,16
1,14
1,12
1,1
1,08
Tongue
Buccal mucosa
OLPTongue
OLPBuccal mucosa

Figure 4