Original Article

Role of fractal analysis in detection of dysplasia in potentially malignant disorders

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Abstract

Background: Fractal analysis is, a noninvasive method, used to determine the intricate characteristics of the matter. Oral leukoplakia (OL), a potential malignant disorder, has definite propensity to turn in to malignancy. In such lesions, fractal dimension analysis (FDA) could be helpful in the early detection of malignant transformation. **Objectives:** To determine the efficacy of fractal dimension analysis in detecting malignancy potential of oral leukoplakia. **Materials and Methods:** After ethical clearance, we enrolled 121 patients in our study. Lesions were photographed before and after toluidine staining. Image J software was used to analyze fractal dimensions (FDs) of digital image and results were compared with biopsy. **Results:** Fractal dimension value is significantly higher in leukoplakia with dysplastic changes. FD values increase as age of patients increases. FD value in leukoplakia with different tobacco products showed more positive correlation with surti/khaini abusers. **Conclusion:** Fractal dimension analysis is a useful method in determination of complication in OL cases and can be used as an effective, noninvasive screening tool at primary healthcare centers for early intervention.

Keywords: Fractal analysis, oral leukoplakia, screening test, toluidine blue

Introduction

The word "fractal" originates from Latin language "fractus," meaning "broken" or "fracture." This has been used to name a form or figures that are self-similar. The significance of fractal analysis is to characterize complex shapes, which are self-similar in nature. Fractal dimensions (FD) are measured as numerical value.[1] In fractal analysis, there is processing of the subject which can be a signal or an image.[1] FD decreases or increases as the complexity of the object decreases or increases.[2]

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Fractal structures exist in nature and in the human body too, e.g. brain convolution[3] (sulci and gyri), blood vessels,[4] etc., The literature suggests that fractal analysis has been used to describe the irregularities in epithelium, connective tissue interface.[5,6] Similarly, various oral pathologies have been diagnosed with the help of fractal dimension analysis.[6] A preliminary study by Demiralp *et al.*[7] suggested its role in bony changes in patients taking bisphosphonates.

Oral leukoplakia (OL) is a potential malignant epithelial disorder of oral mucosa.[8] The frequency of dysplastic change or malignant potential in OL has ranged from 15.6% to 39.2% in several studies.[7] Among general population, the occurrence of leukoplakia varies from 1%–5%.[9]

Early detection of malignant potential is imperative for timely intervention and for prevention of malignant transformation.

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Various techniques and methods have been employed in the early detection of oral cancer[10]; however, biopsy remains the gold standard and confirmatory test. Incisional/excisional biopsy is an invasive procedure and several patients refrain themselves from undergoing procedure which indirectly helps in disease progression and obscures the actual burden of disease.

Fractal dimension analysis is a noninvasive procedure which can be easily carried out on high-quality images of oral leukoplakia as OL has branching pattern with fractal properties. In addition, FD analysis is easy to perform and can be carried out by medical and paramedical staff. Thus, this analysis can be suitably used at primary level of health care to distinguish dysplastic lesions from nondysplastic lesions. Therefore, we planned this study to evaluate the reliability of fractal analysis in the detection of dysplastic changes in oral leukoplakia and in the early detection of malignant transformation.

Materials and Method

Patients

The study was approved with reference no. 89ECMIIBThesis/P74 dated 28/04/2018. The study was conducted in the Oral Medicine unit of Faculty of Dental Sciences, King Georges Medical University. The experimental protocol was approved by Institution's ethical committee. A structured performa was used to gather relevant information from each participant. Informed consent was obtained from all the participants.

Patients reporting to the departmental OPD were screened for oral leukoplakia after taking thorough history and performing clinical examination. A total of 121 individuals (males and females) were included in study group.

Inclusion criteria:

a. Age above 18 years.
b. Clinically evident leukoplakia in oral cavity.
c. Individuals consenting for staining and biopsy procedure.

Exclusion criteria:

a. Medically compromised patients.
b. Patients who are already enrolled/completed treatment of oral leukoplakia.
c. Patients who are allergic to toluidine blue stain.
d. Patients not willing for biopsy procedure.
e. Patients who deny or refuse on informed consent.

Digital images of normal mucosa and lesion were taken with Sony cyber shot digital camera (20.1 MP), before and after staining with toluidine blue. The digital images obtained were preprocessed by cropping in a manner involving keratinized mucosa according to the region of interest (ROI) of size 86 × 124 pixels. ROI was selected on the basis of toluidine dye retention. The area of toluidine dye retention was evaluated for FDA and the same area was biopsied for histopathology examination.

Image analysis

With the help of Image J software version 1.47, the digital images were processed and obtained. Image were converted in to binary as described by White and Rudolf.[11] ROI was duplicated and blurred with Gaussian filter having diameter 35 pixels. The resultant image was subtracted from the original image and grayscale value of 128 was added to it. The image was again converted in to binary and thresholded to the brightness value of 128. Subsequently, binary images were eroded, dilated, and processed as skeletonized images to decrease noise.

Statistical analysis

After image processing, FD of all skeletonized images was calculated using box counting method. A graph plotted between box count and box size showed the resultant FD value. The FD of keratinized/lesion was calculated and compared with the histopathology report of patient with oral leukoplakia. The obtained results were statistically analyzed using SPSS software (students paired t-test)

Observation and Results

The present study was conducted with the sample size of 121 individuals with OL. The Sociodemographic details of study population are summarized in Table 1. Participants were categorized in to group of smokeless and smoke tobacco, which is depicted in Figure 1. Further categorizing the forms of smokeless tobacco, we found that the maximum number of the participants were using tobacco in the form of gutkha followed by surti/khaini and paan/supari chewers [Figure 2]. Right side buccal mucosa was the most common site of leukoplakia. Other than buccal mucosa, mandibular anterior labial vestibule is the next common site [Figure 3].

On analyzing fractal dimension, we found that the association of FD value in images (before and after staining with toluidine blue) was higher in dysplastic cases (mean 1.24 ± 0.16 and 1.18 ± 0.20, respectively) than in nondysplastic (mean 1.12 ± 0.21 and 1.10 ± 0.20, respectively). A highly significant difference was observed in FD values (before and after stain) between dysplastic and nondysplastic cases [Table 2].

We also analyzed fractal dimension values according to age and type of tobacco product used. The FD value (before and after staining with TB) distribution with age showed increasing trend with advancing age. A highly significant difference was observed in FD (before and after staining) values among various age groups [Table 3]. A significant difference was observed in FD values of surti/khaini abusers only. This infers that all products were equally responsible for increased FD values and surti/khaini was more responsible than others [Table 4].

The correlation of FD values (before and after staining with TB) with age and duration of smoking and smokeless tobacco was highly significant [Table 5].

Table 1: Distribution of participants according to demographic profile

Variable		No.	Percentage (%)
Gender	Male	97	80.2
	Female	24	19.8
Place	Rural	58	47.9
	Urban	63	52.1
Economic status	Average	101	83.5
	Higher	3	2.5
	Lower	17	14.0
Total		121	100.0

Table 2: Association of FD value with dysplastic changes

DYSPLASTIC	Nondysplastic		Dysplastic		*t*	*P*
	Mean	SD	Mean	SD		
FD before staining with TB	1.12	±0.21	1.24	±0.16	3.64	<0.001
FD after staining with TB	1.10	±0.20	1.18	±0.20	2.17	0.032

Table 3: Distribution of FD value according to age

Age Group	FD (before staining)		FD (after staining with TB)	
	Mean	SD	Mean	SD
20-29 years	1.07	±0.25	1.01	±0.24
30-39 years	1.14	±0.20	1.06	±0.19
40-49 years	1.20	±0.18	1.11	±0.17
50-59 years	1.24	±0.16	1.22	±0.21
>= 60 years	1.30	±0.09	1.25	±0.08
F	3.97		5.61	
P	0.005		<0.001	

Linear regression models were derived for FD values before and after staining, which are

FD nonstained = 0.955 + 0.001 (age) +0.144 (bidi/cigarette) +0.075 (gutkha) +0.026 (pan/supari) +0.017 (surti/khani) +0.011 (tobacco leaves) – 0.012 (duration) – 0.033 (lower SES) – 0.026 (average SES) [Table 6]

FD-stained = 0.907 + 0.002 (age) +0.100 (bidi/cigarette) +0.058 (gutkha) +0.034 (pan/supari) +0.035 (surti/khani) +0.035 (tobacco leaves) +0.009 (duration) +0.003 (lower SES) – 0.072 (average SES) [Table 7]

Where, the tobacco products value was 1 for particular user and 0 for nonuser. Similar values will be taken for socioeconomic status (SES).

Discussion

The science of texture analysis studies the differential intensity of picture element values by means of mathematical algorithms. Based on this texture analysis, various studies have been published in medical field to study the characteristics of complex structures

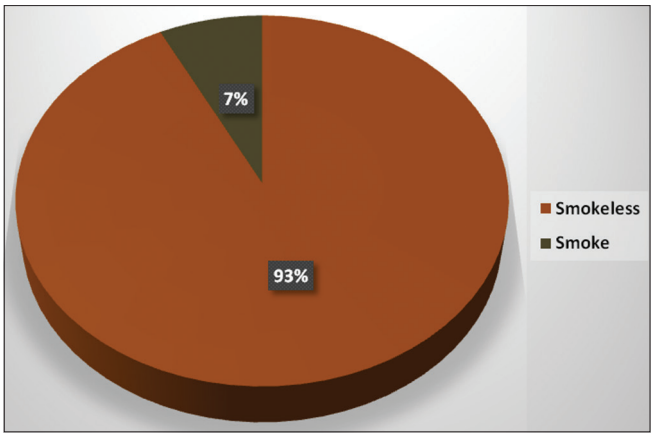

Figure 1: Distribution of participants according to type of tobacco used

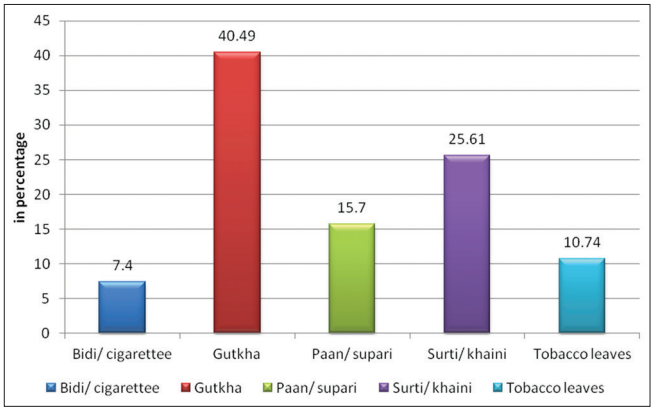

Figure 2: Distribution of participants according to tobacco product used

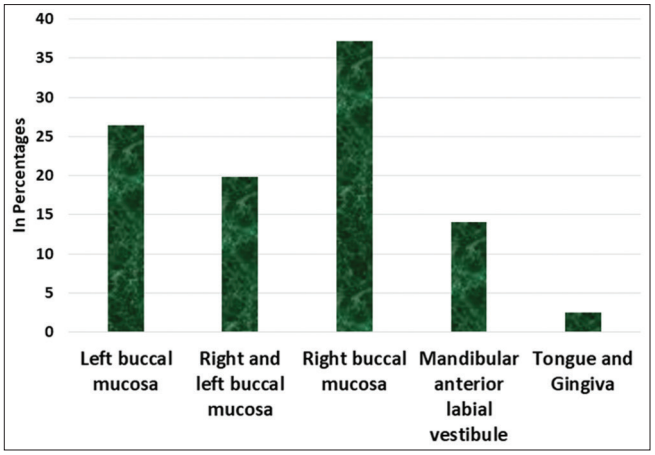

Figure 3: Distribution of participants according to site of lesion

in different areas of human body.[12-14] The most common method of texture analysis is fractal dimension and FD values are consistently higher in malignant regions.[15-17] Recently Metze *et al.*[18] reviewed the role fractal dimension and found that FD increases in carcinogenesis. In addition, FD has been used for the classification of breast tumors,[19] in therapeutic response of small cell lung cancer[20] and to differentiate benign and malignant thyroid nodule.[15]

Researchers have performed fractal analysis in oral cancer to compare the morphometric characteristics or clinicopathological factors in oral squamous cell carcinoma and normal tissue. It has been shown that FD values from digital photograph of oral mucosa in cancer patients were significantly higher than healthy controls. In addition, the measurement of FD has shown higher values in the region of the dysplasia thus greater tissue complexity, as compared with normal mucosa.[21] This hypothesis was further confirmed by Pandey *et al.* who stated the difference in FD value between pre and posttreated lesions and suggested a decrease in complexity/keratinization of the lesion following treatment, i.e. regression of the lesion.[22]

Leukoplakia lesion consists of complicated network of white patch, arranged in a lattice-like network. Age, disease progression, and therapeutic agents could influence the number of elements in this network, their dimension, and connectivity. Villa and Bin described the difference between men and women considering the homogeneity of leukoplakia that female group presented a more complexity and malignant transformation than the male group.[23]

Table 4: Distribution of FD value according to tobacco product

Tobacco Product	Status	FD (before staining)		FD (after staining with TB)	
		Mean	SD	Mean	SD
Bidi/Cigarette	No	1.18	±0.19	1.12	±0.20
	Yes	1.27	±0.20	1.20	±0.19
	t	1.35		1.23	
	p	0.179		0.218	
Gutkha	No	1.20	±0.15	1.14	±0.17
	Yes	1.18	±0.23	1.10	±0.20
	t	0.48		1.29	
	p	0.631		0.199	
Pan/Supari	No	1.20	±0.19	1.13	±0.20
	Yes	1.16	±0.18	1.10	±0.20
	t	0.64		0.52	
	p	0.457		0.605	
Surti/Khaini	No	1.17	±0.20	1.09	±0.20
	Yes	1.25	±0.13	1.21	±0.18
	t	2.06		2.99	
	p	0.041		0.003	
Tobacco leaves	No	1.19	±0.20	1.12	±0.21
	Yes	1.18	±0.13	1.11	±0.20
	t	0.15		0.25	
	P	0.885		0.804	

The present study revealed that the incidence of oral leukoplakia was more in males as compared to females with the majority for participants in 4th and 5th decade. The study had more or less equal number of representations from rural and urban areas. Almost all participants of the study were indulged in consumption of smokeless form of tobacco which shows that preponderance of smokeless form more in this particular region. The most common site of involvement by oral leukoplakia is buccal mucosa with more than 80% affected either unilateral or bilateral.

On analyzing fractal dimension of the study population, we found that FD values were significantly high in dysplastic lesions as compared to nondysplastic lesions. This suggested that complexity of leukoplakia with dysplastic change is more and can be compared reliably for leukoplakia without dysplastic change. Similar results were obtained from Shenoi *et al.*[24] and Deepak[25] studies where tissues with higher dysplasia showed higher FD values as compared to tissues with lesser dysplasia. Goutzenis confirmed that the value of nuclear FD is less in normal mucosa and stage I carcinoma.[26] Similarly, Uma Reddy *et al.*[27] conducted a research on pre and posttreated cases of oral leukoplakia and found a significant difference in FD in pre and post cases of Oral leukoplakia.

FD value (before and after staining with TB) was higher in the participants above 40 years of age. It was clear from paired *t*-test that the difference between FD values of different age groups was significant, and the mean FD value (after staining) was higher as the age increases. Thus, the pattern of complexity in leukoplakia was increased as the age advances. Peterson *et al.* found that age seemed to have some independent influence, which might be explained by the fact that oral leukoplakia is a chronic disease.[28] The prevalence of leukoplakia increased with age advancement. It had been estimated that it mainly affects men over 40 years.[21]

FD values of leukoplakia among different types of tobacco consumers (bidi/cigarette, gutkha, pan/supari, surti/khaini and tobacco leaves) showed the highest value and significant difference in surti/khaini abusers. This suggested that there was more complexity in leukoplakia of participants consuming surti/khaini as a tobacco product. Nonsmokers with leukoplakia had an increased rate of malignant transformation in relation to leukoplakia in smokers.[29]

FD value of leukoplakia showed a strong correlation with the age of participants and the duration for which tobacco products consumed. This infers that complexity of leukoplakia

Table 5: Correlations of FD value with age and duration of tobacco use

Variable	FD (before staining)		FD (after staining with TB)	
	Pearson Correlation	*P*	Pearson Correlation	*P*
Age (years)	.401	<0.001	.430	<0.001
Duration (years)-smoke	.658	.020	.628	.029
Duration (years)-smokeless	.477	<0.001	.491	<0.001

Table 6: Linear regression model showing relationship of FD value (before staining) with study factors

Variables	B	SE	*t*	*P*
(Constant)	.955	.144	6.612	<.001
Age (years)	.001	.002	.628	.531
Bidi/cigarette	.144	.075	1.932	.056
Gutkha	.075	.053	1.412	.161
Pan/supari	.026	.056	.460	.647
Surti/khaini	.017	.057	.293	.770
Tobacco leaves	.011	.004	2.831	.006
Duration (years)	-.012	.124	-0.101	.920
Lower	-.033	.116	-.287	.775
Average	-.026	.043	-.616	.539

Table 7: Linear regression model showing relationship of FD (after staining with TB) with study factors

Variables	B	SE	T	*P*
(Constant)	.907	.149	6.073	.000
Age (years)	.002	.002	1.002	.318
Bidi/cigarette	.100	.077	1.295	.198
Gutkha	.058	.055	1.066	.289
Pan/supari	.034	.058	.586	.559
Surti/khaini	.035	.059	.603	.548
Tobacco leaves	.035	.068	.511	.610
Duration (years)	.009	.004	2.249	.027
SES-lower	.003	.128	.025	.980
SES-average	-.072	.120	-0.600	.550

increased as the duration of the consumption of tobacco product (smokeless and smoke) increased. Correspondingly, Banoczy *et al.* in their study claimed that leukoplakia was higher in tobacco users and the relationship between the tobacco habit and the anatomical location of the leukoplakia was apparent.[30] Therefore, dose-response relationship is evident between tobacco use and oral leukoplakia.

FD values of leukoplakia among participants consuming smoke tobacco also showed higher value in smokers' case than nonsmokers. However, the result showing higher FD value was nonsignificant ($P > 0.05$). Hence, we can rely equally on FD values for studying the complexity of leukoplakia in this group.

Conclusion

The morphology and complexity of leukoplakia is important in the diagnosis of malignancy potential. This complexity can be effectively determined by FD analysis. Therefore, FD analysis could be used as a noninvasive, cost effective diagnostic tool for the early detection of malignant conversion.

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Conflicts of interest

There are no conflicts of interest.

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