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 into 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. In fractal analysis, there is processing of the subject which can be a signal or an image. FD decreases or increases as the complexity of the object decreases or increases.

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

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

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 \cite{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. 89ECM11BThesis/ 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:

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

Exclusion criteria:

- Medically compromised patients.
- Patients who are already enrolled/completed treatment of oral leukoplakia.
- Patients who are allergic to toluidine blue stain.
- Patients not willing for biopsy procedure.
- 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 \cite{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].
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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.[13]

Discussing

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 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.[13]
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. 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.

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.

Table 4: Distribution of FD value according to tobacco product

| Tobacco Product | Status | FD (before staining) Mean ± SD | FD (after staining with TB) 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                                |
| Guttka          | 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. and Deepak 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. Similarly, Uma Reddy and et al. 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. The prevalence of leukoplakia increased with age advancement. It has been estimated that it mainly affects men over 40 years.

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.

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) Pearson Correlation | P       | FD (after staining with TB) 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  |

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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. 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.

### References
1. Anchez I, Uzcategui G. Fractals in dentistry. J Dent 2011;39:273-92.
2. Ang HJ, Jeong SW, Jo BH, Kim YD, Kim SS. Observation of trabecular changes of mandible after orthognathic surgery using fractal analysis. J Korean Assoc Oral Maxillofac Surg 2012;38:96-100.
3. Kiselev VG, Hahn KR, Auer DP. Is the brain cortex a fractal? Neuroimage 2003;20:1765-74.
4. Zamir M. Fractal dimensions and multifractality in vascular branching. J Theor Biol 2001;212:183-90.
5. Landini G, Rippin JW. Fractal dimensions of the epithelial-connective tissue interfaces in premalignant and malignant epithelial lesions of the floor of the mouth. Anal Quant Cytol Histol 1993;15:144-9.
6. Khandekar S, Upadhyaya N, Dive A, Moharil R, Mishra R, Gupta S, et al. Study of epithelial tissue interface using fractal geometry: A pilot study. J Evol Med Dent Sci 2013;2:10041-5.
7. Demiralp KO, Kurşun-Çakmak ES, Bayrak S, Akbulut N, Atakan C, Orhan K. Trabecular structure designation using fractal analysis technique on panoramic radiographs of patients with bisphosphonate intake: A preliminary study. Oral Radiol 2019;35:23-8.
8. Starzynska A, Pawlowska A, Rzepielska D, Michajlowski I, Sobajane M, Blazewicz I, et al. Estimation of oral leukoplakia treatment records in the research of the Department of Maxillofacial and Oral Surgery, Medical University of Gdansk. Postepy Dermatol Alergol 2015;32:114-22.
9. Pindborg JJ, Kaer JP, Gupta PC, Chawla TN. Studies in oral leukoplakias. Prevalence of leukoplakia among 10,000 persons in Lucknow, India, with special reference to use of tobacco and betel nut. Bull World Health Organ 1967;37:109-16.
10. Masthan KMK, Babu NA, Dash KC, Elumalai M. Advanced diagnostic aids in oral cancer. Asian Pac J Cancer Prev 2012;13:3573-6.
11. White SC, Rudolph DJ. Alterations of the trabecular pattern of the jaws in patients with osteoporosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;88:628-35.
12. Raja JV, Khan M, Ramachandra VK, Kadi OA. Texture analysis of CT images in the characterization of oral cancers involving buccal mucosa. Dentomaxillofac Radiol 2012;41:475-80.
13. Rao DN, Shroff PD, Chattopadhyay G, Dinshaw KA. Survival analysis of 5595 head and neck cancers—results of conventional treatment in a high-risk population. Br J Cancer 1998;77:1514-8.
14. Mihara N, Kuriyama K, Kido S, Kuroda C, Johkoh T, Naito H, et al. The usefulness of fractal geometry for the diagnosis of small peripheral lung tumors. [In Japanese.] Nihon Igaku Hoshasen Gakkai Zasshi 1998;58:148-51.
15. Yan Y, Zhu W, Wu Y, Zhang D. Fractal dimension differentiation between benign and malignant thyroid nodules from ultrasonography. Appl Sci 2019;9:1494.
16. Nagamachi S, Nonokuma M, Mizutani Y, Terada T, Yoshimitsu K, Hirai T. The usefulness of fractal analysis in FDG-PET/CT images for diagnosing therapeutic effect of non-small cell lung cancer. J Nucl Med 2019;60(Suppl 1):287.
17. Khundia SS, Bhavthankar JD, Mandale MS, Humbe JG. Quantitating the nuclear discrepancies between
orthokeratotic and parakeratotic epithelial dysplasia using computer aided diagnostics. Int J Innov Sci Technol 2019;4:1016-21.

18. Metze K, Adam R, Florindo JB. The fractal dimension of chromatin-a potential molecular marker for carcinogenesis, tumor progression and prognosis. Expert Rev Mol Diagn 2019;19:299-312.

19. Alvarado-Cruz LB, Delgado-Herrera M, Toxqui-Quitl C, Padilla-Vivanco A, Castro-Ortega R, Arreola-Esquivel M. Fractal analysis for classification of breast lesions. Proc. SPIE 11104, Current Developments in Lens Design and Optical Engineering XX, 111040U (30 August 2019); https://doi.org/10.1117/12.2531201.

20. Mambetsariev I, Mirzapoiazova T, Lennon F, Jolly MK, Li H, Nasser MW, et al. Small cell lung cancer therapeutic responses through fractal measurements: From radiology to mitochondrial biology. J Clin Med 2019;8:E1038.

21. Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: An overview of the literature. J Oral Pathol Med 2008;37:1-10.

22. Pandey PB, Kandakurti S, Saxena VS, Tripathi P, Pamula R, Yadav M. Fractal analysis in oral leukoplakia. J Indian Acad Oral Med Radiol 2015;27:354-8.

23. Villa A, Bin S. Leukoplakia: A diagnostic and management algorithm. JOMS 2017;75:723-73.

24. Shenoi SR, Peshwani P, Garg A, Moharil R. A study of complexity of oral mucosa using fractal geometry. Indian J Dent Res 2017;28:362-6.

25. Deepak P. Fractal analysis in grading of oral leukoplakia. Int J Adv Res Technol 2015;9:26-35.

26. Goutzenis L. Nuclear fractal dimension as a prognostic factor in oral squamous cell carcinoma. Oral Oncol 2008;44:345-53.

27. Uma Reddy C, Anitha M, Feroz A, Chandrashekar L, Sudarshan R, Vigneswary. Digital imaging analysis with fractal dimension in oral leukoplakia. Asian J Res Med Pharm Sci 2017;2:1-5.

28. Peterson BR, Gupta PC, Pindborg JJ, Singh B. Association between oral leukoplakia and sex, age, and tobacco habits. Bull World Health Organ 1972;47:13-9.

29. Waal IVD. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol 2009;45:317-23.

30. Bánočzy J, Gintner Z, Dombi C. Tobacco use and oral leukoplakia. J Dent Educ 2001;65:322-7.