A Study of Complexity of Oral Mucosa Using Fractal Geometry

Abstract

Background: The oral mucosa lining the oral cavity is composed of epithelium supported by connective tissue. The shape of the epithelial-connective tissue interface has traditionally been used to describe physiological and pathological changes in the oral mucosa. Aim: The aim is to evaluate the morphometric complexity in normal, dysplastic, well-differentiated, and moderately differentiated squamous cell carcinoma (SCC) of the oral mucosa using fractal geometry. Materials and Methods: A total of 80 periodic acid-Schiff stained histological images of four groups: normal mucosa, dysplasia, well-differentiated SCC, and moderately differentiated SCC were verified by the gold standard. These images were then subjected to fractal analysis. Statistical Analysis: ANOVA and post hoc test: Bonferroni was applied. Results: Fractal dimension (FD) increases as the complexity increases from normal to dysplasia and then to SCC. Normal buccal mucosa was found to be significantly different from dysplasia and the two grades of SCC (P < 0.05). ANOVA of fractal scores of four morphometrically different groups of buccal mucosa was significantly different with F (3,76) = 23.720 and P < 0.01. However, FD of dysplasia was not significantly different from well-differentiated and moderately differentiated SCC (P = 1.000 and P = 0.382, respectively). Conclusion: This study establishes FD as a newer tool in differentiating normal tissue from dysplastic and neoplastic tissue. Fractal geometry is useful in the study of both physiological and pathological changes in the oral mucosa. A new grading system based on FD may emerge as an adjuvant aid in cancer diagnosis.

Keywords: Epithelial-connective tissue interface, fractal geometry, oral mucosa, squamous cell carcinoma

Introduction

Squamous cell carcinoma (SCC) is the most common type of malignancy affecting oral cavity. Five-year survival rate of oral cancer has not improved over the past several decades and still it remains a serious public health problem.[1]

The tumor, node, and metastasis clinical staging system was proven to be a useful prognostic tool. Nowadays, management by multidisciplinary teams has allowed optimal treatment of cancer patients.[2] However, the biological behavior of individual tumor remains unpredictable. It is unclear why some locally advanced stage disease (stage T3 and T4) gradually results in regional metastasis, whereas some lower stage lesions (stage T1 and T2) show early and aggressive regional metastasis. The ability to predict the primary lesions capable of early metastasis would enable a more specific and aggressive treatment.[3]

Although clinical and histological factors have aided in the estimation of survival rate in patients with oral cancer, there has been a need for more specialized diagnostic and prognostic modalities. Currently, computer-aided image analysis has gained importance. Several researches are being done on biologic markers as well as factors related to the morphology of malignant cells and tissues, which can be studied through image analysis.

Methods of image analysis are broadly divided into two categories:
1. Conventional methods based on the size of the nuclei
2. Modern and accurate methods, which include fractal analysis whose results, are mathematically proven and reliable.[4] These methods assess the complexity of structures of the neoplastic tissue and confirm mathematically the subjective assessment of pathologist.[4]

Optical coherence tomography, confocal, and two-photon microscopy are also noninvasive imaging modalities that are capable of visualizing and assessing alterations in the

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epithelial connective tissue interface (ECTI) geometry.[4] Researches are being done for the early diagnosis of cancer through these imaging modalities.

Classical Euclidean geometry describes properties of smooth-shaped objects such as circles or squares based on their perimeter. However, these Euclidean descriptions are insufficient to describe the complex irregular objects that occur freely in nature (e.g., clouds, coastlines, and other biological structures). Fractal geometry can be helpful to denote such “non-Euclidean” objects. It is an innovation in the field of mathematics; pioneered by Benoit Mandelbrot.[5] The objects characterized by self-similarity are described as “fractal objects.” The assessment of the degree of complexity and irregularity of such kind of objects gives measurements known as “fractal dimensions” (FDs). There are many methods for estimation of FDs. The most commonly used method is box-counting dimension.[1] The number of boxes of size needed to cover a fractal set follows a power-law, \( N = N_d \times R^{-DF} \), with \( DF \leq D \) (D is the dimension of the space, usually \( D = 1, 2, 3 \)) which is repeated with different box sizes.[6]

\[ \text{FD} = \text{slope} = \left( \frac{d \ln N}{d \ln R} \right) / R \]

Where D is the dimension of space

\( N \) is number of boxes

\( R \) is size of boxes

It is helpful for the study of the structural properties of natural objects including specimens of histopathology.[6] Most of the structures of human body such as vessels of the retina and kidneys and bronchial tree of the lungs also follow the natural law of FD. Moreover, fractal geometry is applied in several fields of medicine: cardiology for heart rate estimation, neurology for the analysis of wavering patterns in electroencephalograms, radiology for radiographic analysis, and analysis of mammographic lesions and tomographies.[3]

Fractal analysis has been also applied in histopathology and cytology for the estimation of the FDs of various neoplasms such as carcinomas of the gallbladder, lung, uterus, breast, larynx, and oral cavity.[1,7-11]

**Aim and objective**

The aim of this study is to use fractal geometry to compare the structural complexity in normal, dysplastic, well-differentiated, and moderately differentiated SCC of the oral mucosa. It will be accomplished by estimating the FDs of ECTI profiles obtained from histological sections of the aforesaid entities.

**Materials and Methods**

A total of 80 histopathological wax blocks from the Regional Cancer Institute, Nagpur, were selected from the period January 2013 to January 2016. These specimens which include normal, dysplastic, well-differentiated, and moderately differentiated SCC of the oral mucosa, were verified by the gold standard histopathology and histopathological slides were prepared using periodic acid–Schiff (PAS) staining method. Only one sample of poorly differentiated SCC (PDSCC) of buccal mucosa was found during the above period and hence has been discarded from the analysis. Twenty slides of normal epithelium of buccal mucosa were recently made in the month of January, 2016.

**Sample size calculation**

With a reference to a previous study,[6] the sample size was calculated by mean difference method using openepi.com. The confidence interval and power of the study were set at 95% and 80%, respectively, with the ratio between the groups in the sample being 1. The sample size in each group was determined to be 17 which was rounded off to 20, with a total sample size of 80.

Periodic acid–Schiff-stained histological images of buccal mucosa at 10× (scanner view) for:

1. Normal epithelium [Figure 1a] 20 specimens
2. Dysplastic epithelium [Figure 1b] 20 specimens
3. Well-differentiated SCC [Figure 1c] 20 specimens
4. Moderately differentiated SCC [Figure 1d] 20 specimens.

**Selection criteria**

1. Exclusively buccal mucosa was chosen to standardize our results, as the FDs of different areas of oral mucosa vary considerably
2. PAS-stained slides of all 4 study groups with confirmed diagnosis were included.

**Image J software with plugin for Frac Lac**

We have used open source Image J software (National Institute of Health, USA) with an add-on plugin Frac Lac for the image analysis. We kept the box count from 2 to 64 to standardize our results and to avoid complications of multiple scans. The image size in pixels was also standardized before

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**Figure 1:** Histopathological image of (a) normal mucosa (b) dysplasia (c) well differentiated squamous cell carcinoma and (d) moderately differentiated squamous cell carcinoma of buccal mucosa
processing and analyzing. Image analysis was performed to separate the ECTI using an edge detecting filter software. The image was divided into boxes by the software and output was regenerated in the graph form with the slope of the regression line. The slope represents the FD [Figure 2].

The method was repeated ten times at random places of a single digital image throughout the ECTI and their mean value were calculated. Similarly, all the images were analyzed and the results were tabulated and compared. The image analysis of this study was completed using analysis of variance (ANOVA) and appropriate post hoc tests in 6-month duration. The examiner who did the fractal analysis was blinded to the gold standard of the specimen.

This study was approved by Institutional Ethical Committee of our institute (IEC/VSPMDCRC/35/2016).

**Results**

The mean box FD (D) showed that moderately differentiated SCC was the most complex (D = 1.9327 ± 0.00715) and well-differentiated SCC (D = 1.9468 ± 0.00599), followed by dysplasia (D = 1.9446 ± 0.00587), and the normal buccal mucosa had the lowest FD (D = 1.9327 ± 0.00715) and was found to be statistically different from dysplasia and the two grades of SCC [Table 1 and Figure 3].

A one-way ANOVA of fractal scores of four morphometrically different groups of buccal mucosa was significantly different with F (3,76) =23.720 and P < 0.01 [Table 2].

*Post hoc* test: Bonferroni was also applied and it showed that we can differentiate normal mucosa from dysplasia, well-differentiated SCC, and moderately SCC (P < 0.01) on the basis of fractal analysis of histological images of buccal mucosa. In the current study, we could not differentiate dysplasia from well-differentiated SCC (P = 1.000) and moderately differentiated SCC (P = 0.382) on the basis of FD [Table 3].

**Discussion**

We have excluded PDSCC cases from our study as enzymatic degradation of basement membrane components, and loss of continuity of ECTI is noted in PDSCC and our study is based on FD of ECTI. Incidence of PDSCC is also very low, 121 cases (13.32%) out of 908 cases of OSCC were found at a tertiary level referral hospital in Central India (Vidarbha) in a period of 7 years. There were 499 cases (54.95%) of well-differentiated carcinoma and 252 cases (27.75%) of moderately differentiated carcinoma. The more number of cases of well-differentiated carcinoma might be due to awareness of oral cancer among the residents of this region through cancer awareness programs by the government and various other agencies.

Several similar studies described below were done using H and E stained histopathology slides. However, we in our study have used a special stain: PAS for better visualization of the epithelial-connective tissue junction. The continuity, contrast, and pattern of the basement membrane are better demonstrated by PAS stain followed by the hematoxylin and eosin (H and E) stain.

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**Table 1: Descriptive statistical analysis of the four groups of buccal mucosa**

| Type                      | n  | Mean (D) | SD    | SE     | 95% CI for mean | Minimum | Maximum |
|---------------------------|----|----------|-------|--------|-----------------|---------|---------|
|                           |    |          |       |        | Lower bound     |          |         |
| Normal                    | 20 | 1.9327   | 0.00715| 0.00160| 1.9294          | 1.9360  | 1.92    | 1.95    |
| Dysplasia                 | 20 | 1.9446   | 0.00587| 0.00131| 1.9419          | 1.9473  | 1.93    | 1.96    |
| Well-differentated SCC    | 20 | 1.9468   | 0.00599| 0.00134| 1.9439          | 1.9496  | 1.93    | 1.96    |
| Moderately differentiated SCC | 20 | 1.9485   | 0.00710| 0.00159| 1.9452          | 1.9518  | 1.93    | 1.96    |
| Total                     | 80 | 1.9431   | 0.00895| 0.00100| 1.9411          | 1.9451  | 1.92    | 1.96    |

CI=Confidence interval, SD=Standard deviation, SE=Standard error, SCC=Squamous cell carcinoma
Only buccal mucosa was chosen in our study to standardize the results in all the four study groups, as the FDs of different zones of oral mucosa vary considerably. The most common site of occurrence of SCC is also the buccal mucosa.[15]

Abu Eid and Landini[16] studied changes in ECTI irregularity in normal, dysplastic, and neoplastic mucosa of oral cavity and found that the normal oral mucosa had the lowest mean box FD (1.09), followed by dysplastic ECTI (s) with 1.14, 1.16, and 1.16 values for mild, moderate, and severe dysplasia classes, respectively. The mean FD of SCC was highest (D = 1.23). The increase in FDs is observed as the complexity increases from normal to dysplasia and then to SCC. Our results were also similar to it except some differences in the values of FDs as we have used special stain-PAS rather than the usual H and E stain.

They also analyzed the three grades of dysplasia: mild, moderate, and severe. Although FD increased with severity of the dysplasia, those differences were not statistically significant.[16] Similarly, we analyzed the two grades of SCC and found that FD of moderately differentiated SCC was greater than that of well-differentiated SCC. However, the difference is not statistically significant.[16]

Abu Eid and Landini[17] compared the FD of pseudoepitheliomatous hyperplasia (PEH), normal and dysplastic mucosa of oral mucosa, and concluded that PEH were more irregular than both normal and dysplastic ECTI profiles. In the year 2006, they compared the complexity of SCC and PEH. The results showed that ECTI profiles of granular cell tumor PEH associated with GCT (GCT-PEH) cases were more complex than those of SCC. This may be due to rapid epithelial growth toward the stroma and a coexisting growth by the granular cells toward the epithelium. In GCT-PEH, cell mixing at the ECTI results in different type of structural complexity than those observed in epithelial SCC.[18]

Similar study of structural differences between normal mucosa, dysplasia, SCC, and PEH of the oral mucosa was done in the year 2013 which concluded that FD increases as the complexity and irregularity of ECTI increases from normal to pseudoepithelial hyperplasia then to dysplasia and SCC.[6]

Oral carcinomas are not the only tumors to be described quantitatively by fractal geometry. Fractals have proved propitious in describing the complexity of several tumors affecting various parts of the body such as colorectal polyps (Cross et al., 1994), gallbladder adenocarcinoma (Waliszewski, 1999), basal cell carcinoma of the skin (Miracco et al., 1998), and malignant melanoma (Claridge et al., 1992).[19]

**Conclusion**

Fractal geometry is useful in the study of both physiological and pathological changes in the oral mucosa. This study establishes FD as a newer diagnostic tool in differentiating normal tissue from dysplastic and neoplastic tissue. A new grading system based on FD may emerge as an adjuvant aid in cancer diagnosis. Further researches are required for exploration of the fractal geometry and its application in the field of medical science.

**Limitations**

This study completely relies on software and digital photographic images. The software may give false-positive and false-negative results as it based on color pixel filter. This study is a short-term study and it should be evaluated on a large scale to check the accuracy of the results. The

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Table 2: Analysis of variance between four study groups

| Sum of squares | df | Mean square | F   | P      |
|---------------|----|-------------|-----|--------|
| Between groups | 0.003 | 3 | 0.001 | 23.720 | <0.01 |
| Within groups  | 0.003 | 76 | 0.000 |        |       |
| Total          | 0.006 | 79 |       |        |       |

Table 3: Post hoc Bonferroni test showing correlation between the four study groups

| Type (I) | Type (J)               | Mean difference (I-J) | SE   | 95% CI          | P   | Lower bound | Upper bound |
|----------|------------------------|-----------------------|------|----------------|-----|-------------|-------------|
| Normal   | Dysplasia              | −0.01190*             | 0.00207 | −0.0175* | −0.0063 | <0.01       |
|          | Well-differentiated SCC| −0.01405*             | 0.00207 | −0.0197* | −0.0084 | 0.000       |
|          | Moderately differentiated SCC | −0.01580* | 0.00207 | −0.0214* | −0.0102 | 0.000       |
| Dysplasia | Normal                 | 0.01190*              | 0.00207 | 0.0063*  | 0.0175  | 0.000       |
|          | Well-differentiated SCC| −0.00215              | 0.00207 | −0.0078  | 0.0035  | 1.000       |
|          | Moderately differentiated SCC | −0.00390 | 0.00207 | −0.0095  | 0.0017  | 0.382       |
| Well-differentiated SCC | Normal             | 0.01405*              | 0.00207 | 0.0084*  | 0.0197  | 0.000       |
|          | Dysplasia              | 0.00215               | 0.00207 | −0.0035  | 0.0078  | 1.000       |
|          | Moderately differentiated SCC | −0.00175 | 0.00207 | −0.0074  | 0.0039  | 1.000       |
| Moderately differentiated SCC | Normal | 0.01580*              | 0.00207 | 0.0102*  | 0.0214  | 0.000       |
|          | Dysplasia              | 0.00390               | 0.00207 | −0.0017  | 0.0095  | 0.382       |
|          | Well-differentiated SCC| 0.00175               | 0.00207 | −0.0039  | 0.0074  | 1.000       |

*The mean difference is significant at the 0.05 level. CI=Confidence interval, SE=Standard error, SCC=Squamous cell carcinoma
sample size in this study is too small to draw conclusions, in this regards.

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Conflicts of interest
There are no conflicts of interest.

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