STUDY OF EPITHELIAL CONNECTIVE TISSUE INTERFACE USING FRACTAL GEOMETRY: A PILOT STUDY

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ABSTRACT: Oral squamous cell carcinoma (OSCC) is the most common type of oral malignancy. Despite progress in therapeutic approaches, the five-year survival rate for oral cancer has not improved significantly over the past several decades and it remains about 50%. In clinical practice, the treatment planning and prognosis of oral cancer is mainly based on the histopathological and TNM classifications. However, there is increasing evidence that they are probably insufficient to predict the clinical outcome of patients with oral carcinoma. The research has focused on discovering biologic markers, as well as factors related to themorphology of the neoplastic cells and tissues, which can be studied through computer aidedimage analysis. The aim of this study was to use fractal geometry to compare the morphometric complexity in the normal and squamous cell carcinoma of the buccal mucosa, which was fulfilled by estimating the fractal dimensions of epithelial connective tissue interface (ECTI) profiles, isolated from histological sections of the tissue specimens.**RESULTS**: Fractal analysis of the ECTI may be used as an objective morphometric criteria to distinguishnormal mucosa andsquamous cell carcinoma.
KEYWORDS: Oral mucosa, Squamous cell carcinoma, Fractal analysis, Epithelial connectivetissue interface (ECTI)

INTRODUCTION: Cancer are highly complex disease and heterogeneous in nature regarding site, biology, and treatment response, due to the disruption of tissue architecture. Thus, tissues, and not individual cells, are the proper level of observation for the study of carcinogenesis. The oral mucosa consists of epithelium and connective tissue. The two are connected by basement membrane (ECTI). The structural organization of ECTI is therefore useful in diagnostic histopathology to distinguish between normal mucosa and OSCC and appears to be crucial in tumour invasion and metastasis. However, the description of ECTI has most often been subjective. The use of fractal geometry has therefore been used to describe the irregularities of ECTI profiles taken from the oral mucosa both in normal and abnormal cases. The research has focused on discovering biologic markers, as well as factors related to the morphology of the neoplastic cells and tissues, which can be studied through computer aided image analysis.Fractal geometry is a new development in Mathematics, established by Benoit Mandelbrot. It aids the accurate study of the structural properties of natural objects including histopathology specimens. The assessment and quantification of the degree of complexity and irregularity of these objects gives measurements called "fractal dimensions"(FD). The most widely used method of estimation of the fractal dimension of natural (fractal) objects is the box-counting algorithm used by several computer programs.[1-4]

AIM: The aim of this study was to use fractal geometry to compare the morphometric complexity in the normal andsquamous cell carcinoma of the buccal mucosa, which was fulfilled by estimating the fractal dimensions of ECTI profiles, isolated from histological sections of the tissue specimens.

MATERIAL & METHOD: Histological Hematoxylin& Eosin stainedsections of the tissue specimens were grouped into the following twocategories the Group I(Normal Epithelium) control (20 slides) andGroup II (OSCC)study group (20 slides).These tissue specimens were chosen with confirmed histopathologic diagnosis from buccal mucosa, to standardize the results in both study and control groups, as the fractal dimensions of different zones of oral mucosa vary considerably. The histological pictures were captured at 4 x magnifications (scanner view) by Oylmpustrinocular research microscope Model –BX 51**.**The epithelium connective tissue junctions of each section were evaluated by image analysis using fractal software to quantify FD by the box-counting method. According to this method, a grid with multiple small boxes of particular pixel length is superimposed on the digital image.To standardize our results and to avoid complications of multiple scans, we kept the box count from 2 to 12 only. Image analysis was performed to separate the epithelium from the connective tissue. Irregularity profile image was divided into boxes by the software & output was regenerated in the graph form with the slope of the regression line generated from the software.The slope was the measure of fractal dimension. The method was repeated 10 times at random places of a digital image throughout the epithelial connective tissue junction & averaged to give the Fractal dimension of ECTI profile of a single image. The same method was repeated for all the images and the results were tabulated and compared. [1, 4-6]

The number **N** of boxes of size **R** needed to cover a fractal set follows a power-law, N = N0 *R^(-DF), with DF<=D (D is the dimension of the space, usually D=1, 2, 3), which is repeatedwith different box sizes. "Fractal dimension = slope = (d ln N / d ln R)/ R"[7, 8]

RESULT: In Group I fractal dimensions were ranging from 1.489 to 1.755, with the mean fractaldimension 1.64105. In Group II, fractal dimensions were ranging from 1.689 to 1.911, with the mean fractal dimension 1.82615.(Figure 1). In group II (Squamous cell carcinoma), the mean fractal dimension (1.82615) was more as compared to mean fractal dimension (1.64105)of group I(Normal buccal mucosa) (figure 2).One Way ANOVA test statistics shows statistically significant difference (p=0.0000) for mean fractal dimension between Group I and Group II. (Table 1)

DISCUSSION: Fractal analysis method is able to quantify the irregular structures that are present in tumors, insitu carcinomas which show micro invasion, thinning, fragmentation, interrupted basement membrane staining, where loss of basement membrane continuity cannot be used as a simple criterion of invasion.[5-7]In present days, scientists strongly have opinion that fractal geometry is a revolutionary area of mathematics which has deep impact on every branch of science including medical fields.Derived from the Latin "fractus' meaning fragmented, a fractal is a mathematical object with a fractional dimension.[9, 10]. Several comprehensive reviews of the use of fractal dimensions in pathology have recently appeared in the literature [3, 4, 8, 9.] There are various ways of measurement of FD such as Modified pixel dilatation, Perimeter-area method, Ruler counting method andBox counting method. Box counting method is most commonly used for detection of FD. It is a simple and reproducible way of measuring fractal dimension and isalso used in our study.

There is a growing literature that shows fractals to be useful measures of the pathologies of the vascular architecture, tumor-parenchymal border, and cellular-nuclear morphology.[9]

Rippin (1993) in their studyshowed that the increase of abnormalities observed on this interface(accompanied by severe dysplasia and invasive carcinoma) was followed by an increase offractal dimension. The difference between the two FD values was considerable. 1.00 In the normal epithelium and 1.61 in the invasive carcinoma. In a subsequent study the same investigators found that in specimens from oral lesions diagnosis by means of fractal analysis of the epithelial connective tissue interface may reach an accuracy of 85%. [4]Landini and Rippin went on to describe a more sophisticated multifractal analysis that yields a spectrum of fractal values instead of a single value for each image, which provided a still more reliable discrimination of the pathological state of the tissues. Lefebvre and Benali and Pohlman et al. have demonstrated that fractal methods may also be useful for analyzing digitized mammograms, raising hopes that the number of false positive mammograms might be reduced.[9]Using the box-counting method for the fractal analysis of 359 colorectal polyps Cross et al.(1994) discovered that the mean FD value for tubulovillous adenomas wassignificantly larger than that for meta-plastic polyps or inflammatory polyps.Fractal dimension was proved to be a statistically significant prognostic factor for laryngeal carcinoma in the study of Delides et al. (2005). [4]The same observations were made by other investigators in a recent study of 52 patients with laryngeal carcinomas.[8]In this study a statistically significant correlation between fractal dimension and the cell differentiation was also observed.

In our study, we found that an increase in fractal dimensions was observed as the complexityincreased from normal mucosa tosquamous cell carcinoma and statistics shows statistically significant difference (p=0.0000) for mean fractal dimension betweenthe twoGroups.This finding agrees with the observations of other researchers about different types of neoplasms

CONCLUSION: Fractal geometry is a rapidly growing area of mathematics with immense potential, which provides a new objective assessment method, important for the screening of oral cancer. In oral cancer study, fractal dimension can be used as a quantitative index to discriminate among normal and neoplastic oral mucosa.The methodologies used in this study can be expanded at larger scale and to other pathological entities both inside & outside the oral cavity combined with cellular morphometric analysis & molecular analysis for further understanding of the pathological processes. Although, this study is totally based on software & digital photographic images and limited by theuse of colour pixel filter which may give false positive & false negative results. The use of special stains like PAS or Masson's Trichrome stains may be used to highlight the epithelial connective tissue junction. So further studies are required in this regards, along with a large sample sizeto check the consistency of the results.Within few years it will help us to explore the mystery of morphogenesis, tumorogenesis, angiogenesis, classifying disease entities and many unfold mystery of human life.

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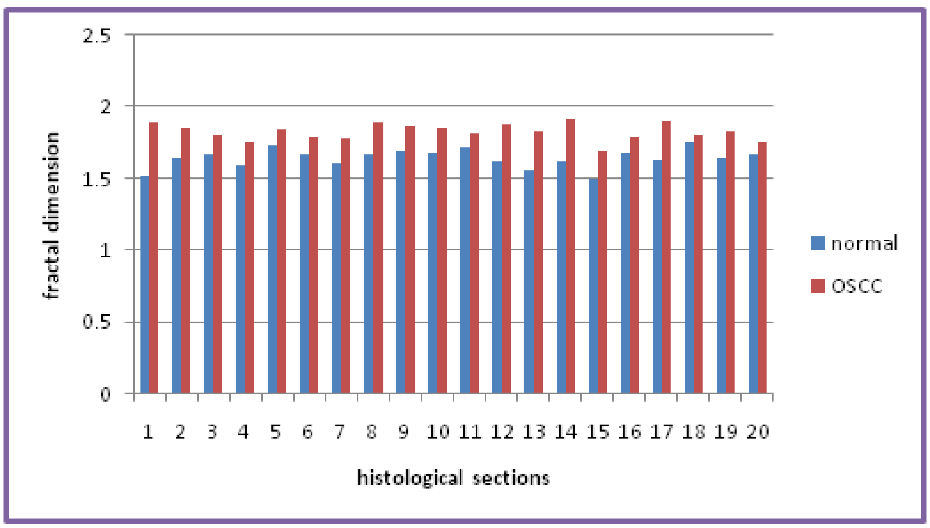

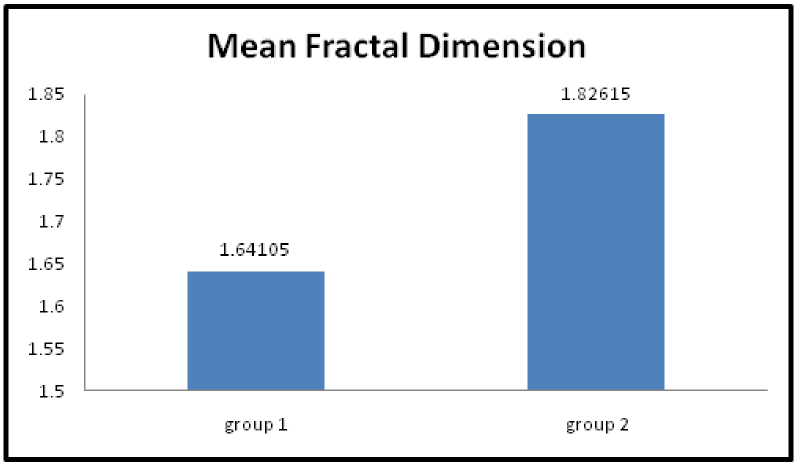

Group	N	Mean FD	SD	P value	Result
Group 1	20	1.64105	0.066663	0.000	significant
Group 2	20	1.82615	0.057286		

Table 1 : comparison of Mean Fractal Dimension between Group I and Group II

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