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Scope of Conventional and Fractal Morphometry in Oral Potentially Malignant Disorders and Oral Cancer

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Abstract

World Health Organization (WHO) defined the terminology 'Oral Potentially malignant disorders' (OPMD) as the presence of risk of malignancy in a lesion or condition either during the time of initial diagnosis or at a future date with the commonly accepted prevalence of 1–5%. All OPMDs may not transform into malignancy, many factors have been explored which effectively assess the risk of malignant transformation in OPMDs including many clinical, pathological and molecular factors. Qualitative & experimental factors of different cells in cytological preparations and/or biopsy specimens are reliable parameters for pathologists. The analysis and assessment of histological units can be enhanced by image analysis assisted by a computer that can be used for statistical comparisons. Fractal geometry is considered to be an ideal method of image analysis in quantitative microscopy & histopathology. Fractal dimension analysis is not only limited to determining cell and tumour types but can also be used for determining cellular behaviours in vitro such as cell migration, apoptosis and cellular differentiation, which can be a useful characterization of oral cancer lines and further help in the treatment planning.

Keywords: Oral Cancer, Oral Potentially Malignant Disorders (OPMDs), Conventional morphometry, Fractal Dimension, Photomicrograph

Introduction

World Health Organization (WHO) defined the terminology 'Oral Potentially malignant disorders' (OPMD) as the presence of risk of malignancy in a lesion or condition either during the time of initial diagnosis or at a future date with the commonly accepted prevalence of 1–5%. (1) Early diagnosis of OPMDs and OSCC can increase survival which may be achieved by both non-invasive and invasive techniques.

However, pathologists diverge in the importance they give to particular histopathologic features and the interpretation of dysplasia varies from one pathologist to another. The wide subjective variation has always sought the necessity of objective means of evaluation(2,3). Recently with the upcoming trends in cytomorphometry and histomorphometry focus on the direction of the diagnostic potential of these techniques in OPMDs and OSCC has been emphasized which

may reduce the subjective variation to a great extent.

A prognostic factor is a clinical or biological feature that is objectively measurable and that gives information on the possible outcome of the disease in an untreated person. All OPMDs may not transform into malignancy, many factors have been explored which effectively assess the risk of malignant transformation in OPMDs including many clinical, pathological and molecular factors. Clinical factors include gender, duration, location, size and nature of the lesion whereas histopathological factors include the presence of dysplasia, DNA ploidy, loss of heterozygosity (LOH) and genes are the molecular factors. (4) Irrespective of histological dysplastic grading, strong evidence suggests that finding of very similar variations in diseases like leukoplakia and erythroplakia are closely linked to neoplastic transformation risk. (5–9). Likewise in 14 out of 42 (33%) cases of oral dysplasia aneuploidy was seen that progressed & 5 out of 44 (11%) cases showed no progression. (10)

A large-scale retrospective study was done in recent times that established dysplasia and aneuploidy can be seen as independent predictive factors for malignant transformation. (10) Many studies were done that demonstrated high-frequency LOH which occurs on 3p and 9p are strongly associated with leukoplakia transformation and the progression or recurrence of oral cancer(11–17). The role of p53 (TP53) has been also established in the malignant transformation potential of OPMDs(18,19). Cervical lymph node metastasis, five-year survival, recurrence are a few well-established prognostic factors in patients diagnosed with OSCC (20–23). A discrete relationship is present between the number & level of cervical lymph nodes metastasized with the epithelial cell and five-year survival (24). Clinical neck dissection has an estimated sensitivity of 75% - 82% and specificity of 80% - 83% (25,26). Evidence of cervical lymph node metastasis is well acknowledged by neck dissection and it's undertaken based on clinical node involvement, the experience of the surgeon with consideration of other predictive and prognostic factors.

Attempts are made for many years to define the parameters and classifications that may enable to segregate the patients into groups of low, intermediate and high risk. Definite factors have been implied like TNM staging, histopathologic malignancy grading system,(27–34) interactions between stroma and invasive tumour front,(35–39) lymph vascular invasion,(40–43), perineural infiltration,(40,41,43), tumour thickness and compromised margins (44,45). A debatable role of the predictive value of proliferation markers in OSCC also exists (30,46–54). However, the utility of these prognostic factors in OPMDs and OSCC has been restricted mostly dues to their semi-quantitative nature and subjective variation. In order to overcome these limitations computers assisted morphometric analysis has emerged as an alternative approach to measure the cellular and nuclear features which further may be correlated with the outcome of OPMDs and OSC.

COMPUTER-ASSISTED MORPHOMETRIC ANALYSIS

Qualitative & experimental factors of different cells in cytological preparations and/or biopsy specimens are reliable parameters for pathologists. Many empirical methods have been backed up by morphometry like an estimation of the surface area, volume, axis ratios, calculation of density of population and various methods which are derived from Euclidean geometry. They find their application to assess the nucleus, nucleoli present, chromatin quantity, abnormalities in the nuclear membrane, perturbations in the cytoplasmic membrane and differentiation degree. Since time immemorial it has been under consideration in mathematics & physical sciences that concepts of Euclidean geometry like length, perimeter, volume or surface area do not give definite answers related to certain objects. The analysis and assessment of histological units can be enhanced by image analysis assisted by a computer that can be used for statistical

comparisons. The advantages of computer-assisted morphometric image analysis are-

i. Quantitative or semi-quantitative description of pathologists' qualitative assessment of cellular architecture.
ii. This objective estimation is not exaggerated by intraobserver or inter-examiner variability.

These advantages combinedly shall have a meaningful predictive value. The morphometry can be studied by conventional and modern methods.

Conventional morphometry, a concept derived from Euclidean geometry has been applied in computer-assisted histopathology image analysis to correlate the size and shape of cell/ nucleus with clinical outcome in cancer. This method has studied its application assuming ideal and regular geometrical shapes of cells. But to great dismay, the chaotic growth in cancer leads to the irregular surface area, uneven contour length and other complex dimensional parameters making the application of conventional morphometry unacceptable. There are some inherent microscopic aberrations in cellular architecture which are neither obvious nor easily appreciated by conventional semi-quantitative morphometric analysis of images. This necessitates the invention of the Fractal dimension and its applicability in cancer.

Recent computational progress related to the field of spatial statistics, artificial nerve network permits the description of cell and nuclear shape irregularity with respect to abnormal chaotic growth in cancer. Chief characteristics of the morphologic complexity of normal & abnormal cells & tissues are variations under scale such as irregularity & self-similarity. No change is seen in the form of a self-similar entity if the measurement scale changes as every part look like the original entity. Minute details and variations in the size & geometrical parameters of an asymmetrical entity are seen upon examining at high resolution. Conventional morphometry uses a single arbitrary measuring scale to analyze the reductive representation or unrealistic approximation of cellular and nuclear irregularities.

MODERN MORPHOMETRY – FRACTAL DIMENSION

Fractal geometry(55) is considered to be an ideal method of image analysis in quantitative microscopy & histopathology(56–60). A sumptuous number of studies have claimed a probable link present between cancer & fractals(56–58). It was stated that the imbalance consisting of diverse biological & chemical reactions is usually related to cancer which could result in chaos and the consequent appearance of fractal geometry. Neoplastic cells lose the ability to control their growth which results in a change in shape. Every cell has a particular fractal geometry which can be called the marker for that type. By using this fractal-mathematical technique along with enhanced and sophisticated picture recognition, we can institute the multi sequential progression of carcinoma in a cell. Statistical intensity & distribution of irregularities present in the cell contour can be measured by Fractal dimension analysis. Fractal nature is demonstrated more by neoplastic cells than the healthy cells because the disordered growth shown by tumors cause uneven convexities of different size over the surface of cell. Fractal dimension helps researchers in the identification of occurrence of tumor cell and to establish with 97% accuracy as to evaluate two different lines of neoplastic pancreas cancer cells to which it belongs.

As told by Joachim Spatz, Fractal dimension analysis is a more accurate, precise & quicker method to determine the cell type of cancer than the conventional procedure. Fractal nature is one of the fascinating patterns in nature seen as 'self-similar' irregular curves or shapes that repeat their pattern when magnified in or out. Being said, fractal patterns are shaped under far-from-equilibrium circumstances or come out from the chaos. The examples of patterns showing fractality range from large scale entities of the universe to the geometry of various biological tissues. Intricate structures in biology do not have definite length and quite often they have

properties of fractal or show scaling properties. Moreover, the structures with fractal nature have profound importance in the human body. To facilitate the gaseous exchanges at the interface of vasculature and alveolar surface, self-similarity of the trachea & bronchi tree offer a vast surface area, coupling of pulmonary & cardiac functions, & fractal branching gives a vast network for the supply of nutrients & oxygen as well for metabolic waste products collection. The fractal arrangement of connective tissue in aortic leaflets provides functions facilitating the proficient circulation of mechanical forces.

Mandelbrot stated that this can be grouped by index like the "fractal dimension" D, which is an analysis of the geometrical complexity & the space-filling properties of a structure. Later on, fractal dimension analysis entered into various parts like pathology, differential diagnosis, prognosis & treatment of the patients. Internal membrane surface of cells or inner lung surface demonstrate properties of fractal geometry section-wise and within certain limits set by deterministic design properties and are a bit tricky to be explained in the terms of classical geometry. This is where the understanding and application concepts of fractal geometry are most beneficial. The phrase Fractal derived from the Latin adjective 'fractus' which means irregular or fragmented comes from the Latin verb 'frangere' which means a break or segregate into irregular fragments as coined by Mandelbrot. Biological & natural objects to be described as fractals must accomplish a specific number of theoretical & methodological parameters as well as a high level of organization, irregularity in shape, functional, morphological & temporal similarity, invariance in scale, iterative pathways & a non-integer fractal dimension.

Mathematical objects are deterministic invariant & self-similar in a wide range of scales and biological components & morphological structures are self-similar within a domain called "scaling window," i.e., only within this particular window, the fractal properties of an irregular object of finite dimension can be seen. Biological & morphological entities require experimental recognition for each of their elements & the scale range has to account for a minimum of 2 orders of magnitude. Principles of fractal and its usage are important to measure dimension properties and spatial measures of biological entities which are irregular which will enable us to a precise understanding of the architectural & morphological organization of tissues and organs, & assess the contrast among delicate morphogenetic changes that occur during the development & progression of physiological, pathologic, and neoplastic processes. Fractal geometry permits structures to objectively quantify the geometric parameter of cell and other tissue architecture even if their form is irregular. Hence, it helps to clarify the doubt of internal gaseous exchange at the surface of the lungs which is homogenous; competently ventilated & perfusion takes place at a low energetic cost. It can now be ascertained that the fractal principle of biological design is heuristically empirical & gives insight into the possibilities of a well-organized genetic study & programming of its outline.

In the world of fractal geometry, the notion of dimension corresponds to 1 for curve, 2 for a surface & 3 for a solid which may seem to be strange but this extension is pretty natural. It is usually anticipated that the number of boxes required to cover a given surface shall be more if boxes that are smaller in size are used. Considering planar entities, it is supposed that when boxes which are $1/3^{rd}$ of their width if used then the number of boxes needed to cover the given object (N) shall be 9 times greater or if stated in equation form, N} L22, wherein L is the width of the box. If in place of a simple, compact entity we calculate the number of boxes required to cover the fractal, we can see that every time the size of the box is decreased by 1/3rd, we shall require 8 (not the expected 9) times as many boxes. We come to know that the quantity boxes required increases slowly with an exponent between 1-2 according to the value N} L2D. For this particular case we can see a non-

integer or fractal dimension (D 5 log(8)/log(1/3) > 1.89).(56)

We have compiled the evidence of the diagnostic and prognostic utility of conventional as well as fractal geometry in potentially malignant and malignant oral disorders.

A detailed analysis of articles related to conventional morphometry in OPMD & OSCC published only in journals indexed in PUBMED has been tabulated in table 1.

Table 1: Available data on conventional morphometry in OPMD & OSCC.

Sample Size (Reference)	Nature of Sample	Cell			Nucleus			Type of Stain	Conclusion
		CD	CP	CA	ND	NP	NA		
97 (61)	Normal	11	31.72	57.63	7.17	19.73	25.01	H&E	Diagnostic value for lesions with a high risk of malignant transformation.
	Traumatic keratosis	11.95	34.03	66.78	7.41	21.32	29.59		
	Lichen planus	13.73	39.62	81.36	8.27	23.12	33.10		
	Leukoplakia	13.56	37.98	76.13	8.33	23.18	33.13		
	Candidal leukoplakia	14.19	39.14	79.63	8.91	24.37	36.40		
	Risk group	15.39	43.73	98.28	9.36	25.81	42.06		
	OSCC	ND	ND	ND	10.28	28.64	54.34		
73 (62)	Normal (Parabasal & Spinous)	-	35.75, 61.02	70.26, 187.85	-	-	-	-	Diagnostic use in Lichen Planus
	Traumatic keratosis (Parabasal & Spinous)	-	37.86 ,62.99	83.46, 205.37	-	-	-		
	Leukoplakia (Parabasal & Spinous)	-	40.24, 57.63	89.42, 178.03	-	-	-		
	Candidal leukoplakia (Parabasal & Spinous)	-	39.92, 53.79	89.52, 157.41	-	-	-		
	Lichen Planus (Parabasal & Spinous)	-	48.38, 74.49	112.51, 227.97	-	-	-		
30 (63)	OSSC (survival <14 months)	-	-	-	11.5	32.9	75.8	H&E	Nuclear dimensions, nuclear contour irregularities & nuclear shape asymmetries were evaluated
	OSSC (survival >36 months)	-	-	-	11.9	33.9	79.9		
28 (64)	Node negative OSCC	-	-	-	-	28.7±0.8	65.3±3.4	H&E	The prognostic factor for metastasis

	Node positive OSCC	-	-	-	-	36.6±1.3	100.4±5.9		
30 (65)	OSCC		-	-	12.8±2 (73.33)	438±150 (70)	80±30(66.67)	H&E	Predictor of survival.
16 (66)	Node negative OSCC	-	-	-	-	3.607	77.997	H&E	Prediction of nodal metastases in OSCC.
	Node positive OSCC	-	-	-	-	3.759	81.701		
60 (67)	Normal gingiva (Parabasal & Spinous)	15.91±1.79, 19.07±4.40	-	187±43.11, 241.04±67.08	8.39±0.78, 9.35±4.53	-	57.05±10.40, 58.74±10.94	H&E	Nuclear parameters showed a statistically significant change than cellular parameters in dysplasia.
	Normal Buccal mucosa (Parabasal & Spinous)	14.75±1.16, 17.35±1.73		159.27±20.92, 226.9±35.89	7.84±0.71, 8.39±0.79		50.13±10.31, 56.51±10.88		
	Leukoplakia on buccal mucosa (Parabasal & Spinous)	17.60±2.91, 17.60±2.91		230.78±72.50, 230.78±72.50	9.40±1.28, 9.40±1.28		73.29±16.92, 73.29±16.92		
	Leukoplakia on gingiva (Parabasal & Spinous)	15.99±2.60, 18.62±2.37		189.09±61.03, 243.99±62.14	9.11±0.95, 9.64±1.08		68.28±10.25, 77.25± 12.78		
60 (68)	Normal	-	-	-	-	29.4±1.5	69.7±6.3	Feulgen	A reliable tool for grading OSCC.
	Well-differentiated OSCC	-	-	-	-	38.5±3.2	111.4±15.1		
	Moderately differentiated OSCC	-	-	-	-	39.2±2.9	116.6±20.7		
	Poorly differentiated OSCC	-	-	-	-	41.1±2.9	122.8±15.5		
70 (69)	Leukoplakia	-	48.3146	123.4443	-	34.2598	59.40263	IHC	Differentiation between normal, leukoplakia and OSCC
	Well-differentiated OSCC	-	52.9577	153.6086	-	40.09	74.47		
	Normal buccal mucosa	-	30.4278	77.86	-	18.2369	27.0177		
25 (70)	Normal oral mucosa (Basal & suprabasal)	-	-	26.8001, 35.6096	4.0649, 4.3477	-	13.2374, 15.1712	H&E	Discrimination between normal and premalignant lichen planus and lichenoid lesions.
	Oral lichenoid lesion (Basal & suprabasal)	-		33.9658, 43.1726	4.3225, 4.7374	-	15.2688, 18.4352		
	Oral lichen planus (Basal & suprabasal)	-		35.8542, 46.9928	4.4517, 4.7651	-	16.4556, 18.7684		
70 (71)	Normal mucosa	-	-	-	3.48±0.81	-	9.30±2.04	Feulgen	Differentiation of grades of dysplasia
	Mild dysplasia	-	-	-	4.86±1.10	-	18.90±5.24		
	Moderate dysplasia	-	-	-	4.90±1.11	-	19.36±5.75		
	Severe dysplasia	-	-	-	5.29±1.42	-	22.21±8.70		

88 (72)	Node negative OSCC	-	-	-	-	32.3±4.8	73.4±19.4	H&E	Predicting lymph node metastasis in OSCC.
	Node positive OSCC	-	-	-	-	37.6±5.6	97.8±29.9		
40 (73)	Normal mucosa	19.5445	-	-	6.5631	-	-	H&E	Cellular and nuclear parameters showed statistically significant changes.
	Leukoplakia	16.6588	-	-	9.1676	-	-		
	Verrucous carcinoma	15.7205	-	-	10.6926	-	-		
	OSCC	14.6909	-	-	11.2179	-	-		
15 (74)	Normal mucosa	-	-	398.8±2.39	-	-	127.6±1.12	H&E	Nuclear size is useful for differentiating normal tissue, leukoplakia and OSCC.
	Leukoplakia with dysplasia	-	-	432.6±3.10	-	-	154.8±2.90		
	Well-differentiated OSCC	-	-	452.5±3.30	-	-	225.7±3.41		
	Moderately differentiated OSCC	-	-	456.6±1.84	-	-	243.7±2.98		
	Poorly differentiated OSCC	-	-	456.6±1.84 457.5±1.88	-	-	258.7±5.13		
30 (34)	Well differentiated OSCC	-	56.798875	218.911250	-	31.164938	89.689875	H&E	Image analysis helps to quantify the nuclear and cell changes associated with malignancy and provide an objective basis for grading dysplasia and tumours.
	Moderately differentiated OSCC		53.016938	217.446125		30.250	93.209250		
	Poorly differentiated OSCC		54.645875	223.020375		30.723250	95.491625		
	Normal mucosa		51.540050	207.1368		16.324000	45.030350		

CD- cell diameter, CP- cell perimeter, CA- cell area, ND- nuclear diameter, NP- nuclear perimeter, NA- nuclear area

FRACTAL DIMENSION IN ORAL POTENTIALLY MALIGNANT DISORDERS AND ORAL SQUAMOUS CELL CARCINOMA

All studies conducted to evaluate the prognostic role of nFD in oral cancer were summarized in table 2.

Table 2: Prognostic role of nFD in oral cancer.

AUTHOR	SAMPLE SIZE (N)	STAINS	PROGNOSTIC ROLE OF NFD
Yinti et al., 2015	14	H & E, Feulgen	Low nFD – increased survival Histological grading

			Staging
Phulari et al., 2016	60	H & E	Distinguish between normal mucosa, dysplasia and carcinoma
Mincione et al., 2015	64	H & E, Feulgen	Staging Grading Low nFD – increased survival
Goutzanis et al., 2007	48	H & E, Feulgen	Carcinomas presented higher mean values of FD compared to normal mucosa. low nFD – increased survival
Bose et al., 2015	107	4′,6-diamidino-2-phenylindole (DAPI)	Pathological tumour-stage Radiation treatment. High nFD of the total tumour microenvironment (stroma plus tumour) with improved disease-specific survival

Discussion

Histopathological evaluation of the lesion is the critical step in the diagnosis of cancer. There occurs significantly inter and intraobserver differences in opinion especially in borderline cases where the wrong diagnosis may misdirect the treatment plan and hence may affect the outcome adversely. The vast expansion of the scope of information technology over the past few decades along with an increase in the incidence of cancer has led to diagnostic medicine amenable to automated computational technology. This will probably lessen the burden of pathologists following the traditional approaches. A three-step process including pre-processing the photomicrograph followed by extraction and analysis of relevant features like fractal dimension, textural features and entropy etc. has proven to diagnose and prognose cancer. We have focused on the fractal dimension as a viable image feature to assess its potential to diagnose malignant oral epithelial cells and predict cervical lymph node metastasis in oral cancer. In cases where sample availability is limited, fractal dimension analysis of nuclei and chromatin can be done, since a miniature amount of tissue is sufficient to determine the phenotype by fractal dimension analysis, which can be compared with conventional pathological methods. (75) Fractal dimension analysis is not only limited to determining cell and tumour types but, can also be used for determining cellular behaviours in vitro such as cell migration, apoptosis and cellular differentiation, which can be the useful characterization of oral cancer lines. (76)

Conclusion

Oral Cancer is a heterogeneous disease that can be classified into many histological and molecular subtypes. Clinically, Oral cancer can be aggressive in terms of primary tumour growth and metastasis to distant lymph nodes and organs. New tools are required for obtaining a better knowledge of the biology of oral cancer evolution, continuation, and molecular heterogeneity. Fractal-based analyses are multifaceted and sensitive tools that have many potential applications in oral cancer research and diagnosis. Considerable attempts are needed to expand the utility of fractal analysis for investigating the unique anatomy and biology of the oral cavity as well as oral cancer.

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