Fractal Analysis in Grading of Oral Leukoplakia
Dr Deepak P

Abstract:-

Oral leukoplakia (OL) is most commonly encountered pre-cancerous lesion in oral cavity with a risk for malignant transformation. Fractal analysis (FA) helps in diagnostic interpretation of images. It is a multi-step task where the aim is the detection of potential abnormalities.

Texture analysis measures fractal dimension (D), where ‘D’ represents the measure of pattern complexity (cell shape, vascularisation, and texture) and is the command used to estimate the number of boxes of increasing size needed to cover a one pixel binary object boundary. Utilizing this concept, the aim of the study was to grade oral leukoplakia and to assess the effectiveness of fractal analysis in differentiating between the various grades of the lesion. TB (Toulinde blue) staining was done in 15 patients with leukoplakia. The site of biopsy corresponded to the ROI (Region of interest) created where there was increased intensity of TB staining. The numerical data obtained from the analysis was compared and correlated with the dysplastic changes confirmed by biopsy. The results of the present study showed that difference between “D” value in the ROI of hyperkeratosis and dysplastic lesion are coming within a range which was found to be statistically significant (p<0.05) suggesting that FA is useful in grading of OL.

Introduction:-

Oral leukoplakia is a premalignant lesion which has 4% risk of transforming into malignancy. It occurs in 1-2% of the population and is most common in patients over the age of 40. It is more prevalent in men. It may affect any part of the oral cavity, but usually presents on the tongue, gingiva and buccal mucosa. Research has shown that oral leukoplakia on the ventral surface of the tongue, floor of mouth and soft palate are more likely to become precancerous / dysplastic. Lesions may also present as red, rough, and warty and have a higher chance of being precancerous / dysplastic (4).

Toluidine blue (TB) is a metachromatic dye of the thiazine group that has been effectively used as a nuclear stain. It is based on the fact that dysplastic and anaplastic cells contain
quantitatively more nucleic acid than normal cells. In vivo, TB stains DNA and/or may be retained in intracellular spaces of dysplastic epithelium and clinically appear as royal blue areas. The staining intensity may provide important data due to binding of TB at sites of molecular changes that predict malignant risk, and it is reported that even weakly stained areas had significantly increased molecular alterations compared to TB negative samples.

In current clinical practice, grading is done with visual examination of biopsy samples by pathologists under microscope. There are several limitations associated with visual evaluations. Firstly, it is often time-consuming and cumbersome for pathologists to review a large number of slides in practice. Secondly, visual evaluations are subjected to unacceptable inter and even intra-observer variations. In order to automate the diagnostic process, recently there has been a great demand for efficient objective methods of evaluation which aim to avoid unnecessary biopsies and assist pathologists in the process of cancer diagnosis. Currently, the challenge remains to develop a technique that not only automates the diagnostic procedure, but also applies the optimum texture feature extraction that better captures and understands the underlying physiology to improve accuracy of cancer recognition (3).

Fractal geometry is widely used in image analysis problems in general and especially in the medical field. It is applied in different ways with different results, the adjective “fractal” to indicate objects whose complex geometry cannot be characterized by an integral dimension, the main attraction of fractal geometry stems from its ability to describe the irregular or fragmented shape of natural features as well as other complex objects(6), based on this

The study was attempted with the following aims and objectives:

1. To investigate the usefulness of fractal analysis as a parameter for numerical expression in the grading of oral leukoplakia.

2. To establish whether fractal analysis could be used to distinguish the various grades of oral leukoplakia (Hyperkeratosis, 1-mild dysplasia, 2-moderate dysplasia and 3-severe dysplasia).
Materials and Methods: -

Ethical aspects:-
All procedures of the study were conducted in full accordance with the ethical principles and received approval from the institutional review.

Case selection and Image acquisition:-

15 patients (14 males and 1 female) between 20–60 years of age were clinically diagnosed as homogenous oral leukoplakia under the following clinical grading criteria (9). These patients then underwent supra vital staining, biopsy and fractal analysis.

1. L1: size of leukoplakia<2 cm
2. L2: size of leukoplakia 2-4cm
3. L3: size of leukoplakia >4cm

All the patients who as been diagnosed as oral leukoplakia underwent TB supra vital staining and for each patient standard photographic technique was followed for image acquisition. The patient was seated in a vertical posture and the head was positioned upright and a sunny orthodontic mouth opener was used to retract the cheeks.

The following camera specifications and settings were used for image capturing:

2. Lens specification – macro
3. Flash – on
4. Resolution – 18.2 mega pixels
5. Magnification – 16x optical zoom
6. Patient to camera distance – 30cm

Inclusion criteria-

Age above 18 years and consenting for biopsy procedure
Clinically evident oral leukoplakia (homogenous type)
Lesions involving buccal mucosa
Can attend follow up

Exclusion criteria-

Medically compromised patients
Patients who have taken Prior treatment for oral leukoplakia
Oral leukoplakia involving sites other than buccal mucosa
Non homogenous leukoplakia
A standard method was used for image cropping and for the selection of region of interest (ROI). The image data were transferred to a computer and images which appeared with the maximum effect of the picture quality was taken for texture analysis.

**Image analysis:-**

Using Image J 1.43 analysis program, the selection of ROI was carried out according to the following criteria:

- Its position was determined manually by an expert Oral physician.
- A square shaped area was selected.
- The region of interest always included the proposed site of the incisional biopsy.
- The size of ROI was selected as 196x156 pixels (a larger ROI, especially in the normal area, would have resulted including under stained area also, which might have altered the results).

All the acquired images were first saved and ROI’s were chosen with the greatest intensity of the TB staining as shown in figure (1).

All the cases were subjected to incisional biopsy for the histopathological confirmation and the following microscopic criteria was followed for the epithelial dysplasia (8).

1) Irregular epithelial stratification,
2) Hyperplasia of basal layer,
3) Drop shaped rete processes,
4) Keratinization of single cells or cell groups in the prickle layer,
5) Loss of intercellular adherence,
6) Increased mitotic activity with occasional abnormal mitosis,
7) Increased nuclear-cytoplasmic ratio,
8) Loss of polarity of basal cells,
9) Cellular pleomorphism,
10) Nuclear pleomorphism, and
11) Enlarged and/or multiple nucleoli.
Image J 1.43 was used for the processing and analysing all images. 16-bit direct digital images were converted to 8-bit images as only 8-bit images can be segmented with Image J. ROI’s with dimensions of 196 x 156 pixels and superior-inferior extension were created in these images at the proposed site of biopsy. While creating ROI’s, the digital images were segmented into binary images. The ROIs were duplicated and blurred by a Gaussian filter with a radius of 2.00. This step removed all fine-scale and medium-scale structure and retained only large variations. The resulting heavily blurred image was then subtracted from the original to reflect the individual variations in the image. The image was then converted to binary with a brightness value of 128 and then inverted (figure no 2) and then the fractal box count was done and the numerical value (table 1) recorded.
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*D* - represents the command used to estimate the number of boxes of increasing size needed to cover a one pixel binary object boundary.

Table 1
Results:

The means, standard deviations of the variables, comparison of fractal dimension between the various dysplastic lesions and the results of unpaired t test is shown in table 2. The difference in the parameters between the two groups were recorded, the mean and the fractal dimension were higher in the greater dysplastic lesion compared to lower dysplastic lesions. The differences in these parameters between the two groups were found to be statistically significant (p<0.05). There was a positive correlation FD between the groups (hyperkeratosis and mild dysplasia) and FD showed an increase in higher dysplasia region, the standard deviation for hyperkeratosis and mild dysplasia is 0.23 and 0.8 respectively (graph 1), and confidence limit for hyperkeratosis and mild dysplasia is 1.58-1.70 and 1.71-1.79 respectively and D value of moderate dysplasia is 1.955. According to the results of this study it can be said that higher the dysplasia greater will be the complex structure when compared to the lesser dysplastic region of interest and falling within a range.

Statistical analysis: Unpaired t test. Statistically significant if P<0.05, *equal variances are not assumed

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Table 2
Fractal and multifractal analyses have been used to study and to characterize a wide range of signals in biology and medicine (6).

Fractal analysis is a method for the quantifying the complex structures by the study of variation in the image pixel intensity under the provided conditions. Various studies have been done in various parts of the body to characterize lesions and differentiate them from normal, through the application of texture analysis. [1]

In various studies, measurement of FD has repeatedly shown higher values in the region of the dysplasia. Similar results were obtained from our study in which tissues with higher dysplasia showed higher FD values thus showing greater tissue complexity, as compared to tissues with lesser dysplasia. Here Texture analysis measures of fractal dimension value ‘D’, where D represents the measure of pattern complexity and as a command used to estimate the number of boxes of an increasing size needed to cover a one pixel binary object boundary and implements the procedural steps previously described. The parameter presented higher D values for the ROI from the lesion when compared with the other grade values, indicating irregularity in the higher dysplastic region. [1]

As the dysplastic changes of oral leukoplakia is largely based on ranking with gold standard biopsy, we compared the texture characteristics on the TB stained photographic images with
the histopathological dysplastic grading. This comparison revealed D value of fractal analysis could effectively discriminate between the grades of dysplasia in oral leukoplakia. It is well understood that the histopathological examination critically depends on the biopsy taken from the most representative area of the TB stained site, which in turn depends greatly on the skill, technique and experience of the dental surgeon. Besides, the histopathological grading by the pathologists is highly subjective with intra and inter-observer variability and has low reproducibility [1]. In this present study, the correlation of fractal analysis and OL grading could be influenced by the possible chances that the site of incisional biopsy represents the most aggressive area of the leukoplakia. Furthermore, histopathological features can differ in different parts of the lesion, and biopsy samples may not always represent the most aggressive area of the whole lesion, so in this study the site for biopsy was chosen from by 5 different observers.

However, drawback of this study is its limited sample size, a retrospective study was done for 13 patients, biopsy followed by histopathological grading later fractal analysis was done to come up with a range of “D” value for dysplasia and in next 2 cases first fractal analysis of the biopsy site and then followed by biopsy for histopathological confirmation and grading in which “D” value was correlating the histopathological findings in both the cases, so in future it can be one of the diagnostic modality using supra vital stained images of oral leukoplakia for dysplasia staging. Future study with more sample size in the technique may give the practical use for treatment planning of oral leukoplakia (1)

**Conclusion:-**

Texture analysis method is helpful in the differentiating between hyperkeratosis and dysplasia in leukoplakia. With further exploration with more sample size we can achieve significant result. Because this procedure is economical and non-invasive it may prove as a helpful chair side adjuvant tool in oral leukoplakia diagnosis, particularly in emergency conditions. Still, use of this technique is under developed and future studies are needed with more sample size to come up with significant statistical values so that it will be used in the diagnosis and treatment planning of oral leukoplakia.
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