Angiogenesis is the new growth in vascular network which is essential for cell proliferation. Since the proliferation of existing capillaries as in physiologic angiogenesis. Tumor angiogenesis occurs by recruitment of endothelial cell precursor or by sprouting of existing capillaries as in physiologic angiogenesis. Angiogenesis (the formation of new blood vessels) is implicated in the pathogenesis of many chronic diseases including cancer. Angiogenesis in the tumor mass permits growth and invasiveness of the cancer cells as it is believed to be essential for the delivery of essential nutrients and oxygen to the tumor microenvironment. Clustered evidence suggests that angiogenesis is a critical event in the progression of solid tumors because tumor growth beyond 2 to 3 mm is often preceded by angiogenesis. Nevertheless, the pattern of tumor blood vessels differ from the normal vasculature by having altered morphology which can be exploited for diagnosis and as a prognostic indicator for premalignant lesions and oral cancers.

Incidence of premalignant lesions and oral cancers is steadily increasing globally. In spite of advancement in early detection, there is increased mortality and morbidity related to oral cancers. Oral cancer is ranked the sixth most common malignancy worldwide and is diagnosed at an increasing rate, with an estimated number of 222,000 new cases of oral cancer diagnosed in men (5% of all cancers) and 90,000 new cases diagnosed in women (2% of all Cancers).

Traditional methods of screening for oral potentially malignant disorders and oral malignancies involve a conventional oral examination with digital palpation. Evidence indicates that the diagnosis of a dysplastic premalignant lesion of the oral mucosa cannot be based solely on clinical findings as the conventional examination is a poor discriminator of oral mucosal lesions. With the aim of improving the efficiency of these diagnoses, number of optical based techniques are being developed to complement clinical examination and to facilitate the identification of early dysplastic changes, initial carcinomas and to differentiate benign lesions from sinister pathology.

At present, though there are simple chairside methods including staining with toluidine blue and exfoliative cytology to aid the diagnosis of such changes, there is a high risk of false positives which can be as high as 30%. Histologic evaluation of a representative biopsy specimen has always been a gold standard for acquiring the final corresponding diagnosis. However, the site for the biopsy is always a subjective choice that sometimes raises doubts about its representativeness. To add to the vast list of our adjuncts to diagnosis colposcopy (direct intra-oral microscopy) that forms the basis of detecting tumor growth i.e. tumor angiogenesis offers advantages in selecting the more representative sites for biopsy than routine clinical examination alone and is a simple, painless, chair side diagnostic method.

Therefore, a technique for non-invasively detecting dysplastic changes or, helping the clinician choose the appropriate site for biopsy can save patients from multiple biopsies and allow a broader range of diagnoses which can aid early detection of oral cancers.

Colposcopic criteria included vascular pattern, inter-capillary distance, surface pattern, color tone, and opacity, as well as clarity of demarcation of the mucosal lesions. The accuracy of colposcopic examination for the detection of mucosal changes approximates between 70% and 98%. Colposcopic Criteria for vascular changes

The vascular changes described in colposcopic literature were used as the criteria for selecting biopsy sites in the oral cavity. These include the vascular pattern, intercapillary distance, surface pattern, color tone, and opacity, as well as the clarity of demarcation of the mucosal lesions.

Two basic types of capillary network can be seen with direct microscopy (ie, colposcopy): network capillaries and hairpin capillaries (Figure a, b). In dysplasia and carcinoma in situ, a specific vascular pattern, punctuation (previously called ground) is common (Figure c). Punctuation is characterized by dilated, often twisted, irregular, hairpin-type vessels. Another pattern of the vessels in dysplasia is called mosaic (Figure d). Like punctuation vessels, true mosaic vessels are usually seen in sharply demarcated areas. When...
it is difficult to describe the pattern of the vessels, the term atypical vessels is used (Figure e). Capillary punctation, mosaic, or atypical patterns are encountered in malignant atypical vessels is used (Figure e). Varied vascular patterns appreciated on mucosal lesion analyses were labelled as per the above mentioned criteria (Figures 1, 2, 3, 4, 5).

Colposcopy is the gold standard tool in gynecology for diagnosis of cervical abnormalities particularly after an abnormal pap smear. It provides an enlarged view of the areas, to visually distinguish the abnormal appearing tissues from the normal to get the best representing biopsy sites for further pathological examination and to aid in the prevention of the premalignant lesions by early detection and treatment. Colposcopy is a well-known medical diagnostic procedure used to examine the tissues of the vagina, vulva, and cervix, carried out under illuminated light with a magnified view of the area of interest (i.e., direct microscopy). Many premalignant and malignant lesions have discernible characteristics which can be detected through this examination, usually done by an instrument known as “colposcope.” An erythematous lesion, homogeneous or not, is considered to be more likely to become malignant than are whitish lesions. The risk is regarded as higher if the lesion is located on the tongue or on the floor of the mouth.

Hence, this study was planned with the aim of analyzing the vascular patterns of buccal mucosa associated frank carcinoma, leukoplakia/erythroleukoplakia and lichen planus/lichenoid lesion, with the help of direct oral microscopy and to assess and compare the colposcopic examination findings with the traditional clinical findings for selection of biopsy site in buccal mucosa associated lesions. In addition we also compared the histopathologically confirmed diagnosis of biopsy specimens that were obtained from direct oral microscopy with those that were selected solely on the basis of clinical criteria.

Materials and methods
The study was conducted in the Department of Oral and Maxillofacial Pathology and Microbiology, I.T.S dental college, hospital and research centre, Greater Noida(UP). Patients referred to the department for diagnosis and treatment of buccal mucosal lesions participated in the present study. A total of 60 patients (Table 1) who participated were clinically evaluated and provisional diagnoses of frank carcinoma, leukoplakia/erythroleukoplakia and lichen planus/lichenoid lesion associated with buccal mucosa were made.

### Selection criteria

Inclusion criteria constituted of patients who were between the age group of 30-70 years and those clinically diagnosed cases of required lesions in the study associated with buccal mucosa; and exclusion criteria included patients with lesions on mucosa other than buccal mucosa, lesions on buccal mucosa with secondary infection; patients having other systemic diseases; and patients priorly undergoing treatment for the lesion.

### Examination using direct oral microscopy (Colposcope)

The oral mucosa of the same participating patients who were clinically analyzed were further examined using direct oral microscopy. An optimum site for biopsy was determined and marked using a stereozoom binocular microscope (Model no. SZMB-1; Leica Microsystems, Wetzlar, Germany) with a mobile floor stand on four castor wheels for easy handling and absolute stability and a CCD digital camera attachment was then used to analyze the buccal mucosa lesions. Varied vascular patterns were recognized and labelled according to the criteria for vascular changes.

Vascular patterns and structure of the mucosal surface and lines of demarcation of whitish lesions were seen better when 4% acetic acid was applied first to the lesion for about 5 seconds and then dried with low-pressure airflow carefully.

After direct oral microscopic examination and labelling local analgesia was administered. Biopsy specimens were taken with 6 mm punches from the representative sites and were histologically analysed. The direct oral microscopies and biopsies were all performed by one of the authors.

### Statistical analysis

The 2-sided binomial test was used for the statistical analysis.

### Results

#### Clinical examination

A total of 60 buccal mucosal lesions including 20 patients of suspected carcinoma, 20 patients of homogeneous leukoplakia/erythroplakia and 20 patients of different types of lichenoid lesions(including lichen planus) were first diagnosed clinically.

#### Direct oral microscopy

The vascular picture depicting the surface pattern, color tone, opacity, and clarity of demarcation were more easily seen with microscopy in almost all the study patients than that when compared with routine clinical examination. 47 of the patients showed changes in the vascular picture on microscopy. Twenty four of these had punctuation vessels (Figure 3), eight had mosaic vessels (Figure 4), and fifteen had atypical vessels (Figure 5). The other lesions showed a normal capillary network.

### Comparative analysis of direct oral microscopic and clinical diagnoses in relation to histopathological diagnosis (Table 2)

All 20 patients with a suspected malignancy on the clinical examination had a histologic diagnosis of epithelial dysplasia (12 patients), or squamous cell carcinoma (8 patients). Nineteen patients had more extensive cytologic and structural abnormalities in the biopsy specimens obtained by direct oral microscopy, but 1 patient had more extensive changes in the specimen obtained after routine clinical examination.

None of the 20 patients with a clinical diagnosis of homogeneous leukoplakia showed histologic signs of epithelial dysplasia. Moreover, none showed histologic differences between the biopsy specimens obtained by direct oral microscopy and those from clinical examination.
In all 20 patients with a clinical diagnosis of lichenoid lesions, the diagnosis was verified by histologic examination. In 14 patients, the histologic changes showed more severe dysplasia or less differentiation in the biopsy specimens obtained by direct oral microscopy, whereas this was true in only 4 samples obtained after the routine clinical examination. 2 patients had no histologic differences between the samples.

In a total of 33 patients (56%), the biopsy specimens selected with direct oral microscopy appeared to be more representative of the histologic findings than those selected with routine clinical examination (0.01, P = 0.05).

Discussion:
In recent years there have been few studies such as by, Pazouki et al. who concluded that there was a close relation between angiogenesis and tumor progression in the oral mucosa. Angiogenesis plays an inevitable role in tumor progression and these changes form the basis of colposcopic literature which can be used as the criteria for screening oral lesions as well as selecting biopsy sites in the oral cavity.10

A study by Fedele in a 9-year randomized controlled trial also revealed that screening via visual examination of the oral mucosa under white light was effective in reducing mortality in individuals exposed to risk factors.11 It is well known that simple visual examination is limited by subjective interpretation and as a consequence, adjunctive techniques have been suggested to increase the ability to differentiate between benign changes of the mucosa from dysplastic/malignant changes as well as to identify areas of dysplasia and early oral squamous cell carcinoma.

In contrast to previous studies none of the studies have described the percentage of positive correlation out of 60 patients between direct oral microscopy and traditional method of clinical examination of patients with suspected carcinoma, homogeneous leukoplakia/erythroplakia and different types of lichenoid lesions(including lichen planus) involving only the buccal mucosa. There was a male predominance with 36 male and 24 female out of total 60 patients, which suggested that the habit of quid and smoking was more common in males. As reported by Silverman,14 Neville,15 and Swango16 our study was consistent with their findings in context with age and gender. Frequently patients with oral squamous carcinomas have a marked inflammatory infiltrate, which interferes with the evaluation of differences however in comparison, biopsy specimens selected by colposcopy rarely show severe inflammation.

With the use of direct oral microscopy in the present study, we observed that the in selecting the most representative site for biopsy, colposcopic criteria appeared to be more representative of the histopathologic findings at least in majority cases compared to those selected by traditional clinical examination. With the correct selection of the biopsy site, it assisted us to reach a more definitive diagnosis and therefore false-negative results are avoided.

The results of our colposcopic examination regarding the selection of biopsy sites for buccal mucosa carcinoma reported a sensitivity of 0.7142 (71%) with a specificity of 0.8524 (58%). The results of our study were similar to previously reported studies, such as a study conducted by Gynther17 who assessed the value of colposcopy in diagnosing mucosal lesions and a study by Shetty who correlated the relevance of tumor angiogenesis pattern with the histopathologic results in oral epithelial dysplasia.18

The progression of the lesion from dysplasia to carcinoma on the basis of clinical findings is not possible. However, such a progression of the lesion in mucosa can be determined by the sequential change or degree of vascular alteration in adjacent affected areas because areas of suspected mucosal change may contain foci of varying degrees of dysplasia and this technique detects signs of progression because at present this seems to be the only way to evaluate vascular changes in the oral mucosa.19 Therefore it is essential in precancerous lesions for regular follow-up.
The complexity and cost are the chief disadvantages of direct oral microscopy however the comparative evaluation studies have proven it to be more specific and inevitable on the grounds of mutilating affect on the patients in case of delayed diagnosis.

Conclusion
The underlying conclusion of the present study is based on vascular and tissue changes. Tumor growth followed by the capillary changes showing characteristic pattern of tumor angiogenesis are different from the usual repair and regeneration associated neo-vascularization. The molecular stages involve various molecules such as vascular endothelial growth factor, basic fibroblast growth factor and transforming growth factor alpha in the pathogenesis. Direct oral microscopic analysis of these patterns would be helpful in the early determination of the underlying pathology and also aid in delineating the biopsy site.

Thus to conclude this study shows that direct oral microscopy can be used to select most representative biopsy sites and allow early screening of the lesion. With this study it is suggested that this method should be evaluated in further clinical studies and analysed along with comparison using various staining methods and also further studies are required to confirm these results. Hence, the most important task is to establish an early diagnosis at the first stages of the disease to avoid unnecessary mutilating effects to the patient in later stages of malignancy.
References


