Abstract

Background: The diagnosis of malignant and potentially malignant epithelial lesions of the oral mucosa cannot be based solely on clinical findings. Therefore, histologic evaluation of a representative biopsy specimen is necessary. However the site for the biopsy is always a subjective choice that sometimes raises doubts about its representativeness. Colposcopy, a well-known gynecological diagnostic procedure, is helpful in the selection of these epithelial dysplasia areas depending upon the vascular pattern. Hence, this study assesses the role of colposcopic examination in the selection of biopsy sites for carcinomas of the buccal mucosa.

Methods: This study included 30 patients between the ages of 30-60 years who were clinically diagnosed with carcinoma of the buccal mucosa and a control group that consisted of 25 healthy, age-matched individuals. For each subject, a thorough clinical examination followed by colposcopic assessment was performed. The most representative site was selected for biopsy from the involved buccal mucosa. The biopsy specimens that measured 6 mm were obtained by punch biopsy and subjected to histopathologic examination. The histopathologic findings were then compared in the two cases.

Results: The sensitivity and specificity of biopsies performed on the basis of clinical criteria was found to be more appropriate compared to biopsies directed through colposcopic examination.

Conclusion: From the study, it was concluded that clinical criterion was found to be more appropriate for the selection of biopsy specimens in cases of carcinoma of the buccal mucosa.

Keywords: Colposcopy, Potentially malignant epithelial lesions, Histopathologic, Epithelial dysplasia, Vascular pattern
**Introduction**

Oral squamous cell carcinoma (OSCC) is the most common cancer of the oral cavity. It accounts for more than 90% of all oral cancers.\(^1\) Each year, globally, there are 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).\(^2\) The incidence of premalignant lesions and oral cancers is steadily increasing globally. Despite advancement in early detection there is increased mortality and morbidity related to oral cancers.\(^3\) In India, the incidence of leukoplakia and carcinoma of the buccal mucosa is 46% as reported by Paymaster.\(^4\) Carcinoma of the buccal mucosa deserves special mention because of the numerous premalignant lesions and conditions that are seen predominantly in this part of the oral mucosa, the most common being leukoplakia and pouch keratosis, which are commonly attributed to the quid habit in the Indian population.

Clinical diagnosis of squamous cell carcinomas of the oral mucosa is not difficult when the lesion is obviously invasive or when the patient experiences pain, functional limitation, or regional lymphadenopathy. Conversely, it is more difficult to diagnose dysplasia and carcinomas mainly in potentially malignant epithelial lesions (PMELs). With the aim of improving the efficiency of these diagnoses, techniques are being developed to complement clinical examination and to facilitate the identification of early dysplastic changes and initial carcinomas.\(^5\)

Biopsy with histopathologic examination is still considered the gold standard in the diagnosis of oral cancer and precancerous lesions and conditions. Selection of the biopsy site is the most important criteria to arrive at a correct diagnosis. However as the biopsy site is a subjective choice, it is possible that biopsy specimens are taken from unrepresentative sites of the lesion or before morphologic changes are detected.

At present, though there are simple chair-side 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%.\(^6\) Therefore a technique for non-invasive detection of dysplastic changes or assisting the clinician in choosing the appropriate biopsy site can save patients from multiple biopsies and allow a broader range of diagnoses which can aid early detection of oral cancers.\(^7\)

Colposcopy (direct intra-oral microscopy) offers advantages in selecting more representative sites for biopsy than routine clinical examination (COE) and is a simple, painless, chair side diagnostic method.\(^8\) Colposcopic criteria include vascular pattern, inter-capillary distance, surface morphology, and surface texture. It is a valuable tool in the early detection of oral premalignancy.
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%.9-11 Various authors have attempted to adapt gynecologic methods of examination to the oral cavity as there is similarity between the two types of mucosa.12 Colposcopy is one such method that visualizes the cervical mucosa for premalignant and malignant changes. Thus far, a few studies have highlighted the value of colposcopy in the diagnosis of oral mucosal lesions.

Hence, this study has been planned to assess the role of colposcopic examination in the selection of the most representative specimen for histopathologic examination from the lesions of buccal mucosa carcinoma. The objectives of the study were to assess the feasibility of using colposcopic examination for buccal mucosa carcinoma; to compare the colposcopic examination findings with clinical criteria for selection of the biopsy site; to correlate the histopathologic findings with colposcopic findings and clinical criteria; and to assess the sensitivity and specificity of colposcopic examination in selection of the biopsy site.

### Materials and Methods

#### Patients

The study was conducted for a period of one year, from May 2010 to May 2011, in the Department of Oral Medicine and Radiology, Government Dental College and Research Institute, Bangalore, Karnataka, India. The study group consisted of 30 cases of clinically diagnosed buccal mucosa carcinoma between the ages of 30-60 years and a control group that consisted of 25 healthy, age-matched individuals.

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![Figure 3. Placement of grid for selection of biopsy site (green color for colposcopy and red color for clinical criteria).](image)

![Figure 4. Carl Zeiss colposcope with a variable magnification index giving a magnification of approximately 6-20×.](image)
Data collection

Patients were selected according to defined inclusion and exclusion criteria. Prior to their participation in the study, we explained the study details to the patients and obtained written informed consent for inclusion in the study. The ethical clearance was obtained from the Ethical Committee appointed by the institution before the start of the study.

Inclusion criteria consisted of a clinical diagnosis of buccal mucosa carcinoma. Excluded were patients with secondary infections; those with other systemic diseases; and patients currently treated for buccal mucosa carcinoma.

Methodology

A total of 30 buccal mucosa carcinoma cases were selected for the study based on inclusion and exclusion criteria. The significance of the number of samples was analyzed statistically before their inclusion into the study. For each subject, a detailed case history and thorough clinical examination was performed. Under good illumination, an intra-oral examination of the lesion was performed. Inspection and palpatory findings were recorded in a prepared proforma. Following clinical examination, the most representative site of the lesion was selected for biopsy according to set clinical criteria for dysplastic changes in leukoplakia and carcinoma of the buccal mucosa.

Clinical criteria for selection of the biopsy site included erythema, induration and ulceration. The outline of the lesion was marked with a black pen and the biopsy site with a red pen with the help of a grid placed on the buccal mucosa. All cases were then subjected to a colposcopic examination. Prior to the colposcopic evaluation, normal colposcopic findings were standardized based on colposcopic criteria. Following colposcopic assessment, the most representative site for biopsy was selected from the involved buccal mucosa according to colposcopic criteria as mentioned by Gynther.13

All patients were subjected to routine laboratory analyses (Hb, BT, CT, RBS, TLC, DLC and ESR) after which routine biopsy for histopathologic examination and punch biopsy following colposcopic evaluation with 6 mm diameter under local anesthesia were performed. The biopsy specimen was immediately immersed in 10% neutral buffered formaldehyde and coded. Later the specimen was embedded in paraffin by routine methods and subjected to histopathologic examination.

Colposcopic examination

Following clinical examination, the mucosa was wiped with saline after which the abnormal epithelium appeared much darker than the normal epithelium (Figure 1). Using a blue or green filter and higher-power magnification (Figure 2); we evaluated the abnormal vascular patterns. Then, 5% acetic acid was applied to the lesion for about 60 seconds. The grid was placed again on the buccal mucosa. We chose the area estimated to have the most extensive cell changes according to colposcopic criteria for biopsy and marked the biopsy site on the grid with a green pen (Figure 3).

We used a Carl Zeiss colposcope SC Media link 100 that had a variable magnification index which gave a magnification of approximately 6-20 times (Figure 4). Colposcopic criteria included vascular pattern, inter-capillary distance, surface pattern, color tone and opacity, as well as clarity of demarcation of the mucosal lesions. In the normal mucosa of the uterine cervix that resembles in histology with the normal oral mucosa, two basic types of capillary networks can be visualized by direct microscopy (i.e., colposcopy), hairpin (Figure 5a) and network capillaries (Figure 5b). In areas of dysplasia and carcinoma-in-situ, a specific vascular pattern, punctuation (previously called ground), is commonly observed. Punctuation (Figure 5c) is characterized by dilated, often twisted, irregular, hairpin-type vessels. Another pattern of the vessels in dysplasia is called mosaic (Figure 5d). If the vessels do not reach the epithelial surface but extend only partially into the epithelium, they appear as red lines surrounding blocks of epithelium. After application of acetic acid, this pattern is even more accentuated because
of acetowhiteness of the atypical epithelium, forming a honey comb pattern. 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 (Figure 5e) is used. Capillary, punctuation, mosaic, or atypical patterns are encountered in malignant lesions.

When the biopsy area according to clinical criteria and that of the colposcopy were superimposed (red and green areas), only one common biopsy sample was obtained. When two different areas were selected from the same lesion, the two different areas which were marked with red and green pen (Figure 3) were biopsied and subjected to histopathologic examination. Biopsy specimens were taken with a 6 mm punch, after which biopsy wounds were sutured (Figure 6) and histopathologic examination of the specimen was performed.

**Histopathologic procedure**

All biopsied tissue specimens were sent for histopathological evaluation. The biopsied tissue was immediately transferred to a bottle that contained a 10% buffered formalin solution. Heamotoxylin and eosin staining was performed for microscopic examination of the sections.

We compared the histopathological diagnosis obtained by COE and direct intra-oral microscopy was performed, after which the data were subjected to statistical analysis.

**Grid preparation**

Printed graph on an OHP sheet was used as a grid in marking the biopsy site. Each lesion was measured and the grid was prepared to the approximate lesion size. The entire lesion was divided into 6×6 mm squares on a transparent grid. The outline of the lesion was marked with a black pen, the red pen was used to mark the area of the biopsy site according to clinical criteria and a green pen marked the biopsy are according to colposcopic criteria.

**Results**

The study group consisted of 30 cases of clinically diagnosed buccal mucosa carcinoma and a control group of 25 healthy individuals.

Table 1 shows the age and gender distribution of the cases and controls. Of the 30 patients, we observed induration in 12 and frank ulceration in 9 patients. Approximately 9 patients had biopsies taken from the adjacent, apparently normal oral mucosa on the basis of the concept of field of cancer. These cases were termed clinically negative, as the biopsy specimens were obtained from apparently normal oral mucosa.

Of the 30 patients, a punctuation vessel pattern was observed in 10 (33%) cases, mosaic pattern in 4 (13%) cases, and an atypical vessel pattern in 4 (13%) cases. In 12 (40%) cases a vascular pattern was not appreciated due to secondary infection and/or ulceration. Of the 25 controls, a network capillary pattern was observed in 12 (48%) cases and hair-pin pattern in 9 (36%) cases. In 4 (16%) cases, a vascular pattern was not well appreciated.

Out of 30 cases, histopathologic reports according to clinical presentation showed that 14
(46.67%) were well-differentiated squamous cell carcinoma, 5 (16.67%) were moderately differentiated squamous cell carcinoma, 2 (6.67%) had carcinoma-in-situ and 2 (6.67%) presented with severe dysplasia.

Histopathologic findings in the colposcopically-directed biopsy specimens showed that 11 (36.67%) cases had well-differentiated squamous cell carcinoma (Figure 7), 5 (16.67%) cases had moderately differentiated squamous cell carcinoma, 1 (3.33%) case was diagnosed with poorly differentiated squamous cell carcinoma, and 2 (6.67%) cases had carcinoma-in-situ.

In approximately 7 cases biopsied on the basis of clinical findings and 11 cases biopsied on the basis of colposcopic findings, the histopathologic picture was found to be negative. Histopathologic findings were not suggestive of dysplastic changes, but were either largely normal or with chronic inflammatory cell infiltration.

As shown in Table 2 the biopsy samples taken from the clinical presentation of cases showed a sensitivity of 0.6842 (68%) and specificity of 0.5455 (57%).

Sensitivity and specificity of colposcopically-directed biopsy specimens had a sensitivity of 0.7826 (78%) with a specificity of 0.5714 (57%; Table 2).

**Discussion**

Oral squamous cell carcinoma is a well-known malignancy which accounts for more than 90% of all oral cancers. The overall five-year survival rate of OSCC has not significantly increased in the last few years despite tremendous advancements made in the plethora of clinical and surgical methods, as well as recent advancements in the field of radiation therapy. Despite these advancements, the survival rates have not significantly improved. This is partly due to the fact that the majority of patients present with advanced stages of the disease. Early detection is crucial for improving outcomes. The aim of this study was to investigate the accuracy of colposcopic-directed biopsies in the diagnosis of oral squamous cell carcinoma, particularly in early stages.

Histopathologic findings in the biopsy specimens showed that:

- 11 (36.67%) cases had well-differentiated squamous cell carcinoma
- 5 (16.67%) cases had moderately differentiated squamous cell carcinoma
- 1 (3.33%) case was diagnosed with poorly differentiated squamous cell carcinoma
- 2 (6.67%) cases had carcinoma-in-situ

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**Figure 5.** Vascular patterns seen in colposcopy.

(a) Hairpin capillary pattern in normal buccal mucosa; (b) Network capillary pattern in normal buccal mucosa; (c) Punctation vessel pattern in buccal mucosa carcinoma; (d) Mosaic pattern in buccal mucosa carcinoma; (e) Atypical vessel pattern in buccal mucosa carcinoma.
of diagnostic and treatment modalities during the previous 2-3 decades. Hence, the most important task is to establish an early diagnosis at the first stages of the disease.\textsuperscript{14}

The present study aimed at assessing the vascular patterns by colposcopic findings and selecting the biopsy site in clinically diagnosed cases of buccal mucosa carcinoma in a comparison of the two methods, clinical criteria and colposcopic examination, for selecting the biopsy site.

In our study most patients were from the 51 to 60 year age group. There was a female predominance in this group, which suggested that the habit of quid was more common in females and smoking more common in males. The findings of our study were consistent with the age and gender of the oral cancer patients reported by Silverman,\textsuperscript{15} Neville,\textsuperscript{16} and Swango.\textsuperscript{17}

In our study, out of 30 patients, 12 presented with indurated lesions while ulceration was noted in 9. These findings suggested that the patients reported to the clinicians only when they developed symptoms of pain invariably associated with ulceration, which confirmed the results of other studies. Bagan\textsuperscript{18} has reported in his study that the clinical presentation of these early malignant lesions was usually seen in the form of well demarcated, indurated erythroleuko-plastic lesions, often associated with pain and ulceration.

A sensitivity of 0.7826 (78\%) and specificity of 0.5714 (57\%) of the biopsy specimens taken with the help of clinical criteria for buccal mucosa carcinoma agreed with the study by Lingen et al.\textsuperscript{19} who reported similar findings. Lingen, in his study, suggested the conventional oral examination (COE) by using normal (incandescent) light as one of the standard method for oral cancer screening. A study by Fedele\textsuperscript{20} 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.

Simple visual examination, however, is well known to be limited by subjective interpretation and by the potential, albeit rare, occurrence of dysplasia and early OSCC within areas of normal looking oral mucosa. 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 OSCC that are not visible to the naked eye.

Hopman\textsuperscript{21} in a study stated that colposcopy was an effective tool for diagnosing cervical intra-epithelial neoplasia. It was suggested that micro-invasive carcinoma was suspected

![Figure 6. Sutured biopsy area in relation to carcinoma of the left buccal mucosa after biopsy of two different sites.](image1)

![Figure 7. A well-differentiated squamous cell carcinoma appreciable in a biopsy specimen obtained by colposcopic examination.](image2)
when mosaic, punctuation and acetowhite epithelium were present with a thick white epithelium that had a clear and elevated margin with an irregular surface contour and raised capillaries.

Shetty, 22 in his study has stated that the histopathologic assessment of a biopsy specimen is regarded as the most reliable criterion for correct diagnosis in cases of epithelial dysplasia; consequently the specimen must be taken from the most representative area of a suspicious lesion for increasing diagnostic accuracy.

In our study we used the criteria for vascular changes described in the colposcopic literature for the selection of a biopsy site. 21, 23, 24 These included the vascular pattern, inter-capillary distance, surface pattern, color tone and opacity as well as the clarity of demarcation of the mucosal lesions. The results of our colposcopic examination regarding the selection of biopsy sites for buccal mucosa carcinoma reported a sensitivity of 0.6842 (68%) with a specificity of approximately 0.5455 (54%). The results of our study were similar to previously reported studies, such as a study conducted by Gynther 13 who assessed the value of colposcopy in diagnosing mucosal lesions and a study by Shetty 22 who correlated the relevance of tumor angiogenesis pattern with the histopathologic results in oral epithelial dysplasia.

The results of colposcopic findings are actually based on vascular and tissue changes. The capillary changes preceding tumor growth with the pattern of tumor angiogenesis are different from the usual neo-vascularization that occurs during repair and regeneration processes. At a cellular level, various molecules such as vascular endothelial growth factor, basic fibroblast growth factor and transforming growth factor alpha are implicated. Direct optical visualization of these patterns would be helpful in the early determination of the underlying pathology and also aid in delineating the biopsy site. 25

In the present study, we found that the biopsy specimens selected according to colposcopic criteria appeared to be more representative of the histopathologic findings at least in certain cases compared to those selected by COE. The altered vascular patterns, in the initial stages of lesion progression, definitely assisted with the correct selection of the biopsy site, which in turn helped us reach a more definitive diagnosis and avoiding false-negative results. Also, the majority of patients with oral squamous carcinomas have a marked inflammatory infiltrate interfering with the evaluation of dysplastic lesions. In comparison, biopsy specimens selected by colposcopic examination overcome this most common cause for the decreased sensitivity of biopsy outcomes in these sets of patients. 13 Colposcopic examination thus aids in the distinction between chronic inflammatory lesions which can then be easily distinguished from dysplastic lesions by the assessment of the features noted in the examination. The study however concludes with the COE having a superior edge in buccal mucosa carcinoma patients compared with colposcopy criteria (Table 2). This could be explained by the fact that keratinization and thickness is more in buccal mucosa carcinoma cases compared to numerous oral pre-cancerous lesions and conditions seen, hence, vascular patterns could not be visualized. However further studies are required to confirm these results.

Conclusion

This preliminary study emphasizes selection of the most representative sites of epithelial dysplasia in frank malignant lesions using colposcopic examination and avoiding those areas as largely affected by chronic inflammatory processes. The study paves the way for future studies with larger sample sizes to reach a definite conclusion regarding the possible use of colposcopic examination as an effective diagnostic adjunct for diagnosing the most representative sites for histopathologic examination and avoiding areas with marked inflammatory changes in oral squamous cell carcinoma patients that could be misleading in early disease stages.
Contributions from the authors

Literature search, manuscript preparation, manuscript editing and manuscript review.

Ethical Declaration

The study has been approved by the Ethical Committee appointed by the Government Dental College and Research Institute, Bangalore and Bangalore Medical College and Research Institute, Bangalore and has therefore been performed in accordance with the ethical standards laid down in the 1975 Declaration of Helsinki and its later amendments in 2000, after obtaining written informed consents from the patients for their inclusion in the study. Details that might disclose the identity of the patients have been omitted.

Acknowledgements

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References
