Colposcopy and carcinoma buccal mucosa: finding significance, a pilot study

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Abstract- Introduction and objectives: 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 gynaecological diagnostic procedure, is helpful in the selection of these sites of epithelial dysplasia depending upon the vascular pattern. Hence, this study was planned to assess the role of Colposcopic examination in the selection of biopsy site for carcinoma buccal mucosa.
Materials and methods: 30 patients between the ages of 30-60 years clinically diagnosed with carcinoma buccal mucosa were included in the study. For each of the subject, a thorough clinical examination followed by Colpospopic assessment was carried out and the most representative site was selected for biopsy from the involved buccal mucosa. The biopsy specimens measuring 6 mm were taken using a punch biopsy and subjected to histopathological examination. The histopathological findings were then compared in the two cases.
Results: The sensitivity and specificity of biopsy done on the basis of clinical criteria was found to be more than as compared to the ones 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 buccal mucosa.

Keywords- Colposcopy, potentially malignant epithelial lesions, histopathological, epithelial dysplasia, vascular pattern.

I. INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common cancer in 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 cancer) and 90,000 new cases diagnosed in women (2% of all cancer).[2] The incidence of premalignant lesions and oral cancers is steadily increasing globally. In spite of advancement in the early detection, there is seen increased mortality and morbidity related to oral cancers.[3] In India, the incidence of leukoplakia and carcinoma buccal mucosa is 46% as reported by Paymaster JC (1962).[4] Carcinoma of buccal mucosa, in particular, deserves special mention with increased incidence because of numerous premalignant lesions and conditions seen predominantly in this part of oral mucosa, the most common being leukoplakia and pouch keratosis, attributed commonly to the quid habit in 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] Though, biopsy with histopathological examination is still considered the gold standard in the diagnosis of oral cancer and precancerous lesions and conditions, the selection of the site for biopsy is the most important criteria to arrive at a correct diagnosis. But, as biopsy site is a subjective choice, it is possible that biopsy specimens are taken from unrepresentative sites of the lesion or, before the morphologic changes could be detected in it.

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%.[6] 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.[7]

Colposcopy (direct intra-oral microscopy) offers advantages in selecting the more representative sites for biopsy than routine clinical examination alone and is a simple, painless, chair side diagnostic method.[8] Colposcopic criteria included vascular pattern, inter-capillary distance, surface pattern, color tone, and opacity, as well as clarity of demarcation of the mucosal lesions.[8] The accuracy of colposcopic examination for the detection of mucosal changes approximates between 70% and 98%.[9-11]

Various authors have tried 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 used to observe the mucosa of cervix for premalignant and malignant changes. So far, a few studies have highlighted the value of Colposcopy (direct intra-oral microscopy) in the diagnosis of oral mucosal lesions. Hence, the study has been planned to assess the role of Colposcopic examination in the selection of biopsy site to aid early diagnosis of epithelial dysplasia in cases of carcinoma buccal mucosa.

II. OBJECTIVES

1. To assess the feasibility of using Colposcopic examination for carcinoma buccal mucosa;
2. To compare the Colposcopic examination findings with clinical criteria for selection of biopsy site in carcinoma buccal mucosa;
3. To correlate the histopathological findings with Colposcopic findings and clinical criteria; and
4. To assess the sensitivity and specificity of Colposcopic examination in selecting the biopsy site in carcinoma buccal mucosa.

III. MATERIALS AND METHODS

A. Source of data

The study was conducted in the Department of Oral Medicine and Radiology, Government Dental College and Research Institute, Bangalore and Kidwai Memorial Institute of Oncology, Bangalore, for a period of 1 year from May 2010 to May 2011. The study group consisted of 30 cases of clinically diagnosed cases of carcinoma buccal mucosa between the age group of 30-60 years and control group consisting of 25 healthy individuals with matched age.

B. Method of Collection of Data

Patients were selected according to the defined inclusion and exclusion criteria. Before selecting the patients for study, details of the study were explained to the patients and written informed consent was obtained for inclusion in the study. The ethical clearance for the study was obtained from the ethical committee of Government Dental College and Research Institute, Bangalore and Kidwai Memorial Institute of Oncology, Bangalore.

C. Selection criteria

i. Inclusion Criteria:
1. Clinically diagnosed cases of carcinoma buccal mucosa; and
ii. Exclusion Criteria:
1. Patients with carcinoma buccal mucosa with secondary infection;
2. Patients with carcinoma buccal mucosa having other systemic diseases; and
3. Patients undergoing treatment for carcinoma buccal mucosa.

D. Methodology

A total of 30 carcinoma buccal mucosa 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 of the subjects, a detailed case history and thorough clinical examination was carried out and under good illumination, intra-oral examination of the lesion was performed. Inspectory and palpatory findings were recorded in a prepared proforma. Following clinical examination of the lesion, the most representative site of the lesion was selected for biopsy based on set clinical criteria for dysplastic changes in leukoplakia and carcinoma buccal mucosa. Clinical criteria for selection of biopsy site for carcinoma buccal mucosa included erythema, induration and ulceration. The outline of the lesion was marked with a black color pen and the biopsy site with a red color pen with the help of a grid placed on the buccal mucosa. All the cases were then subjected to the Colposcopic examination. Before taking up the patients for Colposcopic evaluation, the normal Colposcopic findings were

standardized based on the Colposcopic criteria. Colposcopic assessment was done and the most representative site for biopsy was selected from the involved buccal mucosa depending upon the Colposcopic criteria as mentioned by the author Göran W. Gynther.[13]

All patients were subjected to routine blood investigations (Hb, BT, CT, RBS, TLC, DLC and ESR) before the routine biopsy for histopathological examination and punch biopsy after Colposcopic evaluation with 6mm diameter under local anesthesia were performed. Biopsy specimen was immediately immersed in 10% neutral buffered formaldehyde solution and was coded. Later it was embedded in paraffin by routine methods and subjected to histopathological examination.

E. Colposcopic Examination

Following clinical examination, mucosa was wiped with saline. After the mucosa was wiped with saline, abnormal epithelium appeared much darker than the normal epithelium. (Fig. 1)

Using the blue (or, green) filter and higher-power magnification (Fig. 2), abnormal vascular patterns were evaluated.


Then 5% acetic acid was applied to the lesion for about 60 seconds. The grid was placed again on the buccal mucosa. Area that estimated to have the most extensive cell changes based on Colposcopic criteria was selected for biopsy and the area of the biopsy site was marked on the grid with a green pen (Fig. 3).
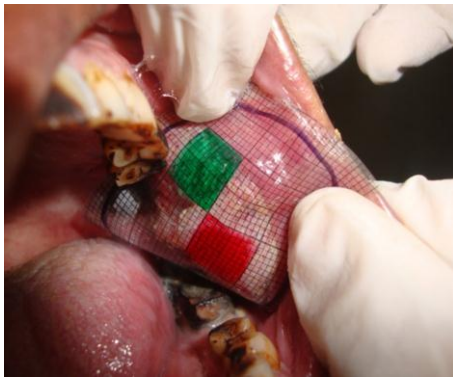
Colposcopic criteria included vascular pattern, inter-capillary distance, surface pattern, color tone, and opacity, as well as clarity of demarcation of the mucosal lesions. In the normal mucosa of the uterine cervix, two basic types of capillary networks can be seen with direct microscopy (ie, Colposcopy): hairpin capillaries (Fig. 4a)


and network capillaries (Fig. 4b).
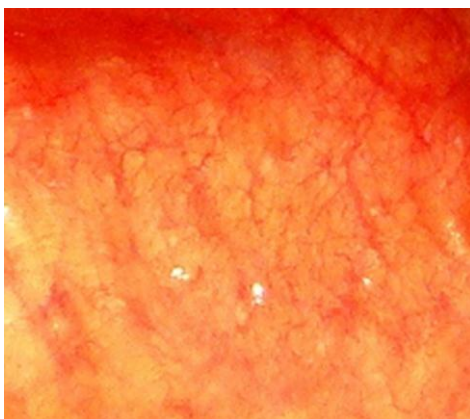
In areas of dysplasia and carcinoma-in-situ of the uterine cervix, a specific vascular pattern, punctation (previously called ground), is seen commonly. Punctation (Fig. 4c) is characterized by dilated, often twisted, irregular, hairpin-type vessels.

Another pattern of the vessels in dysplasia is called mosaic (Fig. 4d). If the vessels do not reach the epithelial surface but extend only partially into the epithelium, they appear as red lines as surrounding blocks of epithelium. The Colposcopic image resembles tiles of a floor. After application of acetic acid, this pattern is 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 (Fig. 4e) is used.
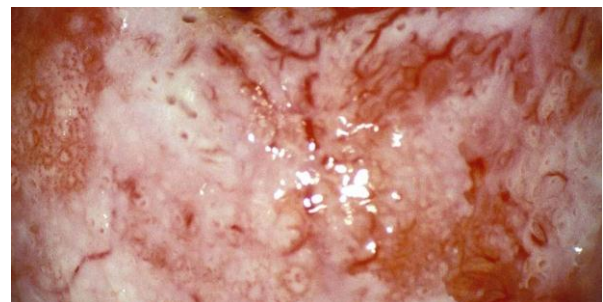
Capillary, punctation, mosaic, or atypical patterns are encountered in malignant lesions. Therefore, if one of them is present, this is an indication for biopsy and histopathological examination.

When the area selected for biopsy by clinical criteria and Colposcopy was superimposed (red and green area), then only one common biopsy sample was obtained. When two different

areas were selected from the same lesion, two different areas which were marked with red and green pen (Fig. 3) were biopsied and subjected to histopathological examination. Biopsy specimens were taken with 6 mm punch, biopsy wounds were sutured (Fig. 5) and histo-pathological examination of the same was performed.

F. Histopathological procedure

All the biopsied tissue specimens were sent for histo-pathological evaluation. The biopsied tissue was immediately transferred to the bottle containing 10% buffered formalin solution. Heamotoxylin and Eosin staining was done for the microscopic examination of the sections.

Comparison of the histo-pathlogical diagnosis obtained with routine clinical examination and direct intra-oral microscopy was performed, and the data was subjected to statistical analysis.

G. Grid preparation:

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

IV. RESULTS

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

- Age and gender distribution: The age and gender distribution of the cases and controls has been illustrated in the figures. (Table 1)
- Commoner clinical presentation observed in cases: Out of the 30 patients, induration in was observed 18 while ulceration was observed in 12 patients.
- Vascular pattern observed in cases: Out of the 30 patients, punctation vessel pattern was observed in 10 (33%) cases, mosaic pattern in 4 (13%) cases, atypical vessel pattern in 4 (13%) cases while in 12 (40%) cases, vascular pattern was not appreciated because of secondary infection and/or, ulceration. In the control group, out of the 25 controls, network capillary pattern was observed in 12 (48%) cases and hair-pin pattern in 9 (36%) cases while in 4 (16%) cases, vascular pattern could not well be appreciated.

Histopathological findings observed in the biopsy specimen obtained from clinical presentation: Out of 30 cases, histopathological report of 19 (66.3%) cases were showing well-differentiated squamous cell carcinoma, 7 (23.3%) cases, moderately differentiated squamous cell carcinoma, 2 (6.7%) cases, carcinoma-in-situ while 2 (6.7%) cases presented with severe dysplasia.

Histopathologic findings in Colposcopically directed biopsy specimens: Out of 30 cases, histopathological report of 15 (50%) cases revealed well-differentiated squamous cell carcinoma, 9 (30%) cases, moderately differentiated squamous cell carcinoma, 3 (10%) cases, poorly differentiated squamous cell carcinoma while 3 (10%) cases were showing carcinoma-in-situ.

Sensitivity and specificity for biopsy samples taken from clinical presentation in cases: A sensitivity of 0.6842 (68%) and a specificity of 0.5455 (57%) was obtained for biopsy samples taken from clinical presentation in the patients. (Table 2)

Sensitivity and specificity of Colposcopically directed biopsy specimens in cases: Sensitivity for Colposcopically directed biopsy specimens in patients came out to be 0.7826 (78%) with a specificity of 0.5714 (57%). (Table 2)

Table 1: Age distribution and Gender distribution in the study sample

	Age in years	Carcinoma buccal mucosa patients		Controls		
		No	%	No	%	
Age	31-40	2	6.7	3	12.0	Samples are age matched between cases and controls with P=0.397
	41-50	5	16.7	12	48.0	
	51-60	23	76.7	10	40.0	
	Total	30	100.0	25	100.0	
	Mean ± SD	54.07±6.55		49.72±7.17		

Gender	Male	10	33.3	15	60.0	Samples are gender matched between cases and controls with P=0.191
	Female	20	66.7	10	40.0	
	Total	30	100.0	25	100.0	

Table 2: Sensitivity and Specificity for biopsy specimens taken from Clinical examination and directed by Colposcopic examination in Carcinoma buccal mucosa patients

		Histopathology			Sensitivity	Specificity	PPV	NPV
		Positive	Negative	Total				
Clinical examination	Positive	18	3	21	0.7826 (78%)	0.5714 (57%)	0.8571	0.4444
	Negative	5	4	9				
	Total	23	7	30				
Colposcopy	Positive	13	5	18	0.6842 (68%)	0.5455 (54%)	0.7222	0.5000
	Negative	6	6	12				
	Total	19	11	30				

V. DISCUSSION

Oral squamous cell carcinoma is a well-known malignancy which accounts for more than 90% of all oral cancers. The overall 5-year survival rate of oral squamous cell carcinoma has not significantly increased in the last few years despite tremendous advancements made in the plethora of diagnostic and treatment modalities in the last 2-3 decades. Hence, the most important task is to establish an early diagnosis at the first stages of the disease.[14]

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

In our study, maximum patients were seen in the age group of 51 to 60 years and there was seen a female predominance in this group. This suggested the habit of quid to be 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 other studies by Silverman[15], Neville[16] and Swango[17].

In our study, 18 patients of oral carcinoma presented indurated lesions while ulceration was noted in 12 out of 30 patients. These findings suggested that the patients reported to the clinicians only when they developed symptoms of pain invariably associated with ulceration in confirmation with the various other studies. Jose Bagan[18] reported in his study that the clinical presentation of these early malignant lesions was usually seen in the form of well demarcated, indurated erytholeukoplastic 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 carcinoma buccal mucosa were in accordance with the studies reported by Lingen MW, Kalmar JR, Karrison TC, Speight PM[19] who reported similar findings in their study. Lingen, in his study, suggested the conventional oral examination (COE) using normal (incandescent) light as one of the standard method for oral cancer screening. A study by Fedele S[20] with 9 years randomized controlled trial also revealed that screening via visual examination of the oral mucosa under white light is 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 oral squamous cell

carcinoma 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/early oral squamous cell carcinoma that are not visible to the naked eye.

Ellen H Hopman[21], in his study stated colposcopy as an effective tool for diagnosing cervical intra-epithelial neoplasia. It was suggested that micro-invasive carcinoma was suspected when mosaic, punctation and acetowhite epithelium was seen with a thick white epithelium that had a clear and elevated margin with an irregular surface contour and raised capillaries.

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

In our study, we used the criteria for vascular changes described in Colposcopic literature for the selection of 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 carcinoma buccal mucosa reported a sensitivity of 0.6842 (68%) with a specificity of about 0.5455 (54%). The results of our study were similar to the previously reported studies including the one conducted by Goran W. Gynther[13] for assessing the value of Colposcopy in diagnosing the mucosal lesions and the one reported by Devi Charan Shetty[22] who correlated the relevance of tumor angiogenesis pattern with the histopathological 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 taking place 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 marking out the site of biopsy.[25]

In the present study, we found that the biopsy specimens selected with Colposcopic criteria appeared to be more representative of the histopathological findings at least in certain cases than those selected with routine clinical examination (COE). The altered vascular patterns, in the initial stages of lesion progression, definitely helped with the correct selection of the biopsy site, which in turn helped us reach a more definitive diagnosis, thus avoiding false-negative results. The study however concludes with the conventional oral examination having a superior edge in carcinoma buccal mucosa patients against colposcopy criteria. (Table 2) This could be explained by the fact that keratinization and thickness is more in carcinoma buccal mucosa cases compared to leukoplakia cases, hence, vascular patterns could not be visualized. However further studies are required to conclude the results.

VI. CONCLUSION

This is a preliminary study that emphasized the selection of biopsy site using Colposcopic examination as a method to select the most representative sites of epithelial dysplasia in frank malignant and potentially malignant epithelial lesions (PMELs).

VII. CONTRIBUTIONS FROM THE AUTHORS

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

VIII. 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 a written informed consent from the patients for their inclusion in the study. Details that might disclose the identity of the patient have been omitted.

IX. COMPETING INTERESTS AND OTHER DECLARATIONS

None

LEGENDS FOR FIGURES

Figure.1: Carcinoma buccal mucosa (nodular variety) on left buccal mucosa before biopsy
Figure.2: Patient positioning for Colposcopic examination
Figure.3: Placement of grid for selection of biopsy site (green color for colposcopy and red color for clinical criteria)
Figure.4a-e: Vascular patterns seen in colposcopy:
Figure.4a: Hair pin capillary pattern in normal buccal mucosa
Figure.4b: Network capillary pattern in normal buccal mucosa
Figure.4c: Punctation vessel pattern in carcinoma buccal mucosa
Figure.4d: Mosaic pattern in carcinoma buccal mucosa
Figure.4e: Atypical vessel pattern in carcinoma buccal mucosa
Figure.5: Sutured biopsy wounds in relation to carcinoma buccal mucosa after biopsy at two different sites

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