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Comparative Study of Magnified Oral Examination and Various Staining Techniques for Detection of Oral Cavity Lesions

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Background: Oral cancer is a frequently encountered entity which can occur even without any signs of dysplasia or carcinoma in situ. Hence histology is mandatory for diagnosis.

Material and Method: In a prospective study, a total of 40 patients in the age group of 30-70 years having oral lesions were included. We compared the results of biopsy after magnified oral examination and also by various staining technique with clinical examination alone and also with each other in 40 patients.

Results: Maximum number (19/35) of malignant cases were detected by direct oral microscopy followed by toluidine blue and double chromoscopy (15/35) each. By clinical examination only 10 malignant cases were detected out of 35 cases. Acetowhite staining and lugol's iodine detects only 9 & 12 malignant cases.

Conclusion: We concluded on the basis of distribution that direct oral microscopy followed by toluidine blue and double chromoscopy were better diagnostic modality than clinical examination alone.

Introduction

In Indian males, oral cancer is a common malignancy. The outcome of oral cavity squamous cell carcinoma depends on early diagnosis. In spite of advanced technique in diagnosis, 2/3 of oral cancer has spread to regional or distant structures at the time of diagnosis [1, 2]. Diagnosis of oral cavity lesions (dysplastic, premalignant and malignant) cannot be based purely on clinical findings.

The objective of this study was to compare the result of biopsy which was taken by various diagnostic modalities (vital dye staining and direct oral microscopy) and by clinical examination alone.

Patients and Methods

This prospective study was carried out in a period of one year in university Hospital, Banaras Hindu University, Varanasi, India. Forty patients presenting with oral cavity

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Table 1: Biopsy result

	Method of Diagnostic					
	Clinical examination **1**	Acetowhite Staining **2**	Toluidine blue staining **3**	Lugol's iodine staining **4**	Double chromoscopy **5**	Direct oral microcopy **6**
No. of patients (having SCC) (y_i)	10	9	15	12	15	19
Total No. of Patients (n)	35	35	35	35	35	35

lesions (suspected malignant or premalignant) were included. Majority of cases were in the age group of 40-70 years. Site of biopsy was determined by clinical examination and direct oral microscopy by measuring lesions on transparent grid divided in 6x6 mm square each of which was given a number.

Out of 40 patients, 5 patients were not biopsied, because of absence of any mucosal lesions. 35 were biopsied clinically in which 19 were suspected malignant and 16 premalignant.

For Acetowhite staining patients were asked to rinse the mouth with clean water for 1 minute then 3% acetic acid was applied over mucosal surface. Whitish opaque areas were taken as positive and biopsy was taken.

For the Lugol's iodine staining mouth was clean by rinsing with 1% acetic acid solution for 30 sec. then lugol's iodine was applied which stained normal mucosa brown while malignant area does not take staining were biopsied.

For double chromoscopy the mouth was clean by 1% acetic acid solution for 30 sec. then toluidine blue 1% was applied which stain abnormal tissue royal blue. Mouth was again rinsed by 1% acetic acid to remove excess stain. Counter staining with lugol's iodine stained normal mucosa brown. Mouth was again rinsed by 1% acetic acid solution to remove excess stain. Appropriate biopsy site from Royal blue stained area was taken.

For direct oral microscopy patients were asked to rinse the oral cavity with 1% acetic acid solution to clear debris over mucosal surface of mouth. Oral examination was done by using steromicroscope with a focal length of 200mm having green filter [3]. Colposcopic vascular changes criteria were used to determine most appropriate biopsy site [4, 5].

Results

The data is detailed in Table 1. Here the number of patients Y_i who to diagnosed malignant with different diagnostic methods are modeled as binomial variable with true success probability P_i. Further, we assume that the success rate across the methods is similar in some way which is equivalent to specifying a random effect model for the true success probability.

To estimate the unknown probability of success P_i we use Bayesian method of estimation and utilized the Win BUGS (Bayesian Inference Using Gibbs Sampling for Windows) software.

For detail information's about how to run the software and diagnostics and their interpretation one can look Ntzoufras, I.

Table 2: Estimate of success rate of different diagnostic modalities

S. No.	Diagnostic Test	Positive Predictive Value (%)
1.	Clinical examination	52.6
2.	Acetowhite Staining	47.4
3.	Toluidine blue staining	78.9
4.	Lugol's iodine staining	63.2
5.	Double chromoscopy	78.9
6.	Direct oral microcopy	100

(2009) [6] and user manual of Win BUGS. After initial burn-in 30000 updates were run and node P_i was monitored.

Table 2 shows that the positive predictive value of first method (clinical examination) is about 53%, the success rate for second method (acetowhite staining) is 47%, third method (toluidine blue) is 79%, fourth method (Lugol's iodine staining) 63%, fifth method (double chromoscopy) 79% and sixth method (direct oral microscopy) has maximum of 100%.

Discussion

Biopsy based on clinical examination of the oral cavity lesions often leads to an uncertain diagnosis and supplementary biopsy is necessary to make a definitive diagnosis. However the site for biopsy is a subjective choice.

Acetowhite staining application leads to white staining of malignant and premalignant lesion. This method also used to detect HPV infection [7]. However the sensitivity and specificity of acetowhite staining to detect HPV infection has not been properly studied. In our study acetowhite detect 9/35 malignant cases. The positive predictive value (Table 2) of this staining method was also found to be lowest (47.4 %) of all the staining methods used in our study. Although acetowhite staining reaction is widely used as a reliable adjunct to the diagnosis of malignant lesions in gynecology, but it was not a sensitive test to diagnose malignant oral cavity lesions as shown in our study. In case of laryngeal lesion acetic acid is also not a useful adjunct to biopsy selection [8].

Lugol's iodine application leads to brown staining of normal mucosa due to high starch content, while tissue suspicious for cancer does not stain and thus appears pale compared to the surrounding tissue. Nakanishi Y *et al*., [9], studied small areas unstained with Lugol's iodine are often observed in the mucosa surrounding esophageal carcinoma, and concluded that Lugol's iodine staining method is useful for detecting group at high risk of multicentric cancer in the upper aerodigestive tract. In our study Lugol's iodine staining detected 12/35 malignant oral cavity lesions with positive predictive value of 63.2 %.

Toluidine blue selectively stains acidic tissue components (carboxylates, sulphates and phosphate radicals) such as DNA and RNA. In addition, malignant epithelium may contain intracellular canals that are wider than normal epithelium; this is a factor that would enhance penetration of the dye [10]. Routine use of toluidine blue in the screening of all patients with oral disease may confuse clinical judgment as a result of the relatively low prevalence of malignant disease in the general population and the possibility of false-positive or false-negative uptake. Our study also in accordance to study done by I.C. Martin *et al*., [11], who suggest restricting the use of vital staining to selective cases (high

risk patients and in suspicious oral lesions). Toluidine blue staining method detected 15/35 malignant oral cavity lesion with high positive predictive value of 78.9 %. So this can be a better diagnostic modality in conjugation with clinical examination for suspected malignant and premalignant cases.

In double chromoscopy along with toluidine blue counter staining with Lugol's iodine also performed. Toludine blue stains malignant lesions royal blue while Lugol's iodine normal mucosa brown giving an added contrast. Epstein *et al*., [12], shown that routine use of double chromoscopy was sensitive and specific. Use of stain provided better demarcation of oral squamous cell carcinoma and dysplastic lesions assisted in site selection for diagnostic biopsy. In our study double chromoscopy detected 15/35 malignant oral cavity lesions with positive predictive value 78.9% same as that of toluidine blue staining. So probably this can also be used as a adjunctive method in conjugation with clinical examination for suspected malignant and pre malignant cases.

In direct oral microscopy we use the stereomicroscope having green filter. Colposcopic vascular changes criteria were used to determine most appropriate biopsy site. Data in our study indicates obvious diagnostic superiority of direct oral microscopy over all other methods including clinical examination as it was able to detect highest number of SCC (19/35) having positive predictive value approaching to 100%.

Conclusion

We conclude that direct oral microscopy followed by toluidine blue and double chromoscopy were better diagnostic modality than other considered diagnostic methods.

Author's Contribution

SK, SP, GK: Conducting the research work, literature search and preparation of the main body of manuscript.
SKT: Conducting the research work, proof reading of manuscript before submission.
AKK: Supervising the research work and proof reading of manuscript before submission.

Conflicts of Interest

None

Ethical Consideration

This study was approved by the institute ethics committee.

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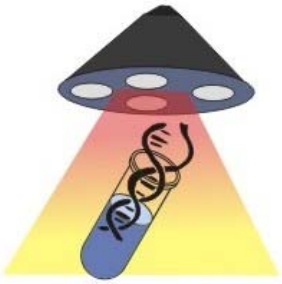

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