



# A Study to Detect Sentinel Lymph Node by Methylene Blue Dye and Histopathological Confirmation of Metastasis in Oral Squamous Cell Carcinoma Patient

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## Abstract

**Background:** Oral Squamous Cell Carcinoma (OSCC) first metastasizes via lymphatic route and the first draining lymph node is Sentinel Lymph Node (SLN). SLN is identified by combine use of nuclear imaging and dye method, as nuclear imaging facility is expensive, not easily available and radioactive materials have more potential complications so dye alone can be an alternative.

**Aims:** To assess the feasibility of using methylene blue dye (MBD) for detection of SLN in OSCC patient and using frozen section analysis to analyze metastatic status of SLN.

**Method:** 20 patients with the histopathological diagnosis of OSCC, irrespective of stage underwent SLN identification by using a peritumoral injection of MBD. Surgery of neck was done first with reflection of subplatysmal flap to identify blue-stained node as SLN in the neck region and sent for frozen section analysis. Surgery was completed with primary resection and neck dissection. Histopathological analysis of SLN was done later.

**Results:** SLN was identified in level Ib and II in 18 cases (identification rate=90%). The accuracy, sensitivity, specificity, PPV and NPV of SLN biopsy with frozen section analysis were 94%, 92.3%, 100%, 100%, 83.3% respectively.

**Conclusion:** Methylene blue dye alone can be successfully used to detect sentinel lymph node in OSCC patient. SLN biopsy with frozen section analysis is sensitive enough to detect the metastatic status of regional lymph node.

**Keywords:** Oral Squamous Cell Carcinoma (OSCC); Sentinel Lymph Node (SLN)

**Received:** February 3, 2018; **Accepted:** March 3, 2018; **Published:** May 12, 2018

**Competing Interests:** The authors have declared that no competing interests exist.

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## Introduction

Oral Squamous Cell Carcinoma (OSCC) is one of the common malignancy in southeast Asian population due to high consumption of tobacco-related products, betel quid with lime and alcohol. OSCC first metastasize to regional cervical lymph nodes before taking another route. The most important prognostic factor is the presence of cervical lymph node metastasis which can decrease the 5-year survival rate lower than 50% [1]. Lymph node metastatic tumors occur in about 40% of patients with oral cancer [2].

Staging of the neck by palpation and imaging techniques are not sensitive enough in detecting micro metastasis resulting in high incidence of occult metastasis in the neck [3]. However, nodes as small as 2 mm can contain micro metastatic disease [4] and the significantly large reactive node might not be metastatic.

Sentinel lymph node (SLN) is the first draining node of carcinoma. Lymphatic spread starts via this lymph node and its biopsy is to remove it for examination to detect presence of cancer cell in it. SLNB has shown the promising result in detecting metastatic regional lymph node in other studies and is accepted as staging for breast carcinoma and malignant melanoma [5]. SLNB with frozen section analysis can help in per-operative histopathological staging of disease thus surgical overtreatment and associated co-morbidities in population with the negative node can be avoided.

Most SLN studies in oral cancers use lymphoscintigraphy with radiolabeled particles and gamma probe localization combined with a blue dye injection [6]. Radiotracer has no significant SLN detection advantage as a single agent over dye [7]. Combination of the two techniques achieves a range of 0% to 18% increase in SLN identification rate over blue dye alone [8].

MBD has shown in numerous other studies to be equally effective in SLN identification in comparison with other blue dyes and is exceptionally associated with potentially life-threatening adverse events and caused fewer changes in oxygen saturation compared with other blue dyes [9]. MBD is cheap, easily available with low molecular weight and hygroscopic property is easily up taken by tissue and washed out by renal system. In this study, we evaluated the feasibility of using MBD alone in identifying sentinel nodes and using frozen section analysis for SLNB in OSCC patient.

## Materials and methods

This was a cross-sectional study done in Oral and Maxillofacial Department, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh, during, September 2016 to August 2017. Twenty patients with the histopathological diagnosis of OSCC irrespective of stage requiring surgical treatment were included in the study. Patient with history of neck dissection, neck irradiation, pregnancy and severe co-morbid conditions like uncontrolled diabetes, nephropathy, malnutrition, respiratory and liver disease were excluded. Ethical clearance was taken from Institutional Review Board, BSMMU, Dhaka and informed written consent was taken from all patients and their attendant.

The objective of this study was to determine the identification rate of sentinel lymph node by use of methylene blue dye, to estimate specificity and sensitivity of SLNB with frozen section analysis in comparison to post-operative histopathology, to find out accuracy, negative predictive value and positive predictive value of SLNB with frozen section analysis.

After selection of patient general, physical and oral conditions were evaluated by history and

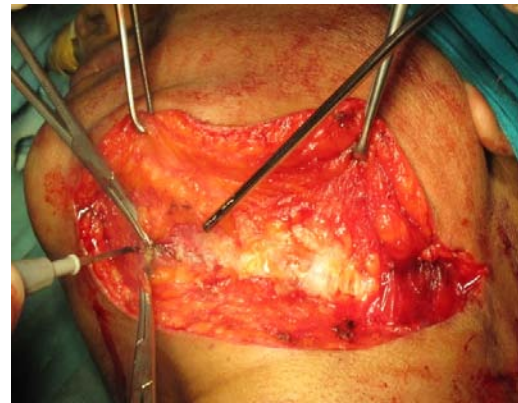
clinical examination. Loco-regional examination findings were recorded. Orthopantomogram, CT-scan of the maxillofacial region with neck, histopathology report of incisional biopsy and pre-anesthetic investigations were confirmed.

Under general anesthesia, after preparation of patient, curvilinear marking was made from menton curving along the upper border of hyoid and midpoint of sternocleidomastoid muscle to mastoid process in lesion side of neck (Figure 1) and 2 markings representing tumor and 1 cm healthy margin marked in lesion area. 1% sterile MBD was injected in the submucosal layer between first and second marking (0.5 ml in each 2, 4, 6, 8, 10 & 12O' clock positions) just before neck incision. Surgery of neck was done first with reflection of subplatysmal flap (Figure 2) for systematic evaluation of level IA, IB, IIA, IIB, III & IV to detect blue stained node (SLN) (Figure 3) and those found within 20-30 minutes were dissected. Then resection of the primary was done. Both specimens were sent for frozen section analysis and findings were recorded.

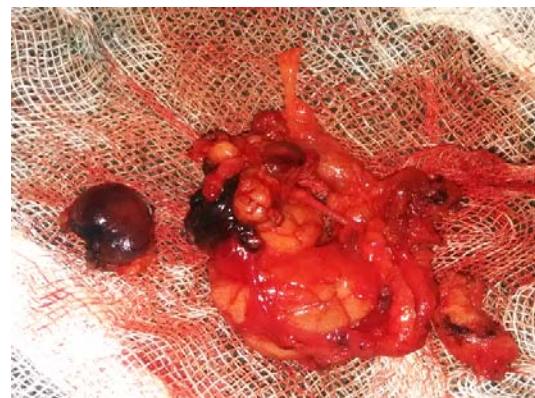
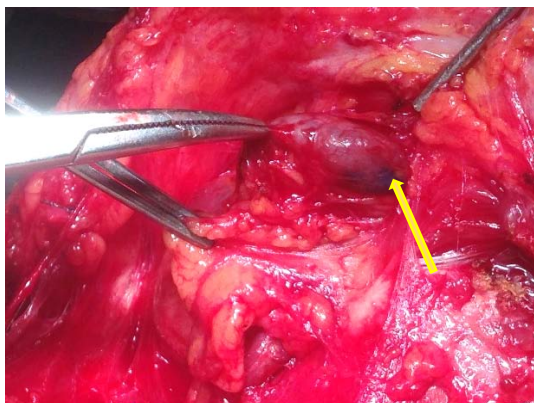
Surgery was completed with Supra Omohyoid Neck Dissection (SOND) when frozen section was negative and functional neck dissection when it was positive. Histopathology of SLN was performed later and findings were recorded. The collected data were summarized in tables and presented in form of tables and graph. Data were analyzed by SPSS 20 (Statistical Program for Social Sciences).



**Figure 1** Marking of incision



**Figure 2** Reflection of flap



**Figure 3** Identification of SLN

**Figure 4** Dissected SLN

## Results

This study was carried out in 20 patients of which 12(60%)were female and 8(40%) were male with Male: Female ratio 2:3. The age range of the patient was 30 to 70 years and mean was 55.75±9.8 years (range: 30-70 years). The most common site was buccal mucosa 7(35%). Most of the cases 13(65%) were of T4 size. other general characteristics of the patient are shown in table 1.

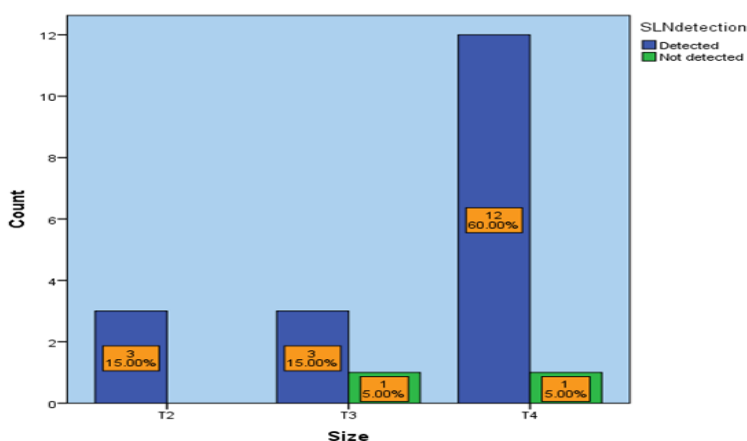
*Table 1* General characteristics of patient

Characteristics		N (%)
Total patients	Male	8 (40%)
	Female	12 (60%)
	Total	20 (100%)
Site	Buccal mucosa	7 (35%)
	Vestibule	5 (25%)
	Retromolar area	5 (25%)
	Lower alveolus	3 (15%)
Size	T2	3 (15%)
	T3	4 (20%)
	T4	13 (65%)

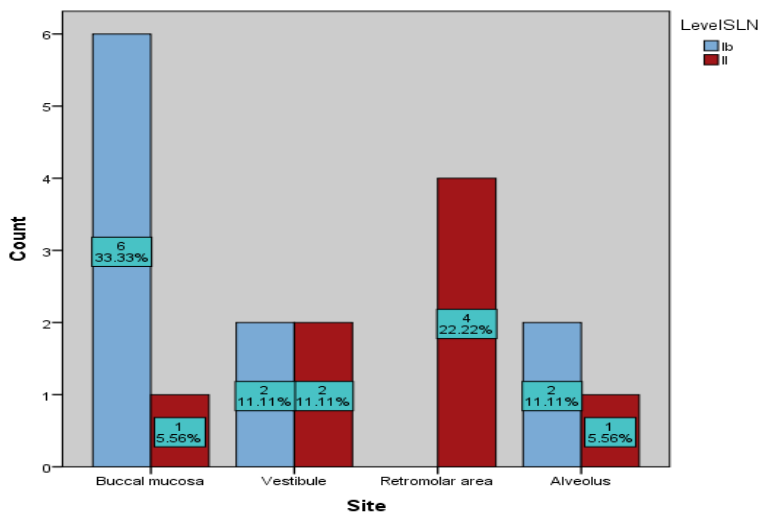
SLN was detected in 18 cases (identification rate = 90%) of which, 3 (15%), 3 (15%) and 12 (60%) cases were of T2, T3 and T4 size respectively (figure 5). Regarding site 7 (38.9%), 4 (22.2%), 4 (22.2%) and 3 (16.7%) cases were of buccal mucosa, vestibule, retromolar area and lower alveolus respectively (Table 2).

In 10 (55.6%) cases, SLN was detected in level Ib which includes 6 (60%), 2 (20%) and 2 (20%) cases of buccal mucosa, vestibule and lower alveolus respectively. In 8 (44.4%) cases SLN was detected in level II which includes 1 (12.5%) case of buccal mucosa, 2 (25%) cases of vestibule, 4 (50%) cases of retromolar area and 1 (12.5%) case of lower alveolus (Figure 6). Out of 18 detected cases all 3 cases of T2 size were non-metastatic, in 3 cases of T3 size 2 (11.1%) were metastatic and in 12 cases of T4 size 11 (61.7%) were metastatic (Table 3).

When frozen section analysis was compared with histopathology as gold standard, there were 12 true positive cases, no false positive case, 5 true negative cases and 1 false negative case. Test of validity for frozen section biopsy was done and observed that sensitivity, specificity, accuracy, positive predictive value and negative predictive value to be 92.3%, 100%, 94%, 100%, 83.3 respectively (Table 4).



**Figure 5** Bar chart showing association of SLN detection with tumor size



**Figure 6** Bar chart showing association of level of detected SLN with site of tumor

**Table 2** Association of SLN detection with site of tumor

			Site			
			Buccal mucosa	Vestibule	Retromolar area	Lower alveolus
SLN detection	Detected	Count	<b>7</b>	<b>4</b>	<b>4</b>	<b>3</b>
		% within SLN detection	38.9%	22.2%	22.2%	16.7%
	Not detected	Count	<b>0</b>	<b>1</b>	<b>1</b>	<b>0</b>
		% within SLN detection	0.0%	50.0%	50.0%	0.0%

**Table 3** Association of lymph node metastasis with tumor size

Size		Histopathology of SLN		Total
		Metastatic	Non- metastatic	
T2	Count	<b>0</b>	<b>3</b>	<b>3</b>
	% of Total	0%	16.7%	16.7%

T3	Count	<b>2</b>	<b>1</b>	<b>3</b>
	% of Total	11.1%	5.6%	16.7%
T4	Count	<b>11</b>	<b>1</b>	<b>12</b>
	% of Total	61.1%	5.6%	66.7%

**Table 4** Test of validity

		Histopathology of SLN		
		Metastatic	Non-metastatic	
Frozen section	Metastatic	Count	<b>12</b> (True positive)	<b>0</b> (False positive)
		% within Frozen section	100% (PPV)	0%
		% within Histopathology	92.3% (Sensitivity)	0%
	Non-metastatic	Count	<b>1</b> (False negative)	<b>5</b> (True negative)
		% within Frozen section	16.7%	83.3% (NPV)
		% within Histopathology	7.7%	100% (Specificity)

## Discussion

Established treatment of OSCC patient is wide excision of carcinoma with elective neck dissection. Treatment of OSCC is influenced by the metastatic status of the regional lymph node. A certain percentage of these metastasis can be detected by modern imaging techniques such as computed tomography (CT), magnetic resonance imaging (MRI), ultrasound (US) with guided fine-needle aspiration, single photon emission computed tomography (immuno-SPECT), or positron emission tomography (PET) [10]. USG guided aspiration cytology are able to detect 75% of metastasis [10] which is not enough to rely on. Lack of reliable diagnostic methods for metastatic lymph node is a major shortcoming in the treatment of cancer. SLN is the hallmark for neck node metastasis as it is the first node where carcinoma drains, detection of SLN and its evaluation for metastatic status can be helpful in deciding management of neck which will affect therapeutic outcome.

In this study methylene blue dye was used to detect sentinel lymph node in 20 patients with histopathological diagnosis of squamous cell carcinoma in oral cavity where all group of clinical size and neck nodes were included. The age range of the patient was 30 to 70 years with mean age of  $55.75 \pm 9.80$  years. Regarding site of the lesion it was found that 7 (35.0%) patients had lesion in buccal mucosa, 5 (25%) had in vestibule, 5 (25%) had in retromolar area and 3 (15%) had in lower alveolus. 8 (40%) were male and 12 (60%) were female patients with Male: Female ratio 2:3. Habib *et al.* [11] in a study of 36 patient with OSCC noted male: female ratio to be 1:1.25 and most common site to be buccal mucosa in 12(33.3%) cases. Byakodi *et al.* [12] noted age range of OSCC patient to be 30-70 years in 112 study subjects and reported that the various studies have shown an increase in evidence of oral cancer in females in Indian subcontinent with the second most common site to be buccal mucosa in 34 (30.35%) cases. This might be due to high consumption of betel quid with lime and tobacco product and keeping them in buccal mucosa for a long time.

This shows the prevalence of OSCC is higher in the female population, old generation, and common site to be buccal mucosa in south Asia.

In present study regarding the tumor size of patients, 3 (15%) cases were of T2 size, 4 (20%) cases were of T3 size, and 13 (65%) cases were of T4 size. The population of T4 is more because majority of patients from rural area where there is lack of diagnostic facilities and health awareness leads to late diagnosis. Many studies didn't include T4 cases because the high probability of clinical node positivity, but clinically positive might be non-metastatic but reactive due to infection from open cancer wound of the oral cavity, that need to be assessed. Calabrese *et al.* [13] stated the risk of neck metastasis depends on the site, size, grading, and depth of infiltration of the tumors. Also, to cross-check the efficiency of MBD those were included in this study.

In this study, among 20 OSCC cases blue stained node or SLN was detected in 18 (90%) cases which includes 7 (100%), 3 (100%), 4 (80%), 4 (80%) cases of buccal mucosa, alveolus, retromolar area and vestibule respectively also 3 (16.7%), 3 (16.7%) and 12 (66.7%) cases were of T2, T3 and T4 size. In 2 (10%) cases SLN was not detected of which each case was of T3 and T4 size, it might be due to the destruction of the lymphatic channel of underlying tissue. Peng *et al.* [14] and Ozdemir *et al.* [15] successfully identified SLN by using dye alone in more than 90% cases. Ramamurthy *et al.* [16] noted the detection rate of 90.6% by use of MB dye alone. In animal experiment did by Wu *et al.* [17] detection rate was 100% by using MBD. Technically surgeon should be sound and swift to access blue colored node in certain time frame because MBD is water soluble and washes out quickly to color successive nodes if times elapse.

The level of SLN was studied in relation to site of the tumor and found that level Ib had drainage in 10 cases which includes 6 (60%), 2 (20%) and 2 (20%) cases of buccal mucosa, vestibule and alveolus respectively. Level II had drainage in 8 cases which includes 1 (12.5%), 1 (12.5%), 2 (25%) and 4 (50%) cases of buccal mucosa, alveolus, vestibule and retromolar area respectively. Which suggest that tumor of buccal mucosa mainly drains in level Ib and retromolar area drains in level II. This study showed that majority of tumor of oral cavity drains in level I and II which is similar to Shah [18] which found that nodal involvement usually occurs at level I-III. In a study did by Peng *et al.* [14] anatomic distribution of SLN of OSCC was as follows: 63.6 % in level IIa, 17 % in level Ib, 11.4 % in level III, and 8% in level Ia.

Association between size of tumor and histopathological finding was studied and found that out of 18 detected cases 3 (16.7%) of T2 cases were non-metastatic, in T3 tumor 2 (11.1%) cases were metastatic and 1 (5.6%) was non-metastatic, in T4 tumor 11 (61.1%) cases were metastatic and 1 (5.6%) case was non-metastatic. This shows as the size increases, the degree of metastasis also increases irrespective of clinical findings and in 1 case of T4 with N0, there was no metastasis at all. Ross *et al.* [19] did upstaging of the clinically N0 neck with SLNB that includes 25% (5/20) of T1 patients, 42% (5/12) of T2 patients, and 45% (5/11) of T3/T4 patients which shows the nodal metastasis is irrespective of size, which was contradictory to this study.

Out of 18 detected cases, 13 (72.2%) cases had metastasis in final histopathology and 12 (66.7%) cases had metastasis in frozen section analysis. There was 1 (5.5%) false negative case and no false positive case. Validity test was carried out for SLNB with frozen section analysis resulting sensitivity 92.3%,

specificity 100%, accuracy 94%, positive predictive value 100% and negative predictive value 83.3%. Sensitivity and NPV is more accurate with immunohistochemistry but it is not possible during operative procedure. In this study, the sensitivity of frozen section analysis is sufficient enough to make real-time decision. Sangwan *et al.* [20] studied 15 patients where sensitivity, specificity, PPV and NPV value to be 40%, 100%, 100% and 77% when compared to HPE. Payoux [21] studied over 30 patients' where sensitivity was 86% and he found one false negative case, Ross *et al.* [19] studied in 48 patients where sensitivity was 95%.

## Conclusion

Methylene blue dye alone can be successfully used for detection of SLN in oral squamous cell carcinoma patients with good accuracy. This method can be used in developing countries and centers where nuclear medicine facilities are not widely available. SLNB with frozen section analysis can be a reliable mean to detect metastatic status of regional lymph node which aid in pathological staging of OSCC and the information can be helpful to surgeons in real time decision making for treatment of neck. For validity of it, multi institutional studies should be done in large number of patients in large scale of time with regular follow up.

## Financial support and sponsorship

No

## Conflict of interest

No any conflict of interest

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