Research Paper

A retrospective analysis of 5-year post surgery recurrence of N0 neck and the role of methylene blue in sentinel lymph node biopsy

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ABSTRACT

The aim of this study is to perform a retrospective analysis of the feasibility of the clinical application of SLNB using methylene blue dye (MBD) for the identification of SLN followed by frozen section biopsy to detect occult metastasis in clinically N0 necks. Hence, to know the reliability of MBD in reducing the need for extensive surgery.

Methods: We retrospectively analyzed the clinic pathological data of 48 patients with early oral cancer. The SLN identification rate (IR) was calculated in SLNB with MBD and the false-negative rate (FNR). Intra operative frozen section biopsy was done for all patients was compared with post-operative paraffin histopathology report and the prognosis of patients was analyzed.

Results: Analysis of the 48 SLNB cases showed that there were significant differences in SLN successful detection rate among patients with different site ($p = 0.043$) and clinical presentation ($p = 0.007$). Similar significant results ($p < 0.05$) were observed with intra-operative frozen and post-operative paraffin histopathology sections. SLNs were successfully detected in 37(77.1%) patients out of 48. The intra operative frozen histopathology completely matched with the post-operative paraffin histopathology showing 39 (81.2%) negative and 09 (18.8%) positive cases. A prognostic analysis of SLN detection based on 48 patients showed that the 5-year survival rate was 100%.

Conclusion: MBD has acceptable SLN identification rate and a low FNR(false negative report) in frozen sections. Stained SLNs with no SLN metastasis are associated with disease-free survival (DFS). Hence, MBD-SLN biopsy has significantly reduced the need for extensive neck dissection in N0 neck with less morbidity.

1. Introduction

The management of N0 neck is currently the most controversial in head and neck cancer. The risk of subclinical metastasis changes according to the anatomic area, histologic type, thickness, and size of the tumor. Therefore, more radical neck dissection in all clinical N0 patients is equivalent to overtreatment of patients with higher surgical morbidity. However, the main problem is the detection of micro metastases (cN0 pN1), which are found in up to 50% of patients with cN0 squamous cell carcinoma of the tongue who undergo neck dissection. For this reason, the surgical protocol N0 neck in for early tongue squamous cell tumors is discussed.¹ Because the procedure is associated with

high surgical morbidity (e.g., bleeding, nerve injury, pain, or lymphedema),² several studies have compared a wait-and-see approach in patients with clinically N0 tumors. In these cases, the results showed a worse prognosis in patients who later develop cN+ and undergo delayed neck dissection. However, improved survival was observed in cN0 tumors that turned out to be pN1 tumors after elective neck dissection.^{3,4} The real problem in these patients with cN0 disease is to know the status of the nodes, whether it is truly negative or whether metastasis is present. From the literature, nodal or occult metastasis is possible in 3% cases of clinically N0-positive oral cavity cancer.² In such cases, patients undergo neck dissection without proper indication. Therefore, neck dissection should be performed only in SLN positive patients and

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the rest should be spared. This would avoid unnecessary surgical morbidity for the patients.⁵ Gamma probes, lymph scintigraphy and radio particles along with injection of blue dye are used to localize the nodes.⁶ The availability and affordability of nuclear medicine and lymphoscintigraphic examinations is limited in developing countries due to financial constraints. An alternative is the use of methylene blue dye, which is less allergic and inexpensive. This dye has shown promising results in breast cancer clinical trials.⁷

The aim of this study is to investigate the reliability of methylene blue in identifying false negative rates in frozen section biopsies. This is a retrospective study that includes the patent data with early oral cancer at our college between 2012 and 2017.

Patient and Methods- Institute Review Board ethical committee approval was taken ((IRB NO:00010,556) - (IORG 0008839)-15/4/2017). There was no choice to take informed consent from the patients as this was a retrospective study.

Patients eligible for methylene blue dye injection with early oral carcinoma between 2012 and 2017 were included in this study (Table 1).

Inclusion criteria: Early oral squamous cell carcinoma (T1-T2) and N0 neck as determined by both clinical examination and preoperative ultrasound (Table 2). The type of neck dissection was planned based on the frozen section report, depending on whether it was positive or negative. Selective neck dissection (SND) was planned for the patients with negative findings and modified Radical Neck Dissection (MRND) for the patients with positive findings.

Exclusion criteria: T3 and T4 lesions. N1 and above, midline crossing lesions, previous surgery, history of radiation, medically compromised patients and patients with missing clinical or pathological data.

SLNB surgery Under general anesthesia, peritumoral methylene blue was injected [Fig. 1] (0.5 ml in each of the 3, 6, 9, and 12 of the clock positions) at a concentration of 0.1 g methylene blue in 100 ml distilled water. The procedure began with dissection of the neck. The incision ran along the middle of the sterno cleido mastoid muscle and the upper

Table 2

Analysis of other variables for SLN detection in oral cancer.

Variables	n (%)	SLN detection		χ^2	p
		Yes	No		
Gender					
Male	23 (47.9)	18 (78.3)	05 (21.7)	0.035	0.852
Female	25 (52.1)	19 (76)	06 (24)		
Age Groups (in years)					
<50	22 (45.8)	19 (86.4)	03 (13.6)	1.980	0.159
≥50	26 (54.2)	18 (69.2)	08 (30.8)		
Site					
Tongue	24 (50)	17 (70.8)	07 (29.2)	11.440	0.043*
Cheek (Buccal Mucosa)	09 (18.8)	09 (100)	00 (0)		
Retromolar Area	04 (8.3)	04 (100)	00 (0)		
Lower lip	04 (8.3)	01 (25)	03 (75)		
Maxillary tuberosity	04 (8.3)	03 (75)	01 (25)		
Alveolar mucosa	03 (6.3)	03 (100)	00 (0)		
Clinical presentation					
Discovered accidentally	25 (52.1)	23 (92)	02 (08)	11.975	0.007*
Bleeding	12 (25)	07 (58.3)	05 (41.7)		
Discoloration	04 (8.3)	01 (25)	03 (75)		
Abnormal sensation	07 (14.6)	06 (85.7)	01 (14.3)

Fig-1. Circumlesion injection of methylene blue.

border of the hyoid bone. Monopolar electrodes were used to elevate the lobes. All nodes of the levels from level 1 to level IV were identified and evaluated.

Fig-2. Identification of sentinel (stained) LN.**Table 1**

Demographic details of the patients (n = 48).

Variables	n (%)
Gender	
Male	23 (47.9)
Female	25 (52.1)
Age Groups (in years)	
<50	22 (45.8)
≥50	26 (54.2)
Detection of SLN	
Yes	37 (77.1)
No	11 (22.9)
Intraoperative frozen histopathology	
Positive	09 (18.8)
Negative	39 (81.2)
Postoperative paraffin histopathology	
Positive	09 (18.8)
Negative	39 (81.2)
Agree with postoperative U/s	
Yes	39 (81.2)
No	09 (18.8)
Site	
Tongue	24 (50)
Cheek (Buccal Mucosa)	09 (18.8)
Retromolar Area	04 (8.3)
Lower lip	04 (8.3)
Maxillary tuberosity	04 (8.3)
Alveolar mucosa	03 (6.3)
Clinical presentation	
Discovered accidentally	25 (52.1)
Bleeding	12 (25)
Discoloration	04 (8.3)
Abnormal sensation	07 (14.6)

The sentinel nodes were stained with blue dye within 20 min (Fig. 2), and the SLN nodes were dissected and sent for frozen section.

Selective neck dissection was performed for the negative findings, and MRND was performed for the positive findings. Crile incision was used for MRND.

Surgery for the primary tumor was then completed, followed by immediate reconstruction. The tumor was sent for frozen sectioning for the safety margins (Table 3). Closure of the wounds was done in layers. All lymph nodes, including SLN nodes (stained with methylene blue), were dissected and sent for histo-pathological examination. Lymph nodes that showed negative micrometastasis underwent immunohistochemistry (IHC) for cytokeratin on paraffin blocks (Table 4). Patients were discharged to the ward to complete postoperative care and treatment. Follow-up was done clinically as well as by radiographs. Follow-up of all patients was done after 1 Month, 3 months, 6 months, 12 months, and 18 months, and then at 5 years postoperatively. During the postoperative follow-up periods, we looked for complications such as dehiscence, residual tumor Complications or dehiscence. SLN collection and pathological evaluation SLN collection procedures were standardized according to the techniques recommended by the College of American Pathologists (CAP).⁸ According to the American Joint Committee on Cancer (AJCC) Cancer Staging Manual (8th edition)⁹ the total number of SLNs collected should be 6. The SLN-FNR in frozen sections = number of MBD -negative frozen sections with positive paraffin result/-total number of positive SLN-paraffin sections.¹⁰ Immunohistochemistry (IHC) and histological grade histopathological evaluation and clinic pathological parameters.

All H & E slides were examined by two pathologists. In difficult consensus cases, the third opinion of an experienced pathologist was sought. All protocols were performed according to the 2017 College of American Pathologist guidelines for oral cavity cancer.¹¹

Immunohistochemical confirmation of cytokeratin was performed. The classification system WHO, revised in 2005 based on Broders classification,¹² included microscopic search for differences between tumor tissue and normal epithelium due to the lack of cellular differentiation. This classification was routinely used in the analysis of biopsies and

surgical specimens.¹³

Data from patients included in this study were collected and analyzed as follows: Detection of SLN, detection of malignant micro metastases, clinical presentation, age at OSCC diagnosis, gender, pathologic AJCC anatomy, and postoperative satisfaction with follow-up. Finally, the data were reexamined, and the authors designed a retrospective observational study.

Late follow-up data were obtained from medical records, emails, and telephone conversations with patients. Follow-up data included disease-free survival (DFS) and overall survival (OS).

Statistical analysis The collected data were entered into a Microsoft Excel spreadsheet, a master file was created, and then transferred to the SPSS version 21.0 data editor (SPSS Inc., Chicago, Illinois, USA) for data analysis. Categorical variables are presented as numbers (percentages) and were analyzed using the chi-square/Fisher's Exact test. Kaplan-Meier Survival curves were used for survival analyzes. For all tests, the confidence interval and P value were set at 95% and ? 0.05, respectively.

2. Results

A total of 48 patients with oral cavity cancer admitted to the Department of Oral and Maxillofacial Surgery Plastic Surgery Department of our college between January 2012 and December 2017 were retrospectively studied. Of them, 23 (47.9%) were male and 25 (52.1%) were female. The patients ranged in age from 37 to 72 years with a mean age of 54.06 ± 11.615 years [Table 1]. SLNs were successfully detected in 37(77.1%)(Table 1) patients. Intraoperative frozen histopathology was in complete agreement with postoperative paraffin histopathology, showing 39 (81.2%) negative and 09 (18.8%) (Table 1) positive cases. These findings were also consistent with the results of preoperative ultrasound.

According to the site of involvement, the majority were found in the tongue ($n = 24$ (50%)), and the clinical presentation was mostly incidentally detected ($n = 25$ (52.1%)). Analysis of the 48 SLNB cases showed that there were significant differences in the successful SLN detection rate between patients with different locations ($p = 0.043$) and clinical presentations ($p = 0.007$) [Table 2]. Similar significant results ($p = 0.05$) were observed for intraoperative frozen sections and postoperative histopathology paraffin sections [Table 3]. A separate table for postoperative paraffin sections is not provided as the values were similar to intraoperative frozen histopathology sections.

In the present study, SLN were detected in patients with oral cavity cancer by intraoperative frozen and postoperative paraffin histopathology. For both methods, the sensitivity, specificity, and accuracy values of SLNB were 66.7%, 100%, and 54.2%, respectively. The false negative rate was 1% for both methods [Table 4]. All 48 patients underwent SLNB successfully and completed the follow-up over a period of 60 months. None of them experienced recurrence or metastasis. A prognostic analysis of SLN detection based on 48 patients showed that the 5-year survival rate was 100%, which was not statistically significant at $p = 0.073$ (Graph 1).

Similarly, a prognostic analysis of postoperative paraffin histopathology detection in 48 patients showed a 5-year survival rate of 100%, which was statistically significant at $p = 0.001$ (Graph 2).

3. Discussion

The concept of sentinel node was first introduced in 1977. It related more to primary malignancies and breast cancer. Sentinel node biopsy is known to be a reliable diagnostic tool for early detection of oral cancer. The use of methylene blue (MBD) in sentinel node biopsy Has been retrospectively studied to observe its meaning. The use of MBD to detect SLNs and micrometastases has significantly reduced the need for more radical surgery. This is consistent with the statement of Antoine E. et al. (3) in his 10-year prospective cohort study. At the same time, some

Table 3
Analysis of other variables for intra-operative frozen histopathology in oral cancer.

Variables	n (%)	Intra-operative frozen histopathology		χ^2	p
		Negative	Positive		
Gender					
Male	23 (47.9)	17 (73.9)	06 (26.1)	1.560	0.212
Female	25 (52.1)	22 (88)	03 (12)		
Age Groups (in years)					
<50	22 (45.8)	20 (90.9)	02 (9.1)	2.634	0.151
≥50	26 (54.2)	19 (73.1)	07 (26.9)		
Site					
Tongue	24 (50)	20 (83.3)	04 (16.7)	20.285	0.001*
Cheek (Buccal Mucosa)	09 (18.8)	08 (88.9)	01 (11.1)		
Retromolar Area	04 (8.3)	00 (0)	04 (100)		
Lower lip	04 (8.3)	04 (100)	00 (0)		
Maxillary tuberosity	04 (8.3)	04 (100)	00 (0)		
Alveolar mucosa	03 (6.3)	03 (100)	00 (0)		
Clinical presentation					
Discovered accidentally	25 (52.1)	20 (80)	05 (20)	10.491	0.015*
Bleeding	12 (25)	12 (100)	00 (0)		
Discoloration	04 (8.3)	04 (100)	00 (0)		
Abnormal sensation	07 (14.6)	03 (42.9)	04 (57.1)		

Table 4
Comparison of SLN detection.

Groups		SLN detected	SLN NOT detected	Sensitivity	Specificity	AR	FNR
Intra -operative Frozen histopathology	Negative (n = 39)	28	11	71.8% (28/39)	100% (28/28)	58.3% (28/48)	01% (11/11)
	Positive (n = 09)	09	00				
Post-operative Paraffin histopathology	Negative (n = 39)	26	13	71.8% (28/39)	100% (26/26)	54.2% (26/48)	01% (13/13)
	Positive (n = 09)	09	00				
Agree with Pre-operative ultrasound	Yes (n = 39)	26	13	71.8% (28/39)	100% (26/26)	54.2% (26/48)	01% (13/13)
	No (n = 09)	09	00				

SLN – Sentinel Lymph Node; AR – Accuracy rate; FNR – False Negative rate.

Graph 1. Kaplan-Meier analysis of SLN detection in 48 patients.

studies reported less than 10% recurrence in oral cavity carcinomas with a follow-up of 5 years.⁷ This is in contradiction with our study where we did not observe any recurrence in any of our cases in the 5-year post-operative period. SND ensures a higher local control rate with cervical node metastasis of 6%(3). In our study, we found that 18.8% (Table 1) of SLNs had positive micrometastases. In all these SLN positive cases were detected intraoperatively after frozen section biopsy. Therefore, it is worth waiting 15–20 min for this frozen section report to avoid unwanted radical neck surgery.

SLNB has been detected in patients with oral cavity cancer by intraoperative frozen section and postoperative paraffin histopathology methods. For both methods, the sensitivity, specificity, and accuracy values of SLNB were 71.8%, 100%, and 58.3%, respectively (Table 4). In 2008, Yuan Liu, found in his randomized study that methylene blue was as effective as the blue dye lymphazurin in SLN detection. Moreover, no allergic reaction was found in the literature in head and neck surgical procedures.¹⁴ He also mentioned cost effectiveness in his study. This is in agreement with our study on the importance of cost effectiveness and no allergic cases were ever reported in our study.

In 2015, Hanwei Peng et al. concluded that near-infrared imaging with indocyanine green combined with methylene blue mapping is a feasible and reliable new method for SN biopsy in cN0 oral/

oropharyngeal carcinoma.¹⁵ This is in agreement with our study in which we detected 77.1% (Table 1) of cases with MBD.

Francisco J. et al in 2002 conducted a study on 18 patients about the use of Lymphoscintigraphy in identifying sentinel lymph node, his reports suggest good prediction of lymphatic involvement by LS/SNB hence reducing the need for extensive neck dissection in N0 neck which is in agreement with our study.¹⁶

Kihito Terada et al. in 2010 has published 5 year follow up study on intra operative frozen section sentinel node biopsy in N0 neck of oral cancer patients, he found that 5 year survival rate was 91.1% and SN positive patients survival was significantly lower than that of SN negative. This is in contradiction to our study as we found the 5 year survival rate of our patients in both groups was 100%.¹⁷

In 2015, Michael Vaiman et al. investigated the utility of MBD in parotid surgery in 148 patients and concluded that its use simplifies the surgery and contributes to better visualization of the nodules.¹⁸ This is in agreement with our study as it reduces the need for more radical surgery in the N0 neck, thus reducing unnecessary surgical morbidity.

Tschopp, L. et al. in 2005 studied the value of frozen section analysis of the sentinel lymph node in clinically N0 squamous cell carcinoma of the oral cavity and oropharynx.¹⁹ They found that 45% of SLN positivity with metastasis occurred intraoperatively after frozen section biopsy.

Graph 2. Kaplan-Meier analysis of post-operative paraffin histopathology in 48 patients.

We found that only 18.8%(Table 1) of our patients were SLN positive after frozen section. Tschopp et al. also had difficulty in detecting SLN in 2 patients because the nodules were in close proximity to the primary tumor. This difficulty was due to the use of a gamma probe. In contrast, no such difficulties occurred in our cases.

The main purpose of the frozen section procedure to analyze SLN is to determine whether occult metastasis is present in C N0 patients. In this way, we could avoid unnecessary surgery and morbidity for patients and improve cost efficiency. The average waiting time for frozen section biopsy in our cases was 20 min, which is very acceptable considering the many advantages it offers. We followed up all our patients for 5 years postoperatively to monitor for possible recurrence. However, we did not detect recurrence in either the SLN-positive group (9 patients with metastases) or the SLN-negative group (39 patients). Selective neck dissection was performed in the SLN-negative group, whereas functional neck dissection was performed in the SLN-positive group. This intra-operative frozen section procedure avoided unnecessary advanced surgical morbidity.

4. Conclusion

The use of methylene blue with a sensitivity of 71.8%(Table 4) is an economical and feasible method for nodule identification as it has an acceptable SLN identification rate and a low FNR(false negative report) in frozen sections.

Stained SLNs with no SLN metastasis are associated with disease-free survival (DFS). The MBD-SLN biopsy and inclusion of conventional 2-part frozen section biopsy with a specificity of 100% has significantly reduced the need for extensive neck dissection in the N0 neck with less morbidity. Hence the reliability of MBD in reducing the need for extensive surgery can be considered as an acceptable investigation option for the economically challenging scenarios.

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Regulatory statement

This study was conducted in accordance with all provisions of the Local Human Subjects Monitoring Committee Guidelines and Institutional Review Board Policies. The approval code for this study is IRB NO:00010,556) - (IORG 0008839)-15/4/2017.

References

- Silverman DA, El-Hajj M, Strome S, Esclamado RM. Prevalence of nodal metastases in the submuscular recess (level IIb) during selective neck dissection. *Arch Otolaryngol Head Neck Surg.* 2003;129:724–728.
- Ambrosch P, Brinck U. Detection of nodal micrometastases in head and neck cancer by serial sectioning and immunostaining. *Oncology.* 1996;10:1221–1226.
- Melkane AE, Mamellet G, Wycisk G, et al. Sentinel node biopsy in early oral squamous cell carcinomas: a 10-year experience. *Laryngoscope.* 2012;122:1782–1788.
- Dias FL, Kligerman J, Matos De Sá G, et al. Elective neck dissection versus observation in stage I squamous cell carcinomas of the tongue and floor of the mouth. *Otolaryngol Head Neck Surg.* 2001;125:23–29.
- Suresh TN, Harendra Kumar ML, Thomas AK, Azeem M. Study of sentinel lymph node in oral Squamous cell carcinoma. *Journal of Biomedical Science.* 2013. 2013;3: 146–149.
- Kurita H, Koike T, Narikawa JN, et al. Clinical predictors for contralateral neck lymph node metastasis from unilateral squamous cell carcinoma in the oral cavity. *Oral Oncol.* 2004;40:898–903.
- Kovács AF, Landes CA, Hamscho N, Risse JH, Berner U, Menzel C. Sentinel node biopsy as staging tool in a multimodality treatment approach to cancer of the oral cavity and the oropharynx. *Otolaryngol Head Neck Surg.* 2005;132:570–576.
- Susan C, Shikha B, Chen YY, et al. *Protocol for the Examination of Specimens from Patients with Invasive Carcinoma of the Breast. V3100.* Washington, DC: College of American Pathologists (CAP); 2012. Available from: www.cap.org/cancerprotocols. Accessed October 20, 2012. Accessed.
- Amin MB, Edge SB, Greene FL, et al. *AJCC Cancer Staging Manual.* 8th ed. New York: Springer; 2017.
- Rubio IT, Klimberg VS. Techniques of sentinel lymph node biopsy. *Semin Surg Oncol.* 2001;20:214–223. <https://doi.org/10.1002/ssu.1036>.
- Seethala RR, Weinreb I, Bullock JM. *College of American Pathologists Protocol for the Examination of Specimens from Patients with Cancers of the Lip and Oral Cavity Version LipOralCavity 4.0. 0. 1.* 2017.
- Broders AC. The microscopic grading of cancer. *Surg Clin.* 1941;21:947–962.
- El-Naggar AK. *WHO Classification of Head and Neck Tumours.* International Agency for Research on Cancer; 2017.
- Liu Y, Truini C, Ariyan S. A randomized study comparing the effectiveness of methylene blue dye with lymphazurin blue dye in sentinel lymph node biopsy for the treatment of cutaneous melanoma. 2008;15(9):2412–2417.

- 15 Peng H, Wang SJ, Niu X, Yang X, Chi C, Zhang G. Sentinel node biopsy using indocyanine green in oral/oropharyngeal cancer. *World J Surg Oncol*. 2015;1–7.
- 16 Civantos FJ, Gomez C, Duque C, et al. Sentinel node biopsy in oral cavity cancer: correlation with pet scan and immunohistochemistry. *Head Neck*. 2003;25(1):1–9.
- 17 Terada A, Hasegawa Y, Yatabe Y, et al. Follow-up after intraoperative sentinel node biopsy of N0 neck oral cancer patients. *Eur Arch Oto-Rhino-Laryngol*. 2011;268(3):429–435.
- 18 Vaiman M, Jabarin B, Abuita R. ScienceDirect Methylene blue staining in the parotid surgery. *Am J Otolaryngol Neck Med Surg*. 2015;37(1):22–26.
- 19 Tschopp L, Nuyens M, Stauffer E, Krause T, Zbären P. The value of frozen section analysis of the sentinel lymph node in clinically N0 squamous cell carcinoma of the oral cavity and oropharynx. *Otolaryngol Head Neck Surg*. 2005;132(1):99–102.