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Accuracy of sentinel lymph node biopsy in accessing neck node status in head and neck malignancies

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ABSTRACT

Head and neck malignancies includes neoplasm arising from oral cavity, salivary glands, pharynx, larynx and nasal cavity. Among them oral cavity cancers are most common. In India, oral cavity squamous cell carcinoma (OCSCC) is amongst the most common malignacies and a leading cause of cancer related death.1-3

Oral malignancies metastasize to cervical lymph nodes commonly. Presence of metastatic cervical lymphadenopathy (specially with extra nodal extension ENE) is associated with poor prognosis. Presence of metastatic cervical lymph nodes on one side of neck reduces the 5-year survival rate by 50% and if present on both sides of neck survival further drops by 25%. Therefore, detection of metastatic cervical lymphadenopathy is of utmost importance for cancer staging and management.4,5

Surgical management of lymphatic basin in oral cavity cancers has been evolved from radical(RND) to modified (MRND) and selective neck dissection (SND) in order to reduce the morbidity associated with radical procedure and to preserve as much function as possible and quality of life while still achieving Oncologically sound results.6.

KEYWORDS: *Technique of sentinel lymph node biopsy (SLNB), Statistical analysis*

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1. INTRODUCTION

Head and neck malignancies includes neoplasm arising from oral cavity, salivary glands, pharynx, larynx and nasal cavity. Among them oral cavity cancers are most common. In India, oral cavity squamous cell carcinoma (OCSCC) is amongst the most common malignacies and a leading cause of cancer related death.[1-3]

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Surgical management of lymphatic basin in oral cavity cancers has been evolved from radical(RND) to modified (MRND) and selective neck dissection (SND) in order to reduce the morbidity associated with radical procedure and to preserve as much function as possible and quality of life while still achieving Oncologically sound results.[6]

The surgical treatment of neck in clinically node negative (cN0) remains controversial. The problem in these patients is to know whether the lymph nodes are actually negative or has occult metastasis. The chances of occult metastasis in these patients can be up to 3%[7,8]. SND was introduced for the management of cN0 disease to lessen the morbidity associated with traditional MRND by limiting the tissue dissection and obtaining adequate nodal sampling which is oncologically sound. Sentinel lymph node biopsy (SLNB) was then adopted to harvest adequate diagnostic material while minimising the morbidity associated with MRND/SND.[9]

SLN identification using methylene dye alone in breast cancer has been widely reported with identification rate comparable to dual technique.[10] Most SLNB studies in oral cavity cancers have used lymphoscintigraphy with radio colloid combined with blue dye and gamma probe localisation.[11-21] However lymphoscintigraphy and gamma probe facilities are not widely available in India therefor alternate technique for SLNB needed to be evaluated.[22]

Methylene blue is a low cost, widely available, hypoallergenic and effective dye. Methylene blue has shown promising results in SLN studies in breast cancer when used in combination with lymphoscintigraphy or used alone.[23,24] In this study we evaluated the accuracy and feasibility of methylene blue dye alone for assessing the neck node status in clinically node positive oral cavity cancers.

2. MATERIALS AND METHODS

The study was conducted in **Department of General Surgery, Vardhman Mahavir Medical College and Safdarjung Hospital, New Delhi**, over a period of **57 months (November 2017 to August 2022)** , after obtaining clearance from "Institute Review Board" and the "Ethical committee" of Safdarjung hospital, New Delhi, India.

Forty one biopsy confirmed clinically neck node positive cases of oral cavity cancers were included in this study who fulfilled the inclusion criteria were informed about the purpose and layout of the study and evaluated after taking informed consent for enrolment in the study. For each patient a detailed history was taken and clinical examination was done. All the findings were recorded in the preform proforma. Imaging in the form of Contrast enhanced compounded tomography (CECT) scan was done from skull base to thoracic inlet in order to stage the disease preoperatively.

The patients were taken up for definitive surgery in the form of surgery for the primary and the neck dissection with a standardised technique by same surgical team each time.

Inclusion criteria:
Biopsy confirmed cases of oral cavity cancers.

Exclusion criteria:
1) Known allergic to methylene blue dye.
2) Previous history of neck dissection or radiation.
3) Recurrent disease.

Technique of sentinel lymph node biopsy (SLNB)
Under general Anesthesia, intra-operatively peri tumoral 1ml[25] of methylene blue dye was injected 0.25ml each at 12,3,6 & 9'O clock respectively (fig 1). Surgery for the neck was done first using modified schobinger's incision. The sentinel node/s (blue node/s) mapped and isolated after raising the flaps (fig 2). The average time taken for the dissection of sentinel node was less than 10min after injection of dye. Only nodes that were stained blue were considered as sentinel i.e. even an enlarged node which did not stain blue was not considered as sentinel. sentinel nodes were resected and sent for histology in a separate container and rest of the neck nodes after conventional MRND(level I to level V) were sent for histology in a separate container (fig 3). The sentinel lymph nodes were assessed for the presence of metastatic deposits and compared with the final histology of all the neck nodes sent after neck dissection along with the final histology of the primary using Hematoxylin and eosin (H&E) staining and serial sectioning of specimens.

Statistical analysis:
Thirty patients of oral cavity cancers were were studies using the descriptive statistics. The Mc Neman's chi square test and pared T test were used to determine association between two variables. P value less than or equal to 0.05 was taken significant.

The values of the diagnostic parameters related to technique of sentinel lymph node biopsy were estimated in term of sensitivity, specificity, positive predictive value, negative predictive value, false negative rate and accuracy on the basis of

distribution of 42 cases into four categories of SLN and Neck expression patterns.

Data analysis was performed by SPSS licensed version 21.0.

Fig 1. Peri-tumoral injection of methylene blue dye.

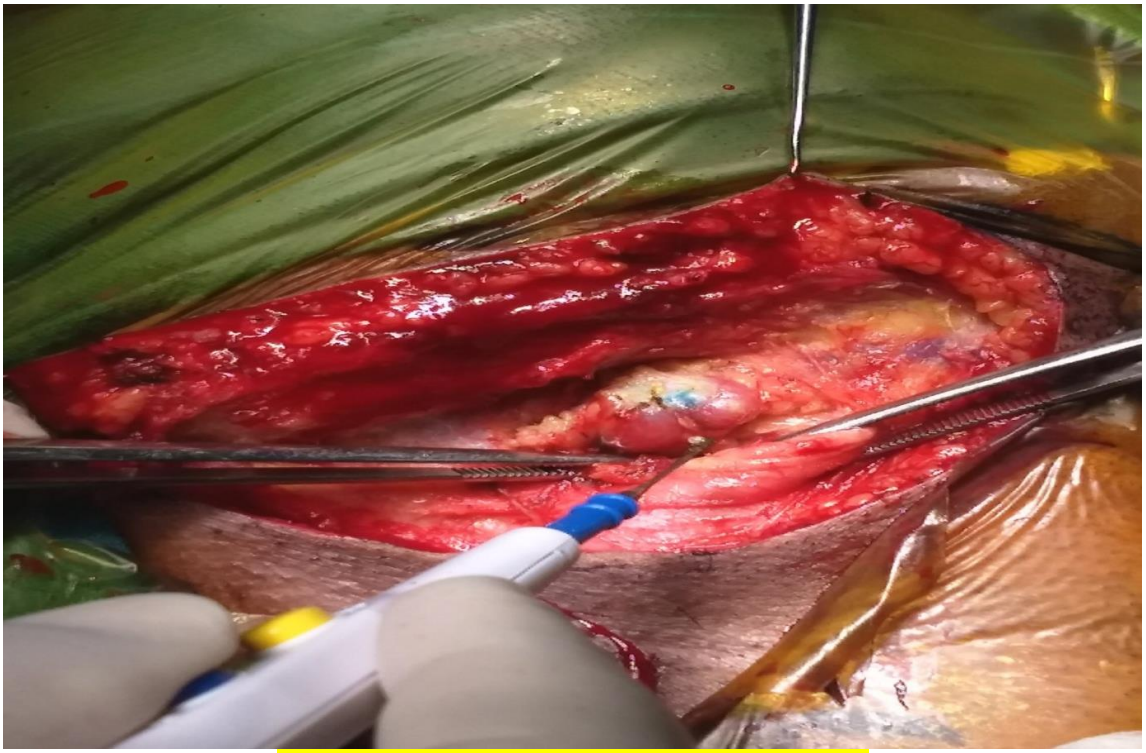
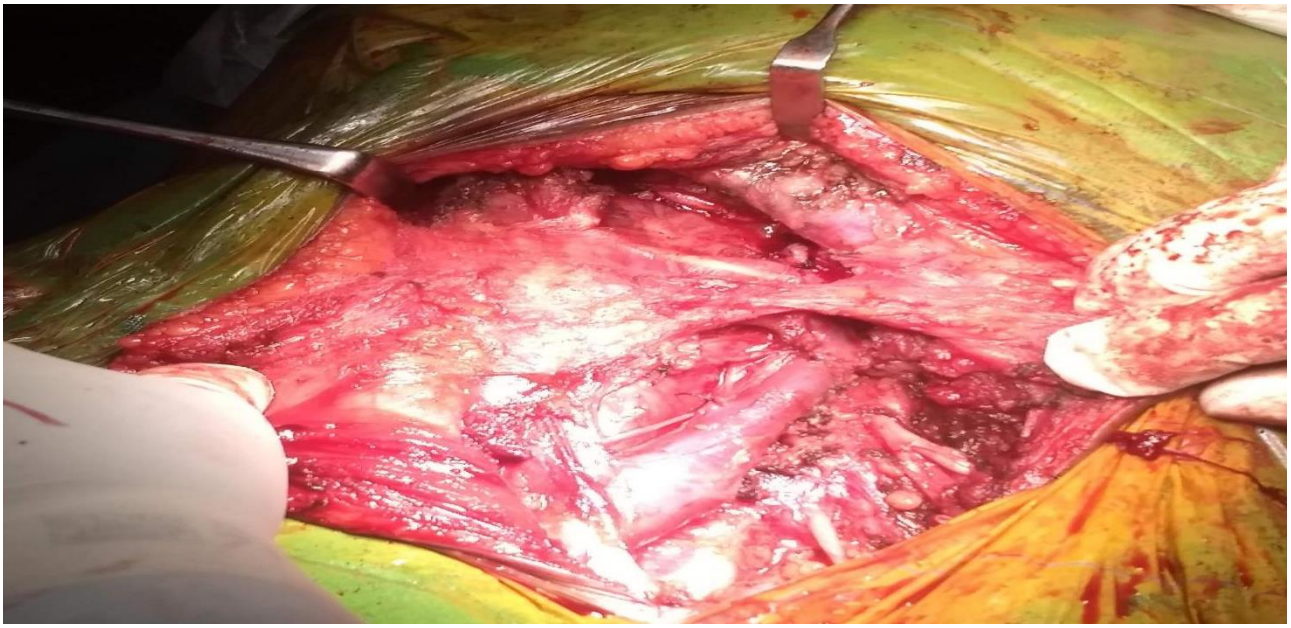


Fig2. Blue sentinel lymph node identified at level Ib.

Fig 3. Complete right modified radical neck dissection (MRND)



3. RESULTS

All 41 cases were biopsy proven oral cavity squamous cell carcinoma (OCSCC). The age of the patients ranged form 25-75 years with mean age of 43.3 years and majority of the patients were in 36-45 years(50%) age group(Table 1). Majority of the patients were male (96.6%) and the most common sub-site of malignancy was Gingivobuccal (GB) sulcus (53.33%) and tongue (26.6%). Most common clinical stage of disease was cT2(50%) and most common histopathological grade was moderately differentiated Squamous cell carcinoma (76.66%).Most common form of addiction was tobacco chewing (60%). General characteristics are given in table 2.

Table 1: Group wise age distribution

Age (years)	No. Of patients	Percentage
25-35	04	9.80%
36-45	17	41.46%
46-55	11	26.80%
56-65	07	17.07%
66-75	02	4.87%

Table 2: General characteristics

Characteristics		N(%)
Total patients	Male	40(97.56%)
	Female	01(2.44%)
	Total	41
Sub-site	Gingivobuccal (GB) sulcus	24(58.54%)
	Tongue	10(24.39%)
	Buccal mucosa	05(12.19%)
	Hard palate	01(2.44%)
	Floor of mouth	01(2.44%)

Clinical T stage	cT1	01(2.44%)
	cT2	21(51.21%)
	cT3	07(17.06%)
	cT4a	12(29.26%)
Differentiation	Well differentiated	11(26.82%)
	Moderately differentiated	30(73.17%)
	Poorly differentiated	0
Substance abuse	Tobacco chewing	28(68.29%)
	Smoking	10(24.39%)
	No addiction	03(7.31%)

The identification rate was defined as total number of cases who underwent successful sentinel lymph node biopsy divided by total number of cases in whom sentinel lymph node biopsy was attempted.

- In this study the **overall identification rate was 95.56%** (SLN couldn't be identified in one case).
- Identification rate for Gingivobuccal sulcus malignancies was 100%
- Identification rate for tongue malignancies was 90% **(SLN couldn't be identified in one carcinoma tongue case).**
- Identification rate for buccal mucosa malignancies was 100%.

Total number of lymph nodes retrieved from 41 necks were 519 out of which 75 (14.45%) nodes were sentinel nodes (blue stained). Total SLN positive for malignancy were 6 (8%). Non sentinel lymph nodes positive for malignancy were 48 (9.25%). Out of all successful SLNBs, in 9 (21.95%) cases three or more Sentinel lymph nodes were identified.

The results from each successfully identified SLN were categorised as true positive, true negative, or false negative, taking the outcome of MRND as reference standard.

- A true negative SLN was defined in this study as a negative SLN and a negative neck after MRND.
- A false negative SLN was defined in as negative SLN with positive lymph nodes in MRND.
- **A true positive SLN was defined as a positive SLN with or without a positive neck nodes after MRND.**

Based on these definitions, there were no false positive cases in this study (P-0.02). Status of SLN given in table 3.

Table 3: Status of SLN.

S.No.	Site	Status of SLN (N=41)						P-value
		Not retrieved	True positive	True negative	False negative	False positive	Total	
1.	Gingivobuccal sulcus	0	8(19.52%)	11(26.82%)	5(12.19%)	0	24	

2.	Tongue	**1(2.44%)**	0	3(7.33%)	6(14.63%)	0	10	**0.02**
3.	Buccal mucosa	0	2(4.87%)	4(9.75%)	1(2.44%)	0	7	
4.	Overall (oral cavity)	1(2.44%)	10(24.39%)	18(43.89%)	12(29.26%)	0	41	

Following parameters were calculated by applying basic descriptive statistical methods;

- Sensitivity of SLN = True positive/(true positive + false negative)
- Specificity of SLN = True negative/(true negative + false positive)
- Positive predictive value (PPV) of SLN = True positive/(true positive + false positive)
- Negative predictive value (NPV) of SLN = True negative/(true negative + false negative)
- False negative Rate (FNR) = false negative/(false negative + true positive)
- Accuracy of SLN = The sum of all true positives and true negatives, divided by the total number of patients with successfully identified SLN.

Overall **sensitivity, NPV and accuracy** was low however, site specific sensitivity, NPV and accuracy was **highest for Buccal mucosa malignancies** (50%,75% & 80% respectively) followed by Gingivobuccal sulcus malignancies (50%,63.64% & 73.35% respectively) and lowest for tongue malignancies (0%,28.57% & 28.57% respectively)with false negative rate of 100% (Table 4).

Table 4: Diagnostic test evaluation.

S.No.	**Characteristics of SLN**	**Site of primary malignancy**			
		Gingivobuccal sulcus	**Tongue**	**Buccal mucosa**	**Overall (Oral cavity)**
1	Sensitivity	61.53%	0%	66.66%	45.45%
2	Specificity	100%	100%	100%	100%
3	Positive predictive value (PPV)	100%	—	100%	100%
4	Negative predictive value (NPV)	68.75%	33.33%	80%	60%
5	False negative rate (FNR)	38.47%	100%	33.33%	54.54%
6	Accuracy	79.16%	33.33%	85.71%	68.57%
7	Identification Rate (IR)	100%	90%	100%	95.56%

Out of these 41 patients. overall sensitivity, NPV, and accuracy were modest. Site-specific analysis showed that SLNB had the best diagnostic performance in Buccal mucosa malignancies (Sensitivity: 66.66%, NPV: 80%,

Accuracy: 85.71%), followed by Gingivobuccal sulcus (Sensitivity: 61.53%, NPV: 68.75%, Accuracy: 79.16%). Diagnostic performance was poor in tongue malignancies, with 0% sensitivity, NPV of 33.33%, and high false negative rate of 100%. The overall accuracy for the cohort was 68.57%.
4 cases (9.75%) had perineural invasion (PNI) and 5 cases (12.19%) had lymphovascular invasion (LVI). None of the patients had extra nodal extension (ENE).

4. DISCUSSION

SLNB is not the standard of care yet in oral cavity malignancies.[24] To our knowledge, The largest meta-analysis done on the diagnostic efficacy of sentinel lymph node biopsy (SLNB) in oral squamous cell carcinoma included 66 studies comprising 3566 patients with cT1-2N0 oral squamous cell carcinoma (OSCC).This study have successfully demonstrated the feasibility of SLNB in early stage OSCC with a pooled identification rate (IR) of 96.3%, sensitivity of 87%, NPV of 94%. In this meta analysis, out of 66 studies only in single study blue dye alone was used for SLN, in rest of the studies various combinations of blue dye, radionuclide and indocyanine green dye was used as SLN tracer and SLN localization was done using combinations of lymphocintigraphy, Gamma probe and Spectrometry.[26]

In our study we used only methylene blue dye as SLN tracer and visual perception of blue stained nodes as SLN localization as it is cheap and easily available and doesn't require any complex facility.

SLNB is technically demanding procedure as it requires expertise and facilities like lymphocintigraphy, gamma camera etc. however, the advantage of SLNB is that it is less morbid with comparable 2-years survival rates in early disease.[23]

In our study, the identification rate (IR) using methylene blue dye alone was 97.56% (40/41 attempted), which is comparable to other studies. Site-specific IR was 100% each for Gingivobuccal sulcus malignancies and Buccal mucosa malignancies; however, for tongue malignancies IR was 90%. The average time taken for SLN localization after the injection of dye was less than 10 minutes, and the most common site for SLN was level I echelon ($P<0.05$). Those studies in which a radio tracer or dual technique was used for sentinel lymph node mapping had better identification rates than methylene blue dye alone (Table 5). However, for Gingivobuccal sulcus and Buccal mucosa malignancies, the IR using methylene blue dye alone in our study was comparable with radionuclide tracers.

One important finding in our study was that SLNB in tongue malignancies had very poor sensitivity (0%) and extremely high false negative rate (100%) (Table 4). Several studies have suggested that squamous cell carcinoma of the tongue is the more common site for skip metastasis. There is lymphatic crossover between the two sides of the tongue, and being a highly active organ during mastication and swallowing, it favors both early and rapid lymphatic spread directly to lower nodal levels, skipping the upper neck nodes 33. Though the number of patients in our study was small, this pattern is clinically significant.

In contrast, Buccal mucosa and Gingivobuccal sulcus malignancies demonstrated better diagnostic performance with sensitivities of 66.66% and 61.53%, and accuracies of 85.71% and 79.16%, respectively. These findings support the potential use of SLNB using methylene blue dye alone in selected sub-sites of oral cavity squamous cell carcinoma in resource-limited settings.

A prospective study done by Bell et.al. Demonstrated that SLNB done using routine hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) accurately predict the nodal stage (N stage) in early oral cavity SCC with negative predictive value (NPV) of 96% and serial sectioning might not be necessary.[34] however, IHC is not feasible for on table diagnosis during SLNB study.

There is limitations of using methylene blue dye alone for SLNB studies. SLN could only be visualised after raising the conventional MRND flaps which could be easily avoided by mapping the SLN prior to incision using lymphocintigraphy and hence SLNB could be done using the small neck incision before converting it to conventional MRND incisions. And also contralateral neck could be assessed accurately which is not possible with blue dye alone. Therefore larger multi institutional trials would be needed for better understanding and to address these drawbacks of methylene blue dye alone where lymphocintigraphy is not widely available.

Table 5: comparison of identification rate with different tracers.

S.No.	Author	Year	Design	Sample size	Site	Tracer used	Ident Rate
1.	Hoft& et.al.[10]	2004	Prospective	20	Oral cavity SCC	Radionuclide	20/20 (100%)
2.	Hart et.al.[27]	2005	Prospective	12	Oral cavity SCC	Radionuclide	12/12 (100%)
3.	Ramamurthy et.al.[28]	2014	Prospective	32	Oral cavity SCC	Methylene blue	29/32 (90.62%)
4.	Sagheb et.al[29]	2015	Prospective	10	Tongue	Tec99 Colloid	10/10 (100%)
5.	Chaturvedi et.al.[30]	2015	Prospective	53	Oral cavity SCC	Radionuclide	51/53 (96.22%)
6.	Sieira et.al[31]	2015	Retrospective	42	Oral cavity SCC	Radionuclide	42/42 (100%)
7.	Schilling et.al.[32]	2015	Prospective	415	Oral cavity SCC	Radionuclide +methylene blue	415/415 (100%)

5. CONCLUSION

Methylene blue dye alone can be used in Gingivobuccal sulcus and Buccal mucosa squamous cell carcinoma with acceptable accuracy (79.16–85.71%) and negative predictive value (68.75–80%). However, in tongue malignancies, this technique is not reliable due to zero sensitivity, poor negative predictive value (33.33%), and a false negative rate of 100%. While the results suggest feasibility in select oral subsites, larger multi-institutional trials are needed to validate the utility of methylene blue dye alone for SLNB, particularly in regions lacking access to lymphoscintigraphy.

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