Original Article

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Dentistry Section

Effectiveness of Lymph Node Revealing Solution in Estimation of Lymph Nodes Yield in Radical Specimen of Oral Squamous Cell Carcinoma: A Preliminary Histomorphometric Observational Study

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ABSTRACT

Introduction: Oral cancer poses a serious health challenge globally, particularly for nations undergoing economic transition. The number and size of lymph nodes obtained, along with nodal involvements, are crucial for proper diagnosis, treatment, and prognosis.

Aim: To assess the number of lymph nodes obtained before and after treating the specimens with the Lymph Node Revealing Solution (LNRS) along with staining characteristics.

Materials and Methods: The present prospective observational study was conducted at the Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, over a period of six months from January 2023 to June 2023 in a tertiary oral healthcare centre in Chennai. Six histologically confirmed Oral Squamous Cell Carcinoma (OSCC) cases with neck dissection were included in the study, involving 32 lymph node levels. Lymph nodes were grossed using both routine procedures of palpation and visualisation and after treatment with LNRS for two days. The number of nodes obtained through both methods was compared along with the staining characteristics. The data were analysed using Statistical Package for Social Sciences (SPSS) software version 26.0. Chi-square and paired t-tests were used to compare the two groups, and any value less than or equal to 0.05 was considered statistically significant.

Results: Out of 134 lymph nodes, 21 were positive with conventional fixation, whereas after the application of LNRS, an additional 41 lymph nodes were retrieved. Two nodes showed metastatic deposits; fortunately, the additional positive nodes did not affect the staging. No statistically significant difference was found before and after immersion in LNRS regarding staining characteristics. The mean area of the yielded nodes was 84.71±4.85 mm^2. There was a statistically significant difference between the size of lymph nodes between the manual grossing method and after immersion in LNRS (p-value <0.001).

Conclusion: The study confirmed that the LNRS technique identified very small lymph nodes in oral cancer patients, which may contain metastatic deposits. This might change the stage of the disease and influence the mode of treatment.

Keywords: Mean area, Metastatic deposits, Node size, Oral cancer

INTRODUCTION

Oral Squamous Cell Carcinoma (OSCC) constitutes 90% of all oral cancers [1,2]. According to the Indian Cancer Society, the country records more than 1,00,000 cases of oral cavity cancers every year [3]. Oral cancer poses a serious health challenge to the nations undergoing economic transition [4,5]. In India, around 77,000 new cases and 52,000 deaths are reported annually, which is approximately one-fourth of global incidences [6]. The prognosis of OSCC depends on various factors, including age, immune response, gender, site, habits, and genetic mutations, reflecting its multifactorial behaviour [7]. Along with these, the management and prognosis of OSCC rely on tumour staging, which further includes tumour size, nodal involvement, and distant metastasis [8,9].

According to American Joint Committee on Cancer (AJCC) 8th edition, the number and size of lymph nodes obtained and nodal involvement are crucial for proper diagnosis, treatment, and prognosis [10]. Drawbacks in the manual grossing of lymph node specimens are time-consuming, missed small nodes, and a lower count following radiation; thus, micrometastasis could be missed in manual grossing and may affect staging and devising adjuvant therapy. The majority of OSCC cases reported to have regional cervical lymph node metastasis. The absence of specific molecular markers [11] and biomarkers for OSCC in diagnosis and treatment also have a crippling effect on the prognosis [12]. Hence, the present study was designed to utilise LNRS to evaluate its effectiveness in cases with OSCC. The objectives of the present study were to prepare an LNRS, to assess its effectiveness regarding staining characteristics and readability, and to assess the number of lymph nodes obtained before and after treating the specimens with the prepared LNRS.

MATERIALS AND METHODS

The present prospective observational study was conducted at the Department of Oral Pathology and Microbiology, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, from January 2023 to June 2023, after obtaining clearance from the Institutional Human Ethical Committee (IHEC/SDC/FACULTY/23/OPATH/240).

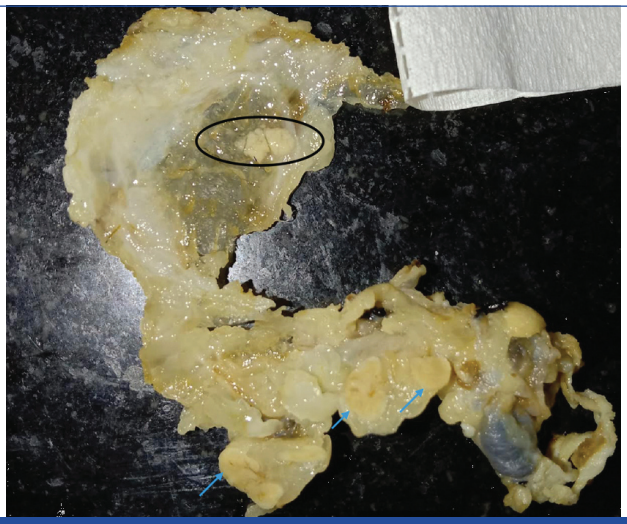
Inclusion criteria: Included six consecutive excised radical specimens of histologically confirmed OSCC cases. The radical specimen included wide local excision with selective/modified lymph node dissection.

Exclusion criteria: Recurrent OSCC cases and cases with a previous history of radiotherapy or chemotherapy were excluded from the study.

Study Procedure

The margins were obtained for clearance intraoperatively on frozen, and the remaining specimen was fixed in 10% neutral buffered formalin. Lymph nodes were grossed using the routine procedure of palpation and visualisation. The lymph nodes were processed, recorded, and sectioned. The remaining specimen was immersed in the LNRS and grossed after two days. The solution was changed every day. The LNRS (per 1 litre) was prepared by adding 650 mL of ethyl alcohol, 200 mL of Diethyl ether, and 50 mL of glacial acetic acid to 100 mL of buffered formalin.

The remaining fibrofatty tissue was re-examined after two days for additional lymph nodes. The number of newly retrieved nodes was recorded. The previously dissected nodes were not counted [Table/Fig-1]. The new nodes were thereafter processed, sectioned, and stained. The data were tabulated for the number of nodes obtained through routine manual grossing versus after LNRS, number of positive nodes obtained through routine manual grossing versus after LNRS, and comparison of staining characteristics between the two groups. All the slides were compared for nuclear staining, cytoplasmic staining, cellular morphology, and uniformity of the staining, in a semi-qualitative manner, and classified into poor (score 0), intermediate (score 1), and good (score 3) as previously mentioned [13]. For assessing the cellular and nuclear morphology, cell outline, cytosolic features, nuclear outline, and nucleolar characteristics were evaluated at high power magnification in all the slides. The size of the lymph nodes was estimated on the mechanical stage of the light microscope, while the mean area was estimated using Image J software [14].



[Table/Fig-1]: Photograph showing the gross appearance of an included lymph node level. Circle- newly identified nodes after clearing of fat and arrows previously cut and processed lymph nodes.

STATISTICAL ANALYSIS

The data were entered in a Microsoft excel spreadsheet (2021). SPSS software (version 26.0, IBM Corp., Armonk, New York) was used for analysis. Chi-square and paired t-tests were used to compare the two groups, and any value less than or equal to 0.05 was considered statistically significant.

RESULTS

Demographic profile: Six patients were included with a male-to-female ratio of 5:1 and ages ranging from 37 to 57 years. Three cases were from the tongue, two cases from the lower posterior gingivobuccal sulcus, and the remaining case involved the upper right maxillary tuberosity. Four cases were graded as Well-differentiated Squamous Cell Carcinoma (WDSCC), and the other two cases were Moderately-differentiated Squamous Cell Carcinoma (MDSCC). Modified Radical Neck Dissection (MRND) was performed for three cases, and Selective Neck Dissection (SND) for the other three cases.

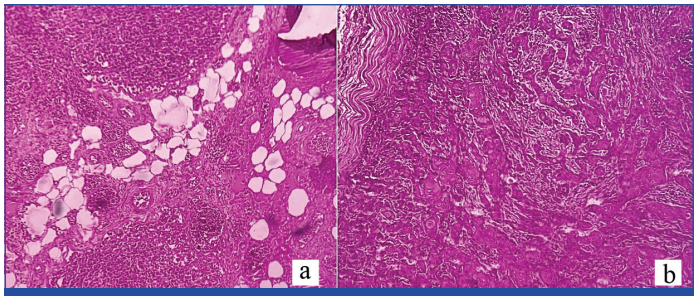
Comparison of node yield by routine manual grossing vs LNRS: Routine manual grossing yielded 134 lymph nodes from 32 lymph node levels, out of which 21 were positive. After immersion in LNRS, an additional 41 lymph nodes were retrieved. Fortunately, two nodes showed metastases, but this did not upstage the tumour staging. The clinicopathological features and nodal yield are detailed in [Table/Fig-2].

Cases	Age (in years)	Gender	Histopathology diagnosis and staging	Level of lymph nodes	No. of lymph nodes without the solution (Positive lymph nodes)	No. of lymph nodes after the solution (Positive lymph nodes)
1	47	M	WDSCC, Stage IVa	L IA	5	0
				Right L IB	0	2
				Right L IIA	2	0
				Right L IIB	7	2
				Right L III	8	1
				Right L IV	9	0
2	37	M	WDSCC, Stage IVa	L IA	19	2
				Left L IB	12 (1)	2 (1)
				Left L IIA	5	0
				Left L IIB	3	3
				Left L III	6	0
				Left L IV	1	3
				Left L VA	9	0
				Left L VB	6	0
3	47	F	MDSCC, Stage IVa	LIA	2 (2)	0
				Left L IB	3 (2)	0
				Left L II	4 (1)	3
				Left L III	5 (4)	1
				Left L IV	5 (2)	0
				Left L V	3	0
4	52	M	MDSCC, Stage IVa	L IA	3	1
				Right L IB	4 (3)	4 (1)
				Right L II	10 (1)	9

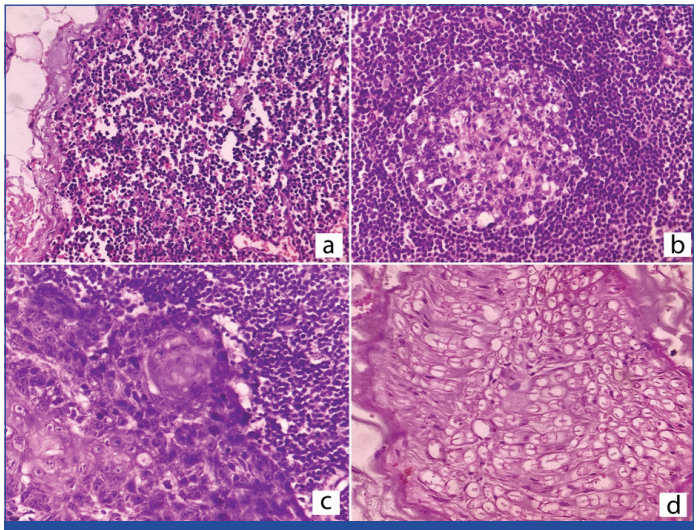
				Right L III	22 (0)	5
5	45	M	WDSCC, Stage IVa	L IA	3	0
				Left IB	6 (2)	0
				Left IIA	10	1
				Left IIB	3 (1)	0
				Left III	5	0
6	57	M	WDSCC, Stage IVa	L IA	5	1
				Left L IB	8 (2)	0
				Left L II	2	0
				Left L III	0	1

[Table/Fig-2]: Clinicopathological features and nodal yield using both routine grossing method and Lymph Node Revealing Solution (LNRS); WDSCC: Well-differentiated squamous cell carcinoma; MDSCC: Moderately-differentiated squamous cell carcinoma. Yellow: Newly yielded lymph nodes, red: New lymph node with metastatic deposits. ()- depict number of positive nodes.

Comparison of staining characteristics: All the sections after the application of LNRS showed good nuclear (p-value=0.268) and cytoplasmic staining (p-value=0.595). No statistically significant difference was found before and after immersion in LNRS. Similarly, no difference was found in the cellular morphology and uniformity of the staining (p>0.05) [Table/Fig-3a,b,4a-d].

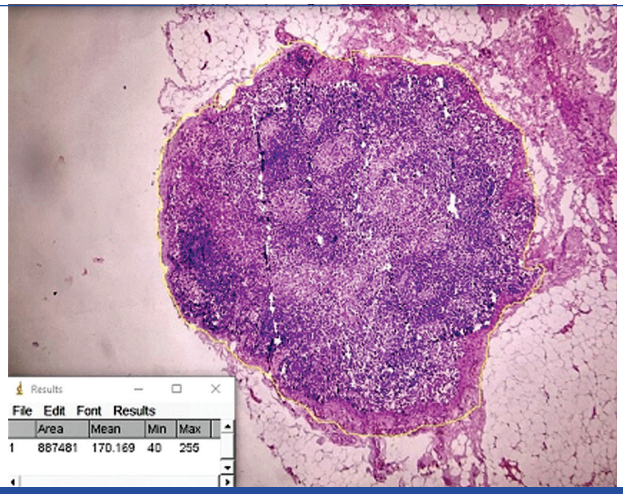


[Table/Fig-3]: a) Photomicrograph of newly identified node demonstrating acceptable staining and maintenance of architecture (H&E, 100X); and b) positive lymph node with metastasis from OSCC effacing the normal architecture of LN (H&E, 100X).



[Table/Fig-4]: Photomicrograph of an H&E-stained section showing nuclear and cytosolic characteristics of cells and tissues of newly identified lymph nodes and structures in extra-nodal areas: a) lymphocytes and lymph node capsule; b) germinal centre; c) cellular and nuclear details of metastatic deposit in lymph node; and d) a nerve bundle in the vicinity, extra-nodally, (400X).

Mean area and size of newly yielded nodes: Additionally, the mean area of the yielded nodes after treating with LNRS was calculated using Image J software. The mean area was 84.71±4.85 mm^2 (±standard deviation) [Table/Fig-5]. The mean area of the lymph nodes prior to LNRS could not be estimated as some large lymph nodes were cut into two and processed as two blocks. However, when the sizes of the lymph nodes were compared, there was a statistically significant difference between the size of the lymph node between the manual grossing method and after immersion in LNRS (p-value <0.001). The mean size of the 134 lymph nodes yielded by the manual visual and palpation method was 13.01±8.16 mm, while after immersing in LNRS, it was significantly lesser (1.99±1.001 mm; p<0.001). The detailed comparative results of staining characteristics, mean area, and size of lymph nodes before and after immersion in LNRS is shown in [Table/Fig-6].



[Table/Fig-5]: Photomicrograph showing whole slide image of a lymph node yielded after immersion in LNRS demonstrating estimation of mean area using Image J software (H&E, 40X).

DISCUSSION

Postoperative adjuvant chemo and radiotherapy, based on tumour staging and histopathological diagnosis, play a crucial role in the patient's recovery [15,16]. It has been reported that the five-year survival rate of cases with lymph node metastasis (54%) is significantly lower than those without lymph node metastasis (87%) [17]. Surgical resection is the conventional treatment option for any OSCC case [18]. Hence, accurate and total lymph node examinations are essential for appropriate management. According to the AJCC 8th edition, the ideal number of lymph nodes in SND and Radical/MRND (RND/MRND) is 10 or more and 15 or more, respectively [10]. It is not always easy to harvest the required number, especially in patients who may have received neoadjuvant therapy, an increasingly common treatment. The use of neoadjuvant therapy is known to further decrease the number and size of identifiable lymph nodes within specimens [19].

The present study harvested new small lymph nodes after immersing them in LNRS. Similar studies were conducted previously in gastric cancer and colon cancer using LNRS, and it was found that the number of lymph nodes increased by 53.0% with LNRS, and the number of metastatic lymph nodes was 28.9% higher than that of the conventional process [19,20]. This is critically important, as node-positive patients (pN1) are considered for adjuvant chemotherapy, whereas node-negative patients (pN0) may not be. The LNRS yields much smaller nodes, which may be missed by manual examination and sometimes are metastatic in nature.

Parameters	Before immersion in LNRS (n=134)			After immersion in LNRS (n=41)			p-value
	Poor (Score 0)	Intermediate (Score 1)	Good (Score 2)	Poor (Score 0)	Intermediate (Score 1)	Good (Score 2)	
Nuclear staining	8 (5.9%)	34 (25.4%)	92 (68.7%)	2 (4.88%)	9 (21.95%)	30 (73.17%)	0.858#
Cytoplasmic staining	7 (5.22%)	30 (22.38%)	97 (72.4%)	3 (7.32%)	7 (17.07%)	31 (75.61%)	0.703#
Cellular morphology	4 (2.98%)	18 (13.43%)	112 (83.59%)	3 (7.32%)	5 (12.2%)	33 (80.48%)	0.462#
Uniformity of staining	6 (4.48%)	19 (14.18%)	109 (81.34%)	0	5 (12.2%)	36 (87.80%)	0.353#
Mean area	NA			84.71±4.85			-
Mean size	13.01±8.16 mm			1.99±1.001 mm			0.001*

[Table/Fig-6]: Comparative evaluation of the staining characteristics, mean area and size of lymph nodes before and after immersion in LNRS.
LNRS: Lymph node revealing solution; NA: Not assessed; *significant, #not-significant

The widely used fixative for routine pathological specimens has disadvantages, such as a slow rate of fixation [21]. In some instances, even after 24 hours, fixation does not provide sufficient firmness to the tissues, especially in cases of large specimens and cystic lesions, resulting in delayed diagnosis [22]. Furthermore, longer exposure of tissues to formaldehyde can have adverse effects on many antigens [23]. Therefore, the combination of various fixatives can mitigate these adverse effects and improve properties. Glacial acetic acid, ethanol, water, and formalin offer advantages over other revealing solutions as they are safe, cheap, easy to use, and relatively quick. The utilisation of LNRS offers many advantages, including lesser time during grossing, an inexpensive technique for detecting very small lymph nodes, ease of working, enhanced visibility, no need for an attentive process, the ability to use immunostains, and clear visibility of the lymph nodes [24].

Simental AA Jr et al., found cervical metastasis from squamous cell carcinoma of the maxillary alveolus and hard palate in 34.6% of patients [25]. A high incidence (20-30%) of cervical metastasis of cancer in the tongue/floor of mouth has also been well studied and reported [26]. Therefore, missing a single positive lymph node in an N0 patient may upstage the tumour staging to N1, which has a significant impact on the outcome. Fortunately, the identification of new positive nodes did not upstage pTNM in any of the present cases.

It has been repeatedly proven and reported that the presence of extranodal extension is proportionately correlated with distant metastasis, locoregional recurrences, and difficulty in obtaining clear margins [27]. Missing a single lymph node with extranodal extension directly upgrades the tumour staging to N3b. Therefore, obtaining more lymph nodes may benefit patients as it allows for accurate cancer staging and appropriate use of adjuvant chemotherapy for node-positive patients. The present study included 32 lymph node levels from six patients with OSCC. Although the results and findings are preliminary, they provide significant insight into the usage of LNRS in neck dissections of head and neck cancers. Further studies with a larger sample size are required to confirm the findings.

Limitation(s)

While the results obtained were promising, there were a few limitations that need to be addressed. Firstly, the sample size was small, with only six patients prospectively recruited. However, the number of lymph node levels was adequate to provide a baseline data for future studies with a larger sample size. Secondly, the mean area of larger nodes could not be assessed for comparison due to the fact that some nodes were too large to be processed as a single block and were thus bisected for further processing.

CONCLUSION(S)

The study confirmed that the LNRS technique identified very small lymph nodes that may contain metastatic deposits. This could potentially change the stage of the disease and influence the mode of treatment. Prospective studies with larger sample sizes are warranted. The authors believe that LNRS does not change the architecture of the nodes and yields a greater number, including the smallest nodes, thus it may be used in routine practice.

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