Original Article

A Study On The Detection Of Micrometastases In The Cervical Lymph Nodes Of Oral Squamous Cell Carcinomas By Serial Sectioning

Pathology Section

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ABSTRACT

Background: Metastatic deposits in the regional lymph nodes have always been a subject of great interest in oncology. There is a need for the detection of micrometastases in the lymph nodes in oral carcinomas by serial sectioning, so that any missed tumour cells in the routine single sectioning technique could be detected by this. The present study was aimed at the detection of micrometastases in the cervical lymph nodes in oral squamous cell carcinomas- by serial sectioning. **Materials and Methods:** This study was done on 16 cases with 119 lymph nodes which measured 1cm or less in greatest diameter, by a single section routine method and this was compared with serial sectioning at 100µm intervals. All the 2269 sections were stained with the routine Hematoxylin and Eosin staining for the detection of micrometastases. **Results:** The detection of micrometastases by the serial sectioning method was 2.03%. The percentage was the same for both the one section and the serial sectioning methods. **Conclusion:** The serial sectioning of the lymph nodes did not reveal any other detection than that was revealed by the one section method. But definitely, the serial sectioning method appears to be the best feasible method to evaluate micrometastases.

Key Words: Carcinoma, Lymph nodes, Micrometastases

INTRODUCTION

Metastasis, the spread of tumours, is one of the important characteristics of malignancy. Regional metastasis to the lymph nodes, which has a definite bearing on prognosis, is a widely debated topic and controversies exist with regards to its assessment and the line of treatment in oral squamous cell carcinomas. Regional metastasis to the cervical lymph nodes also reflects this confusing and conflicting scenario.

Accessing the lymph nodes in the absence of clinical enlargement is challenging. Occult metastases, which is otherwise known as micrometastases, are the microscopic foci of the metastasis in the nodes, which do not clinically show detectable enlargement. Specific limitations in the histological evaluation of nodes are dictated by the variables of the lymph nodes, the size of the metastatic lesion, as well as the method and number of the sections taken from the node.

Studies on micrometastasis have been done in gastric cancers [1], breast cancers [2], colorectal cancers [3] and in the carcinomas of other sites and their results have shown that the detection of micrometastasis has a significant difference in the recurrence and survival rate. Very few published data are present on the detection of micrometastasis in the cervical lymph nodes of oral squamous cell carcinomas [4] by serial sectioning.

This study was planned to evaluate the micrometastases in the cervical lymph nodes, which were equal to or smaller than 1cm, by serial sectioning of 100µm and to compare them with those which were detected by the routine one section technique in both node positive and node negative cases of oral squamous cell carcinomas.

MATERIALS AND METHODS

This study involved the retrieval of the cervical lymph nodes from formalin fixed, post operative specimens of the oral squamous cell carcinomas of 16 patients.

Lymph nodes which are equal to/or less than 1cm at their greatest diameter, were taken from the cervical region of Radical Neck Dissection (RND) specimens with the diagnosis of oral squamous cell carcinoma of different histological grades. Both node negative and node positive cases were considered for the study. No levels of the lymph nodes were considered for the study, as they were taken from the archives of the department.

The present study was carried out by harvesting a total number of 119 lymph nodes from the RND specimens of 16 patients, in which 3 were node positive and the other 13 were node negative cases. Each of these lymph nodes which were obtained, were evaluated both by the routine one section method and by serial sectioning at every 100µm and a total of 2269 sections were evaluated. The usefulness of serial sectioning in detecting micrometastases, when compared to the one section method, was evaluated. Each lymph node was cut at its greatest diameter.

The two halves of the lymph node were processed routinely and they were embedded in paraffin wax separately. The lymph nodes were then sectioned for obtaining single sections and then the whole lymph node was cut at every 100µm till it was exhausted. All the sections were stained by the routine H and E staining and they were checked for micrometastases by oral pathologists.

RESULTS

[Table/Fig 1]: The ages ranged from the third to the seventh decade. Out of 16 patients in the study, 5 were females and 11 were males. Seven were of the well differentiated, eight were of the moderately to well differentiated and one was of the moderately differentiated pathological types (grades?).

[Table/Fig 2]: Out of the 119 lymph nodes which were examined, 20 were of 0.6cm and 173 sections were made from it, 19 were of 0.7cm and 395sections were made from it and 42 were of 1.0cm and 850 sections were made from it.

Case no	Age	Sex	Location of the lesion	TNM staging	Histologic grading
1	45	M	Left buccal mucosa	T3 N2 Mo	Moderate
2	63	M	Right alveolus	T3 N1 M0	Moderate to well
3	35	M	Left angle of the mouth to retromolar area	T3 N3 M0	Well
4	35	F	Left lower region from 34 - 38	T3 N1 M0	Well
5	52	M	Left alveolus, midline to 3rd molar region	T2 N1 M0	Well
6	40	F	Lower lip	T3 N1 M0	Moderate to well
7	45	M	Angle of mandible to lobule of ear	T3 N2 M0	Moderate to well
8	25	M	Left retromolar region	T2 N1 M0	Moderate to well
9	32	F	Anterior 2/3 to base of tongue	T3 N2 M0	Well
10	50	M	Left alveolus and retromolar region	T2 N2 M0	Moderate to well
11	48	M	Left upper retromolar region	T2 N1 M0	Moderate to well
12	43	M	Left alveolus	T3 N2 M0	Well
13	56	F	Right retromolar region	T3 N2 M0	Moderate to well
14	49	M	Lower lip	T2 N1 M0	Well
15	39	M	Right alveolus and retromolar region	T2 N2 M0	Moderate well
16	64	F	Right buccal mucosa	T2 N2 M0	Well

[Table/Fig 1]: Clinical details, staging and grading of selected cases

Micrometastases was detected in only two nodes of size- 1cm of a node negative case by the one section method and in the same case, by serial sectioning. No other lymph nodes by the one section method or by serial sectioning proved to be positive for micrometastases.

When comparison was made between the one section method and the serial sectioning method, identical results in detecting micrometastases were observed in both the methods, i.e. 2 positive lymph nodes out of 119 lymph nodes (2.03%) [Table/Fig 3].

DISCUSSION

Metastatic deposits in the regional lymph nodes have always been a subject of great interest in oncology. The term 'micrometastases' itself has been defined by different authors differently. Black et al [5] defined micrometastases as tumours occupying <20% of the sectioned area. De mascarel et al [3] defined micrometastases as metastatic deposits which measured less than 0.5mm in diameter. Tumour deposits within the lymph nodes were classified and staged according to the revised guidelines which were set by the International Union against Cancer (UICC). According to this classification system, the metastases measuring 0.2mm to 0.2cm were considered as micrometastases. In our study, we considered the 0.2mm – 0.2cm deposits of malignant cells in the lymph nodes as micrometastases. The isolated tumour cells (ITC) are the individual malignant cells which are seen in lymph nodes, which can be detected only by immunohistochemistry.

Many authors have carried out studies on the evaluation of micrometastases of the lymph nodes in gastric cancers (Mahera Y et al [6], Ishii et al [7]), colorectal carcinomas (Davidson BR et al [8], Isozaki et al [9]), breast cancers (Tsuchiya et al [10], Harry D Bear et al [11], Hainsworth et al [12]), cervical cancers (Okamoto et al [13]), vulval cancers (Narayansingh G V et al [14]) and oesophageal carcinomas (Natsugoe S et al [15], Stephen McGrath et al [16]). There are very few published articles on oral squamous cell carcinomas (Joes E et al [17], Ferlito A et al [4].

Case no.	No. of LN evaluated	Distribution of LN according to their sizes	No. of sections evaluated	Metastases in 1 section method	Metastases in serial section method
1	11	1.0cm – 4 LN 0.8cm – 5 LN 0.6cm – 2 LN	76 96 20 = 192	Nil	Nil
2	8	1.0cm – 2LN 0.7cm – 6LN	56 116 = 172	Nil	Nil
3	11	1.0cm – 6LN 0.8cm – 5LN	164 128 = 292	Nil	Nil
4	2	1.0cm – 1LN 0.6cm – 1LN	16 20 = 36	Nil	Nil
5	8	1.0cm – 3LN 0.8cm – 1LN 0.6cm – 4LN	36 4 28 = 68	Nil	Nil
6	7	1.0cm – 4LN 0.7cm – 3LN	55 30 = 85	2	3
7	9	1.0cm – 4 LN 0.8cm – 2 LN 0.6cm – 3 LN	44 28 16 = 88	Nil	Nil
8	9	1.0cm – 2 LN 0.8cm – 6 LN 0.6cm – 1 LN	50 155 24 = 229	Nil	Nil
9	4	1.0cm – 1LN 0.8cm – 3LN	16 100 = 116	Nil	Nil
10	8	1.0cm – 2LN 0.7cm – 6LN	45 164 = 209	Nil	Nil
11	7	1.0cm – 3LN 0.7cm – 4LN	84 85 = 169	Nil	Nil
12	7	1.0cm – 3LN 0.8cm – 4LN	80 76 = 156	Nil	Nil
13	6	0.8cm – 3LN 0.6cm – 3LN	64 20 = 84	Nil	Nil
14	9	1.0cm – 2 LN 0.8cm – 6 LN 0.6cm – 1 LN	40 124 24 = 188	Nil	Nil
15	6	1.0cm – 3LN 0.8cm – 3LN	48 76 = 124	Nil	Nil
16	7	1.0cm – 2LN 0.6cm – 5LN	40 21 = 61	Nil	Nil
Total	119	119	2269		

[Table/Fig 2]: Details of microscopic evaluation of lymph nodes

Node positive cases		Node negative cases	
No. of cases with No. of nodes in parenthesis	Micrometas-tases with No. of nodes in parenthesis	No. of cases with No. of nodes in parenthesis	Micrometas-tases with No. of nodes in parenthesis
3 (18)	Nil	13 (101)	2 (2)

[Table/Fig 3]: Micrometastases in node positive and node negative cases

Different methods were employed to detect micrometastases, in which serial sectioning was the most commonly used method. Many controversies exist on whether serial sectioning is useful or not.

In breast cancers, Nasser et al [18] employed serial sectioning at 150μm intervals and he detected a 31% significance rate. M C

Guckin et al [19] did serial sectioning at 100µm intervals and detected a 25% significance rate. Thus, it can be seen that the detection rate varied from 9 – 33%, and that it had no correlation with the serial sectioning intervals. The present study detected micrometastases in only two nodes out of 119 nodes of the 16 cases, of which 96 were from node negative and 16 were from node positive cases.

The detection rate was 2.03% and when compared to the one section method, the results were identical. The rate of detection of the micrometastases in breast cancer varied from 9 – 33%, and even recent studies by Jose E et al [17] 2003, showed that the detection of micrometastases by serial sectioning in oral cancer is beneficial.

However, there are no studies on oral squamous call carcinomas, where they selected only lymph nodes of size less than 1cm and did serial sectioning at 100µm intervals. The lesser value of detection could be attributed to a limited sample size of 119 lymph nodes of 16 cases.

Other researchers used a much larger sample (88 – 3349). The sample size had to be curtailed because of the constraint of time and due to the selection of lymph nodes of size 1cm or lesser than that. Other studies had included all the lymph nodes of all sizes and so, the probability of detecting metastases in larger lymph nodes was higher.

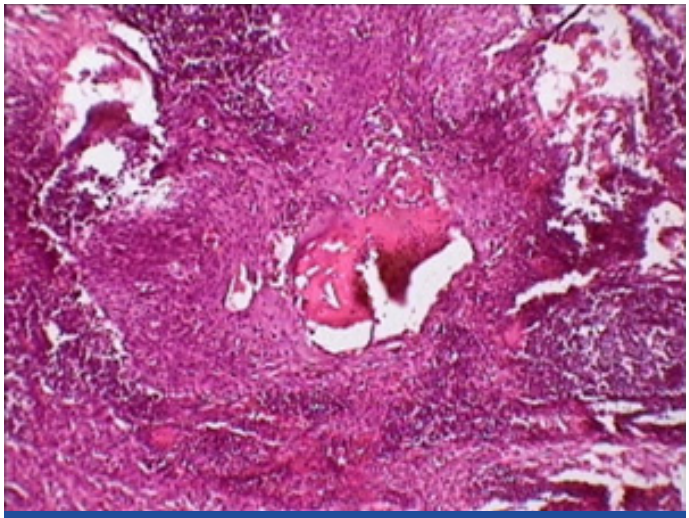
The selection of lymph nodes of size 1cm or less in this study had clinical importance, as they usually escape clinical detection. Therefore, the evaluation was restricted to lymph nodes which were sized 1cm or less, so that the detection of micrometastases in these could be of clinical significance.

Due to limited positivity and identical results, no definite comment can be made about the advantages of serial sectioning over the one section method. However, the opinions vary between some researchers, (Nasser et al [18], Jose E et al [17]) claiming the superiority of serial sectioning over the one section method, while others (Wilkinson et al [20], Hartviet et al [21], De Mascarel et al [3]) claim that it is not so.

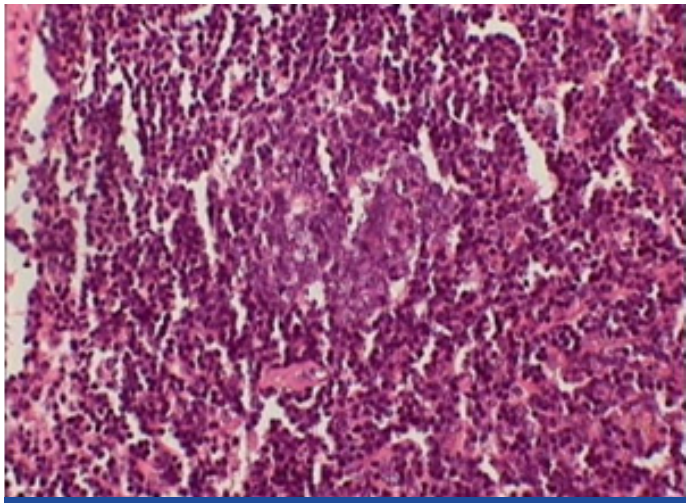
In the present study, we faced some problems in the detection of the malignant cells within the parenchyma of the lymph node. Great difficulty was experienced in differentiating a single cell or a group of malignant cells which invaded the lymph nodes in the H and E sections, because the endothelial cells and the histiocytes closely resembled the malignant squamous cells [Table/Fig 4, 5, and 6]. However, the comparison of these cells with the endothelial cells in forming a lumen elsewhere in the same section and the absence of hyperchromatism, great variation in size, shape and keratin formation, helped to differentiate these from the malignant squamous cells.

This difficulty was reported by Nasser et al [18], where there was confusion between the epithelial cells, the benign nevus cell nests, the sinus histiocytes laden with keratinous debris and the keratin positive reticulum cells of the lymph nodes. Therefore, it might be possible that in this study, a few foci of malignant squamous cells may have been missed, though such sections were examined and discussed with qualified oral pathologists for a consensus.

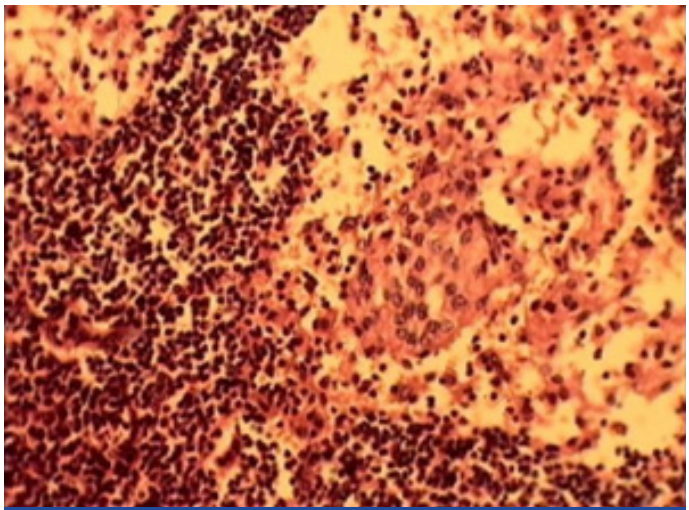
The impact of micrometastases on prognosis has been an issue of great debate. It is a common experience that node negative patients come back with metastases after few years of therapy and that in these patients, the cause is often undiagnosed, untreated



[Table/Fig: 4]: H&E 10X showing malignant cell island



[Table/Fig: 5]: H&E 40X endothelial cells mimicking the malignant cells



[Table/Fig: 6]: H&E 40X endothelial cells mimicking the malignant cells

micrometastases of the lymph nodes (Fisher et al [22], Rosen P P et al [23-26])

Immunohistochemistry may help in differentiating the endothelial cells from the epithelial cells and also, the smaller foci of epithelium in the back ground of the highly cellular lymphocytes. However, the use of immunohistochemisty was not resorted in our study. The present study was limited to the evaluation of lymph nodes by serial sectioning and the routine H and E staining.

CONCLUSION

The present study did not reveal much change between the 1 section technique and the serial section method. As metastases are a significant feature in the treatment and prognosis of tumours, we suggest that serial sectioning is definitely one of the best feasible methods to evaluate micrometastases, and it is advised to evaluate the micrometastases by serial sectioning for at least suspicious, small lymph nodes, if all the nodes were not excised.

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