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ORIGINAL ARTICLE

IMPRINT CYTOLOGY VERSUS IMMUNOHISTOCHEMISTRY OF SENTINEL LYMPH NODES IN BREAST CANCER WITH CLINICALLY NEGATIVE AXILLA

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Aim: *The success of sentinel lymph node (SLN) biopsy in determining axillary lymph node status necessitates an accurate and rapid method for intraoperative examination of the nodes. The aim of this study was to evaluate the feasibility and accuracy of immunohistochemistry (IHC) of touch imprints in detecting axillary nodal metastasis.*
Methods: *Sentinel lymph node biopsy was performed in 50 patients with clinical T1-2 N0 breast cancer. After harvesting, the SLN were bisected, imprinted and subjected to IHC. Results were compared with those of routine hematoxylin and eosin (H & E) and IHC examination of the same node.*
Results: *The SLN was the only site of metastasis in 15 patients (37.25%). IHC staining of the imprinted SLNs is more accurate than H&E imprint or paraffin sections H&E and IHC stained. Immunohistochemistry was capable to detect micrometastasis in 4 paraffin sections of SLN.*
Conclusion: *IHC of touch imprint is feasible and can provide reliable results for intraoperative evaluation of SLN in patients with breast cancer. It is also more sensitive for detection of micrometastasis in paraffin sections.*

Keywords: *Patent blue dye, lymphatic mapping, Micrometastasis.*

INTRODUCTION

Breast cancer is a common disease causing considerable morbidity and mortality in female population. It is the second highest cause of cancer mortality in women accounts for 18 percent of all female malignancies.(1)

Axillary lymph node dissection (ALND) is a standard operative procedure which provides precise staging and excellent local control in the treatment of patients with breast cancer.(2) However, it is associated with considerable postoperative morbidity in number of patients.(3) The early postoperative complications are pain, numbness and paraesthia of the upper arm as well as later motor dysfunction and lymphedema of the arm. Recently, sentinel lymph node biopsy has emerged as promising procedure in the treatment of breast cancer.(4)

The sentinel node is the first axillary lymph node draining the site of primary tumour.(5) Accurate intraoperative examination of sentinel nodes would enable the selection of candidates for ALND during the initial operation eliminating the need for second surgery in patients with false negative results in intraoperative SLN examination.(6)

Three different methods have been used to identify the SLN in patients with breast cancer: vital blue dye,(7) lymphoscintigraphy with intraoperative use of a γ probe.(8) and a combination of these two methods.(9) Two techniques are employed for intraoperative examination of SLN, imprint cytology and frozen sectioning. Frozen sectioning is often used but this technique has the disadvantage that the sections may undergo artifacual changes, tissues are consumed and the procedure is time consuming. On the other hand imprint cytology can provide a clear cytological details and quick diagnosis, preserving tissue for permanent section and is more accurate than frozen-section analysis.(10) Staining touch imprints with anticytokeratin

immunohistochemistry might improve the accuracy of imprint cytology in the intraoperative evaluation of SLN.[11]

Cytokeratin immunohistochemical staining of the SLN has found micrometastatic disease previously undetected by routine hematoxylin and eosin staining[12] and can facilitate the detection of clinically meaningful micrometastasis and it can reduce the false-negative rate.[13]

The aim of this study is to evaluate the feasibility of SLN identification in breast cancer patients by intraoperative peritumoral injection of patent blue dye and to assess the sensitivity of this technique to detect axillary lymph node metastasis and to correlate the results of the imprint cytology of SLN with hematoxylin and eosin staining versus immunohistochemical staining using a low molecular weight cytokeratin marker (CK19).

PATIENTS AND METHODS

Fifty patients with breast cancer (stage T1 or T2)[14] with malignant breast mass (proved by FNA, core biopsy or an excisional biopsy) and clinically negative axillary nodes underwent sentinel lymph node biopsy at the department of surgery, Assiut University Hospital between December 2003 and March 2005. All patients have been consented for the study. The study was approved by the medical ethics committee of Faculty of Medicine, Assiut University and all patient supplied informed consent for this study

Careful history was taken from each patient and clinically assessed as regards the site, size of the mass, condition of the nipple, areola and skin of the breast and the axillary lymph nodes were examined.

All patients were scheduled for mammography and breast ultrasonography to assess the breast mass and condition of the axillary lymph nodes. Patients with tumour stage more than T2 and/or clinically affected lymph nodes were not included in the study.

All patients scheduled for modified radical mastectomy or conservative breast surgery and were subjected to an intraoperative lymphatic mapping using patent blue dye 2.5% (Blue patent V of sodium; Guerbet).

Technique:

In the operative room: Ten to fifteen minutes prior to induction of anaesthesia, 2ml of vital blue dye were injected peritumorly at 2-4 injection sites at 3,6,9,12 o'clock with a sterile 25 gauge sterile syringe. Gentle massage of the breast was done after injection of the dye for 2-3 minutes to help migration of the dye through the lymphatic channels. Any blue stained lymphatic channel coming out of the breast was carefully dissected and followed to reach the blue stained node which was picked up and labeled as "the sentinel lymph node". Any other blue stained nodes were also picked up and labeled.

All retrieved lymph nodes were washed in distilled water to remove blood cells then dried by wrapping in dry gauze.[15]

In the histopathology lab: SLNs smaller than 5 mm are bihalved, while bigger nodes were serially sectioned at 2 to 3 mm intervals to maximize the surface area for evaluation. The fresh cut surface imprinted on 4 coated slides, air dried for 2 min, fixed and two slides stained with H&E. The SLNs and the non-sentinel nodes were fixed in formalin, embedded in paraffin and 3-4 serial sections were cut and stained with H&E.

For each patient two imprinted slides of SLNs subjected to immunohistochemical (IHC) staining using avidine biotinylated peroxidase complex technique with mouse monoclonal antibody against cytokeratin (Neomarker Keratin 19 Ab-4). If sentinel node was tumour negative on paraffin section, an additional 4 µm section was cut and subjected to immunohistochemical staining.[16]

(A) Immunohistochemical staining of the imprints was performed using ABC method using staining kit (k150 DAKO). Briefly, after fixation for 2 min, the slides then washed in PBS, for 30 sec three times, then incubated with the primary antibody CK19 (Labvision MS) for 10 min at room temperature at a dilution 1/100, then washed again in buffer for 30 sec three times and incubated with avidine-biotin peroxidase complex for 10min. then after washing the slides incubated the DAB chromogen for 5 min, washed in running water, counterstained with hematoxylin.

(B) Immunohistochemical staining of paraffin section antigen retrieval is required by microwaving in citrate buffer for 10 min, together with prolonged exposure with the CK to 30 min.

An immunostained imprint was considered positive if at least one group of no fewer than six coherent cancer cells were seen within the node tissue or imprint.

The status of each sentinel lymph nodes examined by H&E immunohistochemical staining using cytokeratin Ab was compared with that of imprint.

The statistical analysis was done using Bayes's rule formulation.

RESULTS

The fifty patients recruited for this study were females. Their age range (30 to 79 years) with mean age 43 years. A summary of the clinicopathological features is shown in Tables 1,2. The tumour was located in the right breast in 56% of the patients and in the majority (64%) was located in the upper outer quadrant. Tumour size ranged from 1-5 cm. Successful localization of a blue stained SLN was obtained in 48 patients with a success rate 96 %. A single SLN was detected in 46 cases and in the remaining two cases 2 SLNs were obtained.

The majority of the SLN were found at level 1, in 4 cases it was found at level II and it was apical (level III) in only one case. The number of dissected axillary LNs ranged from 6-20 LNs in each case. Thirty eight women had invasive ductal carcinoma (IDC) (76%), two cases had invasive lobular carcinoma, eight cases were IDC with intraductal component and in two cases it was dutcal carcinoma in situ only.

Table 1. The clinicopathological features of the studied cases.

Item	Number and percentage of cases
(1)Side	No (%)
Rt	28 (56%)
Lt	22 (44%)
(2)Site	
UOQ	32 (64%)
UIQ	10 (20%)
LOQ	1 (2%)
LIQ	5 (10%)
C	2(4%)
(3)Size	No (%)
T1 (<2 cm)	20 (40%)
T2 (2-5 cm)	30 (60%)
(4)Level of LNs	
Level I	43 (86%)
Level II	4 (8%)
Level III	1 (2%)
Failure	2 (4%)
(5)Histological type	
IDC.	38 (76%)
ILC.	2 (4%)
DCIS	2 (4%)
IDC &DCIS	8 (16%)
(6)No of LNs	
6-10	10 (20%)
11-15	25 (50%)
16-20	15 (30%)

UOQ: upper outer quadrant. IDC: invasive ductal carcinoma.
UIQ: upper inner quadrant. ILC: invasive lobular carcinoma.
LOQ: lower outer quadrant . DCIS: ductal carcinoma in situ.
LIQ: lower inner quadrant.
C: central.

Table 2. Pathologic status of SLN versus ALN.

Item	Number of cases	Percentage
Negative SLN with negative ALN	30	62.5%
Positive SLN (only affected one)	15	31.25%
Positive SLN with other ALN involvement	3	6.25%
Negative SLN with positive ALN	0	0%
Total	48	100%

SLN: sentinel lymph node.
ALN: axillary lymph node.

Among the 48 cases, 14 cases showed metastatic deposits in the smears of SLNs using H&E with 100% specificity and 77.7% sensitivity, but using immuno-histochemical staining revealed micrometstasis in extra 4 cases in addition to the above with 100% specificity and sensitivity Table 3. However, in the paraffin sections eight cases showed metastatic deposits using H&E with 100 % specificity and 66% sensitivity but IHC for the negative cases revealed micrometastasis in another 4 cases with 100% specificity and sensitivity Table 4, (Figs 1-4).

Table 3. Comparison for detection of SLNs metastasis using imprint cytology.

Method	Positive	Negative	Total	Specificity	Sensitivity
H&E	14 (29%)	34 (71%)	48	100%	77.7%
IHC	4 (12%)	30 (88%)	34	100%	100%

SLN: Sentinel lymph node.

Table 4. Comparison for detection of SLNs metastasis using paraffin sections.

Method	Positive	Negative	Total	Specificity	Sensitivity
H&E	8 (17%)	40 (83%)	48	100%	66.6%
IHC	4 (10%)	36 (90%)	40	100%	100%

SLN: Sentinel lymph node.

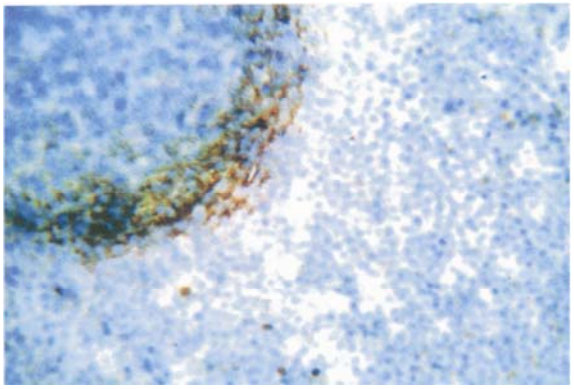

Fig 1. A large cluster of malignant epithelial cells showing diffuse cytoplasmic stain for CK-19 in imprint cytology (X200).

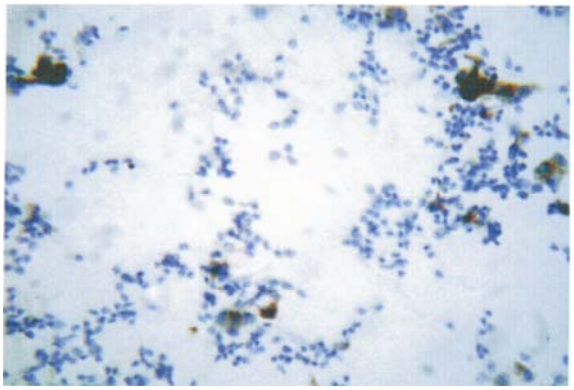

Fig 2. A Small cluster of malignant epithelial cells strongly stained with CK-19 using immunohistochemistry (X200).

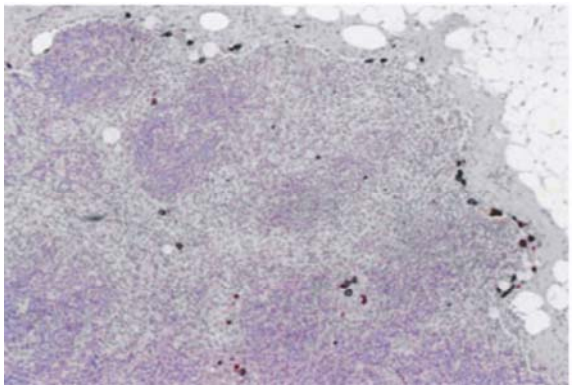

Fig 3. A paraffin section of a sentinel LN showing a small cluster of tumour cells stained with CK-19 (X100) that have been missed with H&E staining same paraffin section.

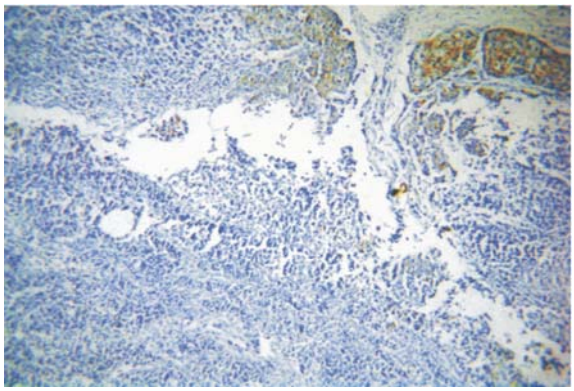

Fig 4. A sentinel LN paraffin section showing subcapsular tumour cells positive for CK-19 immunohistochemical stain (X100).

DISCUSSION

The status of axillary lymph nodes remains the most reliable prognostic indicator and predictor of survival in breast cancer and axillary lymphadenectomy, although considered the most reliable method for axillary staging, compared to physical examination and different imaging modalities, is often associated with significant morbidity which affects the patients quality of life.[17]

Accurate identification and meticulous histological examination of the SLN allows accurate prediction of the tumor status of the rest of the axillary lymph nodes, and thereby avoiding the morbidity of an unnecessary complete axillary dissection in node negative patients.[7] The SLN biopsy for breast cancer staging has been widely accepted because it is more sensitive and less morbid than axillary dissection.[13]

In this study intraoperative mapping using Patent Blue Dye showed an overall successful localization rate of 96%. Some authors reported 98% successful localization of SLNs by patent blue dye in 2 separate studies involving 98 and 51 patients respectively,(18,19) others showed a success rate of localization of 66% in their initial studies,[7] which improved to 93% in subsequent studies.[20,21]

In this study 46 out of the 48 cases showed only one blue stained SLN and only 2 cases showed 2 closely related blue SLNs. Using the blue dye 1 or 2 lymph nodes were detected with a mean number of 1.1 SLNs.[22] Others reported that 15% of patients having SLN biopsy for breast cancer have multiple SLNs.[23] These data suggest that there is no absolute upper limit for the number of SLNs that should be removed.

Intraoperatively, the level of the SLN was determined, 89.6% of SLNs were detected at level I, SLNs skipping level

I and detected at level II in 8.3% of cases and level III SLNs were detected in 2.1% of cases (Only one case). Others noted level II SLNs in 12% of cases and not detect level III SLNs.[9]

In this study all imprinted SLNs were subjected to pathological examination using H&E. 14 cases showed metastatic deposits (29%) and it was absent in 34 cases (71%). Immunohistochemistry (IHC) was capable to identify micrometastasis in 4 other cases than these 14 positive SLNs, adding 12% to the positivity of SLN metastasis. Also in this study, all paraffin sections of SLNs were subjected to pathological examination using H&E eight cases showed metastastatic deposits (16.6%) and 40 cases (83.3%) showed no metastasis by H & E staining. Immunohistochemistry (IHC) was capable to identify micrometastases in four other cases in addition to eight positive SLNs. Investigators using only routine H & E staining tend to find lower rates of SLN positivity in the range of 17 to 32%.[24] In accordance with our results, studies using IHC stains have some higher rates of nodes positivity, ranging from 42-62%[25] with an increase of metastasis detection by approximately 11%. Also other studies showed that, immunohistochemistry up stages 2% - 20% of H & E-negative sentinel nodes.[13] Thus IHC staining should be a part of any standard protocol for the evaluation of the SLNs.[26]

Among the 18 cases of metastatic SLNs found in this study, 15 SLNs were the only affected lymph nodes in the axilla (83%), and only 3 cases (17%) showed metastatic deposits in the axillary dissection specimen. Others showed 10% metastatic deposits in axillary dissection specimens.[13] While, other studies showed that SLNs were the only site of metastasis in 66% (12 out of 18) and in 52% (12 out of 23) respectively.[9,27]

In this study all cases of negative SLNs (30 cases) showed no metastatic deposits in the axillary dissection specimen by both H&E staining as well as IHC, with a sensitivity and a negative predictive value of 100%, which is compatible with the results of many studies.[21,9,28] Recently, it has been found that touch imprint cytology is cost effective for assessing SLN metastasis intraoperatively.[29] Also, it has been concluded that anticytokeratin immunohistochemistry enhances detection of occult metastases, particularly micrometastases or isolated tumor cell clusters.[30]

We concluded that IHC of touch imprints of axillary SLNs during surgery for breast cancer is a feasible and could yield reliable results if done and interpreted carefully.

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