Original Research Article

A study on accuracy of sentinel lymph Node biopsy using methylene blue in clinically No. breast carcinoma

Joel John[1*], Sansho EU [2*], Sheeja S [3], John S Kurien[4]

[1] Senior Resident, Department of Surgery, Government Medical College, Kottayam, India
[2] Associate Professor (CAP), Department of Surgery, Government Medical College, Kottayam, India
[3] (CAP) Professor, Department of Pathology, Government Medical College, Kottayam, India
[4]Professor and HOD, Department of Surgery, Government Medical College, Kottayam, India

Received: 21-04-2021 / Revised: 05-06-2021 / Accepted: 09-08-2021

Abstract

Background: Lymphatic mapping of breast has the potential of changing the standard of surgical care in carcinoma breast patients. The continued interest in less extensive surgery , the need for faster recovery and the increasing demand for early discharge have brought the value of axillary lymph node dissection (ALND) into close scrutiny and led to the emergence of less invasive procedures like sentinel lymph node biopsy as an accepted means of staging the axillary lymph nodes. Our study aims at bringing this method more accessible at affordable cost at suburban areas by using methylene blue dye in place of costlier dyes. **Methods**: We injected 3ml of methylene blue subareolarly in three places depending on the quadrant in which tumour is situated, before putting incision for Modified Radical Mastectomy and thorough massaging was done for 5-7 minutes in clockwise manner. After the procedure the pathologist segregated the stained lymph nodes and studied and reported them separately along with all the dissected axillary lymph nodes. Once the histopathology report was obtained, we studied the incidence of positive lymph nodes in stained nodes and also incidence of positive nodes in unstained nodes. Then we calculated sensitivity, specificity, false negativity, positive predictive value, negative predictive value and accuracy of sentinel lymph node biopsy with methylene blue staining. **Results**: Of the 40 patients, Sentinel Lymph node was identified in 34 patients (85%). Among the 6 unidentified patients 5 were T1 disease. i.e. <2cm and had lump in the inner quadrants. Average time for detection of sentinel lymph identification after injecting dye was 41.5 minutes (20-75 min). False Negative rate, defined as proportion actually positive among those test negative was 4.34% in this study. In 1 of the 23 patients the sentinel node was negative for metastasis but axilla was positive. Total number of cases with positive axillary nodes was 22, i.e. both SLN and rest of the axilla also being positive for metastases, and SLN was negative for metastases in 1 cases (false negative rate of 4.34%). Total number of cases with negative axillary nodes was 17 i.e. with no evidence of metastasis of which 5 were unstained. With above mentioned results, the overall Sensitivity, Specificity, Positive Predictive Valve (PPV) And Negative Predictive Valve (NPV) of SLNB in predicting axillary node status was 95.65%, 29.41%, 64.71% and 83.34% respectively. The overall accuracy was 67.50% .None of the 40 patients in this study had any kind of adverse reactions like anaphylaxis or eczema due to the Methylene blue dye. **Conclusion:** SLNB with methylene blue is effective in Indian set of patient population especially in resource scarce regions, thus reducing the cost of the procedure and avoiding all the morbidity associated with unnecessary axillary lymphadenectomy, that too at a lower cost.

Keywords: Methylene blue, sentinel lymphnode biopsy

Introduction

Breast cancer is a heterogeneous disease, and current treatment is guided by the individual characteristics of the tumour as well as the size and location of the tumour. Survival rates in women with carcinoma breast have improved steadily over the last several decades. The decreased mortality from breast cancer is thought to be the result of early diagnosis via mammographic screening, and improvements in the treatment modalities. The current treatment of carcinoma breast is guided by histopathological staging, and more recent insights into breast cancer biology. There is an increased emphasis on defining disease biology and status in individual patients, with the subsequent tailoring of treatment modalities. However, lymphatic mapping of breast has the potential of changing the standard of surgical care in carcinoma breast patients.

**Correspondence*
Dr. Sansho EU
Associate Professor(CAP), Department of surgery , Government medical College Kottayam, India.
E-mail: elavumkal@gmail.com

The continued interest in less extensive surgery, the need for faster recovery and the increasing demand for early discharge have brought the value of axillary lymph node dissection (ALND) into close scrutiny and led to the emergence of less invasive procedures like sentinel lymph node biopsy as an accepted means of staging the axillary lymph nodes.

Our study aims at bringing this method more accessible at affordable cost at suburban areas by using methylene blue dye in place of costlier dyes.

Methodology

Our study is a prospective observational study conducted in the Department of General Surgery, Government Medical College, Kottayam 12 months after getting Institutional review board approval, from 21/12/2017 .Study population includes all cases of clinically N_0 carcinoma breast undergoing modified radical mastectomy performed in the Department of General surgery during the study period and the sample size calculated and was found to be 40. Proforma questionnaire form was included as study tool.

Study procedure

We conducted the study in the Department of General Surgery during period of 1 year after obtaining written informed consent from 40

clinically node negative carcinoma breast admitted for Modified Radical Mastectomy.

We injected 3ml of methylene blue subareolarly in three places depending on the quadrant in which tumour is situated, before putting incision for Modified Radical Mastectomy(Fig1A), for example if the tumour is in the upper outer quadrant ,dye is injected at 9oclock ,12oclock and in the midpoint of that quadrant(1ml each in each point). In clockwise manner thorough massaging was done for 5-7 minutes. Then skin flaps are raised and mastectomy is performed and axilla is entered for the dissection. After complete axillary dissection we sent the specimen for histopathological analysis. The pathologist segregated the stained lymph nodes and studied and reported them separately along with all the dissected axillary lymph nodes(Fig1B). Once the histopathology report was obtained(Fig1C&D), we studied the incidence of positive lymph nodes in stained nodes and also incidence of positive nodes in unstained nodes. Then we calculated sensitivity, specificity, false negativity, positive predictive value, negative predictive value and accuracy of sentinel lymph node biopsy with methylene blue staining. Incidence of skip metastasis is also calculated after the final histopathological report is obtained.

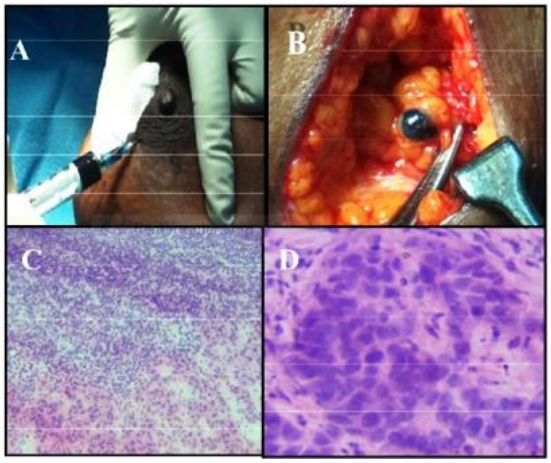

Fig 1A: Injection of the dye in Sub areolar region. Fig. 1B: Intra operative figure showing blue node in Axilla
Fig1C: Light microscopy (low power field) showing subcapsular foci Fig. 1D:Light microscopy (high power field) showing metastasis.

Inclusion criteria

All patients with clinically node negative carcinoma breast undergoing modified radical mastectomy in the department of General surgery during the study period of one year.

Exclusion criteria

All post-neoadjuvant therapy patients, all breast cancer patients with clinically palpable lymph nodes and patients with active skin lesions over the breast and axilla were excluded from the study population. We do not expect any limitations and the principal investigator is responsible for the data collection and for data analysis. There was no funding agency and the analysis was done with the help of SPSS.

Data management and analysis

We have done Diagnostic Test evaluation and the gold standard in our study is histopathological report and the diagnostic test being evaluated is sentinel lymph node biopsy using Methylene Blue. The final data was collected and analysed by the investigator himself and put in a 2x2 box from which we calculated sensitivity, specificity, false negativity, positive predictive value, negative predictive value and accuracy of sentinel lymph node biopsy with methylene blue staining(Table1). Of the 40 patients Sentinel Lymph node was identified in 34 patients.(Table2) Total number of cases with positive axillary nodes was 22.(Table3) 34 out of 40 patients had Bluish discoloration of urine in the post-operative period(Fig2)

Table1:2x2 box of final observations.

Statistic	Value	95% CI
Sensitivity	95.65%	78.05% to 99.89%
Specificity	29.41 %	10.31% to 55.96%
Positive Likelihood Ratio	1.36	0.98 to 1.86
Negative Likelihood Ratio	0.15	0.02 to 1.15
Disease prevalence	57.50% (*)	40.89% to 72.96%
Positive Predictive Value	64.71% (*)	57.13% to 71.61%
Negative Predictive Value	83.33% (*)	39.08% to 97.50%
Accuracy	67.50% (*)	50.87% to 81.43%

Table 1: *Results* **(*) Note** ;If the sample sizes in the positive (Disease present) and the negative (Disease absent) groups do not reflect the real prevalence of the disease, then the Positive and Negative predicted values, and Accuracy, cannot be estimated and you should ignore those values.

Table 2: Frequency chart of malignant cells in Sentinel Lymph Nodes detected

	NUMBER OF CASES
Sentinel node identified	34
a)Total no of cases with positive axillary nodes	22
b)Total no of cases with negative other nodes and negative SLN	17

Table 2 showing frequency chart of malignant cells

Table 3: Sentinel lymph nodes identified.

		HPR		
STAINED		Present (1)	Absent(0)	Total
	Present (1)	22 TP*	12 FP**	34
	Absent (0)	1 FN#	5 TN##	6
	Total	23	17	40

Table 3 showing identified Sentinel lymphnodes ,*true positive,**False positive, #false negative, ##true negative

Adverse reactions

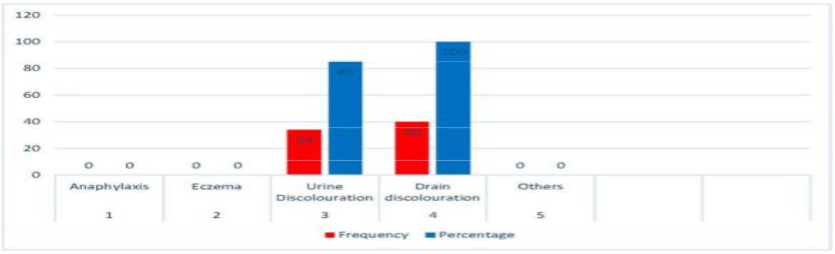

Fig 1: Chart showing frequency of adverse reactions

Of the 40 patients, Sentinel Lymph node was identified in 34 patients(85%).A total 6 patients of the study sample had no blue node in the axilla. The probable reasons for the same after analysis of variables in these cases may be; Size of the tumour influenced the identification of Sentinel Lymph node. Among the 6 unidentified patients 5 were T1 disease. i.e. <2cm and Site of the tumour also may have influenced the identification of Sentinel Lymph node, as 6/6 unidentified patients had lump in the inner quadrants. Average time for detection of sentinel lymph identification after injecting dye was 41.5 minutes (20-75 min). In 3 of the 6 negative Sentinel lymph node patients, time taken to identify the node was >50 minutes. False Negative rate, defined as proportion actually positive among those test negative was 4.34% in this study. According to this study the false negative rate in sentinel lymph node biopsy can be minimised by reducing the number of coloured nodes taken for sampling, i.e. the higher number of nodes taken as SLN, greater will be the False Negative Rate. In 1 of the 23 patients the sentinel node was negative for metastasis but axilla was positive. Total number of cases with positive axillary nodes was 22, i.e. both SLN and rest of the axilla also being positive for metastases, and SLN was negative for metastases in 1 case (false negative rate of 4.34%). Total number of cases with negative axillary nodes was 17 i.e. with no evidence of metastasis of which 5 were unstained. This can be improved by gradually mastering the technique of SLNB. With above mentioned results, the overall Sensitivity, Specificity, Positive Predictive Valve (PPV) And Negative Predictive Valve (NPV) of SLNB in predicting axillary node status was 95.65%, 29.41%, 64.71% and 83.34% respectively. The overall accuracy was **67.50%**.None of the 40 patients in this study had any kind of adverse reactions like anaphylaxis or eczema due to the Methylene blue dye.34 out of 40 patients had Bluish discoloration of urine in the post-operative period, which subsided by itself by Post-operative day 3.All the patients invariably had bluish discoloration of drain fluid, at least for 3 days.

Discussion

Breast cancer has ranked number one cancer among females in India with age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women[1]. Data from various national cancer registries were compared for incidence and mortality rates. The age adjusted incidence rate of carcinoma of the breast was found as high as 41 per 100,000 women for Delhi, followed by Chennai, Bangalore and Thiruvananthapuram. A statistically significant increase in age adjusted rate over time (1982–2014) in all the population based cancer registries namely Bangalore (annual percentage change: 2.84%), Chennai (2.44%), Bhopal (2.00%), Barshi (1.87%),Delhi (1.44%) and Mumbai (1.42%) was observed. Mortality to incidence ratio was found to be as high as 66 in rural India whereas it was as low as 8 in urban areas. Besides this, in Indian women, young age has been found as a major risk factor for breast cancer. Breast cancer projections for India during time periods 2020 suggests the number to go as high as 1797900. Better health awareness and availability of breast cancer screening programmes and treatment facilities would cause a favourable and positive clinical picture in the country[2].Histopathological examination of lymph nodes retrieved by axillary lymph node dissection (ALND) is currently the gold standard for staging carcinoma breast. Advocates of ALND contend that the procedure benefits patients by producing regional control of disease and they also argue that the removal of microscopic nodal metastasis is often curative without adjuvant chemotherapy in certain patients. There are many pitfalls of SLNB in breast cancer . Sentinel lymphnodes may be absent in 5-10% of patients may be either due to reduced functional capacity of the nodes as a result of extensive metastatic infiltration or due to the decrease in the amount or disruption of lymphatics. Preservation of functional capacity of the lymphnodes are necessary for the uptake ot the dye/radioactive colloid. Fatty degeneration of the Sentinel lymphnode can also lead to reduced functional capacity. The decrease in absolute amount of lymphatics in the breast has been noted in elderly patients as a part of their involutional and atrophic changes. Following core biopsy or excision biopsy, SLN identification would be more difficult, owing to disruption of the lymphatic architecture and localised inflammation. Another factor for concern is the occurrence of skip metastasis[3,4].If the SLN is tumour free using both haematoxylin and eosin staining and immunohistochemistry, the probability of non SLN involvement is less than 0.1%. This tends to suggest that axillary nodal metastasis in carcinoma breast occurs in an orderly fashion according to the lymphatics anatomical drainage as proposed by Reintgen et al, So what was previously described as “skip metastasis” may be variation in local lymphatic anatomy rather than non-sequential spread of

tumour cells. The incidence of skip metastasis in several studies range from 1 - 42 %. This variation may be due to varying techniques of axillary dissection, individual anatomical variation and failure to identify micro metastasis in lower level nodes. Another explanation is that excessive infiltration of the lymph node can lead to directional flow changes of lymph leading to "skip phenomenon", resulting in an alternative node being labelled as the SLN, thus increasing false negatives. another pitfall is the presence of non axillary sentinel nodes[5,6]. Lymphatic mapping in patients with breast cancer can reveal sentinel lymph nodes that are not in axilla. The most common site is the internal mammary nodes, other site being nodes in breast parenchyma, nodes in deep interpectoral fossa. Detection of internal mammary node can be identified in lymphoscintigraphy. Site of lesion is another area of concern[7]. Tumour in the upper outer quadrant can pose some difficulty during gamma probe localisation. This is due to the close proximity to the site of injection leading to the "shine through phenomenon". To overcome this problem retraction of breast downward and medially and use of collimator during probe guided surgery is recommended. This phenomenon can also occur in inner quadrant tumours when SLN is internal mammary nodes. Routine staining is currently the method on which treatment planning is based. There is definite error rate in routine pathological assessment of axillary dissection specimens which may underestimate metastatic disease by 11% to 20%. Giuliano et al have found that immunohistochemical studies of sentinel nodes showed micro metastasis in an additional 11% of women whose sentinel node was tumor negative on light microscopy[8].But ALND is potential for producing a wide spectrum of complications, such as paraesthesia due to intercostobrachial nerve injury, drain complication,wound infection, seroma ,acute and chronic upper limb lymphoedema[9]. It can also result in delayed postoperative adjuvant therapies. Historically, approximately 40% of patients treated with complete ALND (defined as dissection of all nodes in levels I, II and III) develop acute lymphoedema, and approximately 5% to 10% of the patients experience chronic lymphoedema. New data suggest that although limiting axillary dissection to levels I and II has not changed the 40% incidence of acute lymphoedema, the incidence chronic lymphoedema has decreased to 5%. The increased scrutiny given to axillary dissection is due, in part, to the lack of effective treatment for chronic lymphoedema. Routine axillary dissection is not beneficial in 70–80% of early breast cancer patients who are clinically node negative (N0)[10].Interestingly, the most significant complaint by patients, following breast cancer surgery is the morbidity associated with axillary dissection. Though the removal of axillary contents provides better local control of the disease, that may not in itself offer a survival advantage. The overall survival depends on the development of distant metastasis and is not influenced by axillary dissection in most of the patients. Mapping of the breast is clearly changing this long held paradigm and has the potential of changing the standard of surgical care of the breast cancer patient. A promising alternative to assess axillary lymph node status in early breast cancer patients is Sentinel Lymph Node Biopsy (SLNB). The sentinel lymph Node (SLN) concept represents a biological model that assumes the presence of a specific afferent lymphatic drainage pathway from a primary tumour to a principal, "sentinel", lymph node in the regional lymphatic basin[11,12]. Also axillary lymphadenectomy results in significant morbidity like chronic lymphoedema of ipsilateral extremity frozen shoulder syndrome and long term sensory abnormalities[13]. The definition of SLN in breast carcinoma is the node most likely to harbour metastases if they are present. The assumption is that if SLN is negative, all other axillary lymph nodes will be negative. Thus the technique of SLNB was developed to provide surgeons with information that allows axillary dissection to be avoided if SLN is negative, which is less invasive and with least morbidity. The concept of sentinel node biopsy is based on two basic principles: the existence of an orderly and predictable pattern of lymphatic drainage to a regional lymph node basin, and the functioning of a first lymph node as an effective filter for tumour cells. With the widespread use of sentinel node biopsy, sufficient data was provided to prove that sequential lymphatic dissemination and entrapment of tumour cells in first draining lymph nodes occur[14,13]. The sentinel node concept is actually based on the Halsted theory that stressed the importance of locoregional cancer treatment because of the step-wise spread. The spectrum and systemic hypotheses, however, suggest that lymph node involvement can be an indicator of distant disease and therefore sentinel node biopsy is also a staging tool to select patients for adjuvant systemic therapy. Though different types of blue dyes are available ,we have performed SLNB study using methylene blue dye in clinically N0 breast cancer patients as it is more readily available, safe, less expensive and equally effective [15-20].We conducted the study of SLNB in breast cancer patients using methylene blue dye alone method. Though methylene blue dye demonstrated poor lymphatic uptake in initial studies recent work by Blessing et al shows that it can be substituted for isosulfan blue[20]. We used methylene blue dye to identify SLNs as it is less expensive and more readily available as proven by studies conducted by Gold HT et al[9], according to which assuming approximately 136,000 cases of Stage I breast cancer diagnosed in women in 2004 in the US, over $14.7 million dollars could have been saved by using methylene blue instead of isosulfan blue if all cases undergo SLN biopsy. Including the 62,600 Stage II breast cancer patients potentially eligible for SLN biopsy would yield a total savings of $21.5 million. In our study we found that it readily reaches SLN and stains the node deeply blue. Various sites of injection of the mapping agent are peritumoral, intratumoral, intradermal over the tumour site and subareolar. Intradermal or subareolar injection appear to increase the identification rate when compared to peritumoral injection in most studies[21,22], although the two methods have been found equivalent in other studies .Thus here we have used the more effective subareolar site of injection. 1.6% of patients experienced allergic reactions due to use of isosulphan blue in a large MSKCC study[23]. However none of our patients had any of the above reactions proving safety value of the methylene blue dye[24,19]. The administration of isosulfan blue dye has been shown to artificially decrease the intraoperative pulse-oxymetric readings without any associated ill effects or changes in arterial oxygen tension. In this series none of the patients had any interference during intraoperative monitoring. Skin lesions at the site of injection of methylene blue is seen in 2% of patients. There is no blue tattooing effect on skin. Fat necrosis at the injection site has also been described following use of methylene blue dye. None of the patients in our study population had any fat necrosis. Though literature states that SLN identification was higher when combination was used (86–98%) rather than when dye alone was used (74 – 94%)[20,24].In this study the identification rate was 85 % which is in par with combination method. False negative rates varied between 0–19 percent, higher when radio colloid was not used. In this study SLN false negative rate is 4.34%. 1 case of skip metastasis was found to be in a patient where the time required for axillary dissection was maximum. Thus the incidence of skip metastasis may be avoided by faster lymphadenectomy. The accuracy of 67.5 %, though satisfactory, may be improved in a larger study with more number of study population and also improving and mastering the technique of sentinel lymph node biopsy. With the advent of frozen section, the surgeon has the luxury of deciding upon the axillary dissection, depending upon the results of sentinel lymph node. Studies by P J Van Diest et al have proven the reliability of frozen section in cytological investigation of sentinel lymph node in carcinoma breast. Therefore, in the large majority of patients with clinically N0 Breast Carcinoma Methylene Blue can be used to locate the sentinel node in the axilla, and thereby provide important information about the status of axillary nodes. Patients without clinical involvement of the axilla should undergo sentinel-node biopsy routinely, and may be spared complete axillary dissection when the sentinel node is disease-free. Results of sensitivity, specificity, positive and negative predictive value and overall accuracy of this method are comparable and compatible with results from oncological breast cancer centres and allow introduction of SLNB in routine surgical practice in our clinical practice as the alternative for ALND for N0 Breast carcinoma.

It also contributes to better co-ordination between specialist of surgeon and pathologist.

Conclusion

Methylene blue dye is an effective, cheap and safe blue dye for SLNB. SLNB using methylene blue dye can stage the axilla with high accuracy and low risk of false negativity in breast cancer patients SLNB with methylene blue is effective in Indian set of patient population especially in resource scarce regions, thus reducing the cost of the procedure and avoiding all the morbidity associated with unnecessary axillary lymphadenectomy, that too at a lower cost. A very good coordination between members of multidisciplinary team including surgeons, and pathologists is required for a successful SLNB.

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Conflict of Interest: Nil

Source of support: Nil