

A study of lymph node retrieval in resected specimens of Breast carcinoma using GEWF Solution

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Abstract

Introduction: Breast carcinoma is the 2nd most common cancer in women in India and forms a major burden of disease on the health system. By performing this study, one can decide whether the standardized method of serial sectioning, inspection, and palpation is enough in the staging of a cancer, or if visual enhancement techniques (GEWF) should be employed to improve the staging of cancer.

Aims and Objectives: This study evaluated the usage of GEWF solution as a visual enhancement medium for retrieval of lymph nodes from resected modified radical mastectomy (MRM) specimens of Breast carcinoma for staging. Basing on the results, which are expected to be encouraging, this method can be recommended as a routine procedure for lymph node retrieval in cases of Breast carcinoma by using GEWF solution.

Materials and methods: This study was performed over a period of two months—from 1st August to 31st September, 2012—in GSL Medical College, Rajahmundry, Andhra Pradesh. 6 MRM specimens of Breast carcinoma were included.

All the specimens of Breast carcinoma during the period of study were processed according to the conventional methods.(1-5) All the lymph nodes that were not identified by the initial grossing and those that have been enhanced visually by the treatment with GEWF solution were also removed and processed.

Discussion: A total of 6 cases were utilized in this study; the conventional and GEWF solution methods were utilized. The results of the 6 cases can be used to conclude: Despite the GEWF solution increasing the yield of Lymph Nodes in 5 out of the 6 cases, there was no difference in the staging by using standard method and GEWF solution. GEWF increased the yield of LNs from breast cancer specimens. However, the utilization of GEWF solution did not change the staging of the cancer.

Keywords: Breast carcinoma etc.

Introduction

Breast carcinoma is the second most common type of cancer in women in India and forms a major burden of disease on the health system. "Staging is an appropriate indication of the extent to which a person has been effected by cancer; an essential means to determine the extent of spread including distant metastases. Pathologic Staging is the most important factor in assessing prognosis in patients and lymph node status has a pivotal role in the staging process" (Newell). TNM Staging is the most commonly used method of staging in cancer and this method is acknowledged by the International Union Against Cancer as well as American Joint Committee on Cancer. It requires appropriate and accurate staging of the disease for definite management of patients and one of the major criteria is the assessment of lymph node

status. As per the latest protocol of TNM staging, the clinical stage of the disease is based on the number of lymph nodes involved.⁽¹⁾⁽²⁾ It is also important to find small lymph nodes of less than 0.5 cm in size, as they may also harbor metastatic disease. Such lymph nodes are difficult to dissect from the adipose tissue of the axillary fat.

There's has been an ongoing debate on the total number of lymph nodes required for the staging of a certain type of cancer. "Some authors have prescribed that a minimum number of lymph nodes (ranging from 6-17 per case) is required for adequate staging. Others have recommended simply that as many lymph nodes as possible should be examined" (Newell) These results suggest that the use of adjunctive techniques to identify lymph nodes may lead to improved staging" (Newell).

Lymph Node status is an important prognostic factor" (Gregurek). After a carcinoma has been detected, one of the first techniques used to determine the extent of the carcinoma is the retrieval of lymph nodes. The first

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approach, made by physicians, to retrieve lymph nodes is by the standardized method of serial sectioning, inspection, and palpation. However, “The standard method (serial sectioning, inspection and palpation) may be regarded as arduous, and lymph nodes may be missed, especially those 5 mm. or less in size” (Newell).

As a result, it is important to determine whether techniques other than the standard method should be employed to increase the overall number of lymph nodes retrieved. “There have been many techniques developed to make LN retrieval more efficient. These methods are usually referred to as visual enhancement techniques. These methods usually cause either a color change of the LN from the surrounding fat or cause the fatty tissue to dissolve or weaken allowing the LN to be more easily identified” (Gregurek). The different visual enhancement techniques present are: fat stretching, alcohol treatment, xylene clearance, cedar oil clearance, etc.

Out of the various visual enhancement techniques present, GEWF solution is easily prepared, and is cost efficient. In order to determine the accuracy of GEWF solution in the staging of breast cancer, this study will be performed. With the foregoing, it is evident that there is a need for use of such a solution that helps in visually enhancing the lymph node identification for a better yield, is least noxious, is of low cost, takes less time for processing and is equally effective that ultimately helps in managing the cancer better. GEWF is one such solution that helps in this regard.⁽¹⁻⁵⁾

According to the available literature there is no well conducted and organized study regarding usage of the GEWF solution for retrieval of lymph nodes in resected specimens of the Breast except one pilot study done on 11 cases.⁽⁴⁾ Most of the studies are in colorectal cancers and there is a uniform positive response for usage of GEWF solution for increased pick up rate of lymph nodes in the resected specimens.⁽¹⁻⁵⁾ Hence it is imperative to study the utility of simple GEWF solution in breast cancer specimen.

By performing this study, one can decided whether the standardized method of serial sectioning, inspection, and palpation is enough in the staging of a cancer, or if visual enhancement techniques should be employed to improve the accuracy of the staging of a particular cancer. With the increasing prevalence of Breast Cancer across the world, this study will also be of great importance in the prognostic and diagnostic aspects.

TNM Staging

The TNM system is based on the extent of the tumor (**T**), the extent of spread to the lymph nodes (**N**), and the presence of distant metastasis (**M**). A number is added to each letter to indicate the size or extent of the primary tumor and the extent of cancer spread.

Primary Tumor (T)

TX	Primary tumor cannot be evaluated
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T0	No evidence of primary tumor
Tis	Carcinoma in situ (CIS; abnormal cells are present but have not spread to neighboring tissue; although not cancer, CIS may become cancer and is sometimes called preinvasive cancer)
T1, T2, T3, T4	Size and/or extent of the primary tumor
Regional Lymph Nodes (N)	Lymph
NX	Regional lymph nodes cannot be evaluated
N0	No regional lymph node involvement
N1, N2, N3	Involvement of regional lymph nodes (number of lymph nodes and/or extent of spread)
Distant Metastasis (M)	
MX	Distant metastasis cannot be evaluated
M0	No distant metastasis
M1	Distant metastasis is present

1. For example, breast cancer classified as T3 N2 M0 refers to a large tumor that has spread outside the breast to nearby lymph nodes but not to other parts of the body. Prostate cancer T2 N0 M0 means that the tumor is located only in the prostate and has not spread to the lymph nodes or any other part of the body.
2. For many cancers, TNM combinations correspond to one of five stages. Criteria for stages differ for different types of cancer. For example, bladder cancer T3 N0 M0 is stage III, whereas colon cancer T3 N0 M0 is stage II.

Stage	Definition
Stage 0	Carcinoma in situ.
Stage I, Stage II, and Stage III	Higher numbers indicate more extensive disease: Larger tumor size and/or spread of the cancer beyond the organ in which it first developed to nearby lymph nodes and/or organs adjacent to the location of the primary tumor.
Stage IV	The cancer has spread to another organ(s).

Review of Literature

Lymph node (LN) assessment is a crucial part of the histopathologic staging of Breast cancer. Stage I and II cases need no further therapy after oncologic resection. In contrast, stage III cancers, which are defined by LN metastases, are generally treated with adjuvant chemotherapy. There is a lack of consensus in the literature on how many LNs should be assessed. Inadequate lymph node evaluation is associated with worse outcome in terms of tumor recurrence and patient survival, particularly in patients with stage II cancer. The actual basis for this association though is not known, but it is most likely that it reflects inaccurate staging and the resulting lack of adjuvant therapy.⁽⁷⁾ In cases of Breast carcinoma, clinical staging of stage II tumors is based on detecting 1-3 positive lymph nodes. However detection of

just another positive lymph node changes the stage to stage III. In fact, some authors go so far as to suggest that patients who are deemed lymph node negative on the basis of a low number of retrieved lymph nodes should be considered to be at a high risk of recurrence and thus are suitable candidates for adjuvant therapy. The retrieval of a low number of lymph nodes is also likely to be an indicator of poor-quality surgical or pathologic care.

The GEWF solution is a modification of a fixative described in 1949 by Lillie.⁽⁶⁾ According to the available literature there is no well conducted and organized study regarding usage of the GEWF solution for retrieval of lymph nodes in resected specimens of the Breast except one pilot study.⁽⁴⁾ This study though was conducted as a pilot project with a very few cases (11 cases) has in the first instance concluded that use of GEWF solution is useful in enhancing lymph node pick up rate from resected axillary tail of breast cancers. However, in case of Colorectal cancers there is a uniform positive response from various workers for usage of GEWF solution for increased pick up rate of lymph nodes in the resected specimens.⁽¹⁾⁽²⁾⁽³⁾⁽⁵⁾⁽⁶⁾

Aims and Objectives

This study evaluated the usage of GEWF solution as a visual enhancement medium for retrieval of lymph nodes from resected modified radical mastectomy (MRM) specimens of Breast carcinoma for appropriate staging of tumor.

This study is going to be the first study on the subject which includes a significant number of cases of Breast carcinomas. Barring the pilot study, no large scale study has been done in this regard till now. Since the results of the pilot study are encouraging, it is expected to obtain similar results in this study also. "Increased LN retrieval is linked to increased survival" (Gregurek). Although the accuracy of the GEWF solution is not yet completely confirmed, GEWF solution is an advantageous visual enhancement technique, and is presumed to increase the total yield of lymph nodes obtained; this therefore greatly increases the accuracy of staging of breast cancer, allowing for proper and precise treatment to be given to patients.

Basing on the results of this study, which are expected to be encouraging, this method can be recommended as a routine procedure for lymph node retrieval in cases of Breast carcinoma by using GEWF solution.

Materials and methods

This study was performed over a period of two months - from 1st August to 31st September, 2012- in GSL Medical College, Rajahmundry, Andhra Pradesh. 6 MRM specimens of Breast carcinoma received during this period were included.

GEWF (Glacial acetic acid-ethyl alcohol-distilled water-formalin) solution was used as a fixative which is

prepared by mixing the reagents in the following order and quantity for making 16L of solution: Absolute Ethyl Alcohol (10L), Distilled Water (3.4L), Formalin (1.6L) and Glacial Acetic Acid (1L).⁽²⁾ Absolute ethyl alcohol is a fat solvent, whereas distilled water is used to make up the volume. Formalin is a fixative and glacial acetic acid is a dehydrating agent and makes capsule of the lymph nodes to stand out chalky white. Preparation of this solution does not require additional equipment, is least noxious, doesn't involve volatile chemicals and also less expensive and hence suits the purpose.

Table 1 Materials Used in the Preparation of GEWF*

Reagent	Volume (L.)
Absolute ethanol	10.0
Distilled Water	3.4
Formaldehyde (40%)	1.6
Glacial acetic acid	1.0

*The reagents are mixed in the order listed

All the specimens of Breast carcinoma during the period of study were processed according to the conventional methods.⁽¹⁻⁵⁾ The lymph nodes identified were dissected out *in toto* and processed. After that, the specimen of adipose tissue was separated and kept in the GEWF solution for a period of 72 hours. This made lymph nodes to stand out as "firm chalky white nodules".⁽²⁾ All the lymph nodes that were not identified by the initial grossing and those that have been enhanced visually by the treatment with GEWF solution were also removed and processed. The extra yield was noted. Sections were cut at 5µm and stained with hematoxylin-eosin and were examined for metastasis as per the standard protocols.

Observations and Results

The following is a microscopic picture of a negative Lymph Node:

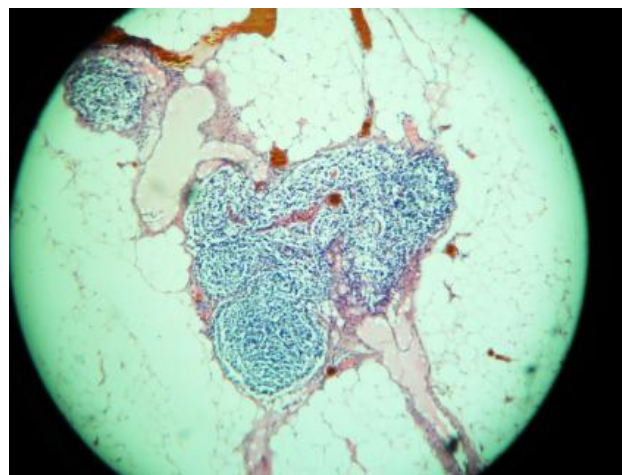


Figure 1

The following is the microscopic picture showing a positive Lymph Node:

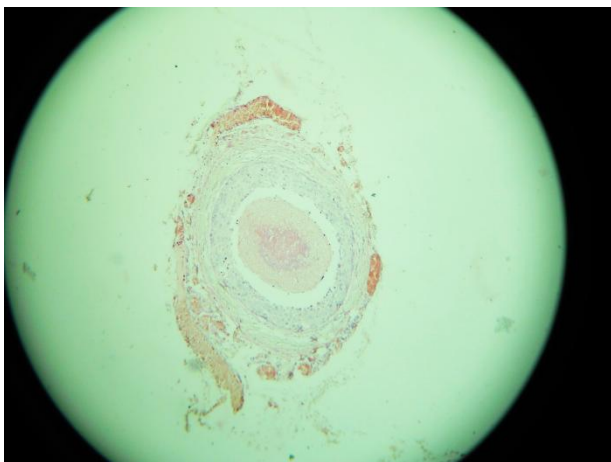


Figure 2 (10x)

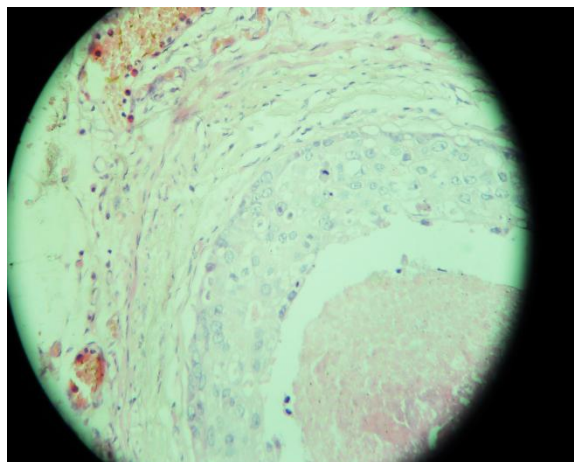


Figure 3 (40x)

The following is the gross picture showing the Axillary Tail containing Lymph Nodes:

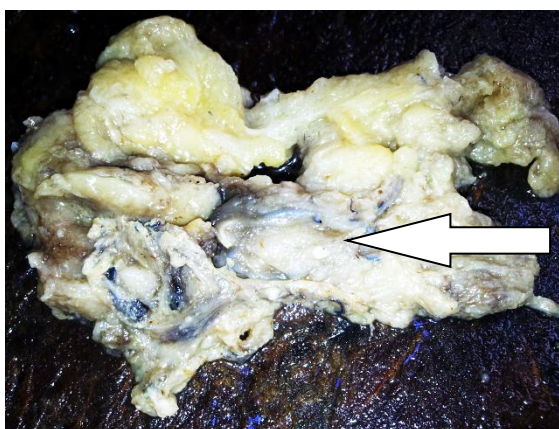


Figure 4

- The arrow above refers to the visual appearance of Lymph Nodes, after GEWF solution is used.

Table 1: Data of the 6 cases

SL. No	1	2	3	4	5	6
Biopsy No. –Date	5134-2/8/12	5214-6/8/12	5377-13/8/12	5573-22/8/12	5658-25/8/12	5925-5/9/12
OP/ IP No.	652/12	153/12	161/12	722/12	735/12	790/12
Name	P.Anapoorna	N.Appayama	M.Sarada	K.Nagamani	K.Satyavathi	K.Vijayalakshmi
Age/ Sex	74/ F	60/F	69/F	46/F	46/F	40/F
Clinical Diagnosis	Carcinoma of Breast - Right	Carcinoma of Breast - Right	Carcinoma of Breast - Right	Carcinoma of Breast - Right	Carcinoma of Breast - Right	Carcinoma of Breast - Right
Nature of Specimen	MAC	MAC	MAC	MAC	MAC	MAC
FNAC Report	Fn- 2871/ 12, IDC	Fn-2716/12, Suspicious morphology, B-4791/12, IDC	Fn-3165/12, IDC= LN metastasis	B- 2412, IDC	----	Fn – 3348/12, IDC
Histopathology	IDC, NST, 2+2+1= 5, Grade 1	IDC, NST, 2+2+1= 5, Grade 1	IDC, NST, 2+2+1= 5, Grade 1	IDC, NST, Paget Disease	IDC, NST, 3+2+3= 8, Grade 3	IDC, NST, 2+3+2= 7, Grade 2, Posterior Margins Invaded (T2N0M0)
Pre GEWF LN Status	15/13	17/0	20/5	19/13	15/4	13/0
Post GEWF, Supposed LN	4	11	5	2	2	3
Post GEWF, LN	3	11	3	2	0	2
LN Status	0/3	0/11	0/3	2/2	0	0/2
Pre stage GEWF	N3	N0	N2	N3	N1	N0
Post stage GEWF	N3	N0	N2	N3	N1	N0

Table 2: Sizes of the LN of the 6 cases (Post GEWF)

Biopsy No. – Date	Post GEWF, Total LN - positive LN	Size of Positive LN (Millimeters-mm.)
5134- 2/8/12	3	1, 1, 1 mm.
5214- 6/8/12	11	4,4,3,3,3,3,2,2,1.5,8, 0.5 mm.
5377- 13/8/12	3	1,1,1 mm.
5573-22/8/12	2	1.5, 4 mm.
5658 – 25/8/12	0	---
5925- 5/9/12	2	2, 0.5 mm.

Table 3: Findings of the Study

Period of Study	August -September 2012		
Total Number of Cases	6		
Number of cases yielded extra Lymph Nodes	5		
	Standard	GEWF	Total
Total Number of Lymph Nodes	99	21	120
Total Number of Lymph Nodes Positive	35	2	37
Number of Cases Lymph Node positive (pre GEWF)	4		
Number of Cases Lymph Node positive (post GEWF)	1		
Mean Number of Lymph Nodes	Standard	GEWF	Total
	17	4	20
Mean Size of Lymph Nodes	2.28 mm		
Smallest Size of Lymph Node (showing metastasis)	1.5mm		

Discussion

It is essential to retrieve the adequate number of Lymph Nodes for accurate tumor staging, choice of the treatment and determination of prognosis. In this study, 2 different methodologies were utilized. One was the standard method, which primarily depends on visual isolation and palpation. The second was a visual enhancement technique, GEWF solution. GEWF solution is beneficial as the chances of tiny lymph nodes are quite less with the standard method; the GEWF solution ensures the resection of even tiny lymph nodes.

A total of 6 cases were utilized in this study; first Lymph Nodes were resected using the conventional method, and then later, GEWF solution was employed, and more Lymph Nodes were resected from each of the 6 cases. Out of the 6 cases, the number of Lymph Nodes that were resected using the standard method was 99, and 21 using the GEWF solution, making the total number of Lymph Nodes, 120. The number of Lymph Nodes that were positive using the standard method was 35, and 2 using the GEWF solution; therefore the total number of positive lymph nodes in the 6 cases was 37. As demonstrated in Table 1, overall, the usage of GEWF Solution has allowed for an increase of 3.5 Lymph Nodes, as compared to the normal conventional method (pre GEWF). However, the overall number of Lymph Nodes resected was more by using the conventional method, than with GEWF solution. Regardless of this increase in Lymph Nodes, out of the 6 cases, only 1 (as shown Table 1 - Biopsy No. 5573) is positive in its Lymph Node Status after GEWF retrieval, revealing positive for metastatic tumour deposits. Out of the 6 cases, 4 cases (as shown in Table 1) showed positive in its Lymph Node Status, with the standard methodology, therefore revealing positive for metastatic tumour deposits. As shown in Table 3, the

smallest size of Lymph Nodes (showing metastasis) is 1.5 mm. As shown in Table 1, GEWF solution increased the yield of Lymph Nodes in 4 out of 5 cases or 80% of the cases.

Despite the fact that GEWF solution has increased the yield of Lymph Nodes in 5 out of the 6 cases, there was no difference in the staging by using standard method and GEWF solution, as revealed in Table 1.

Conclusions

GEWF increased the yield of LNs from breast cancer specimens. However, the utilization of GEWF solution did not change the staging of the cancer.

Summary

Due to rapidly increase prevalence of Breast Cancer across the world, this study is absolutely important in both prognostic and diagnostic aspects. The resection of Lymph Nodes using the conventional method is not completely accurate, as small ones can be easily missed. For this, visual enhancement techniques should be used. In this study, one such visual enhancement technique, namely the GEWF solution was employed, in an attempt to increase the yield of Lymph Nodes, and therefore improve the staging. As shown in Table 1, GEWF solution increased the yield of Lymph Nodes in 4 out of 5 cases or 80% of the cases. However, there was no improvement in staging by using the GEWF solution.

Suggestions

Other visual enhancement techniques such as Alcohol treatment, fat stretching, etc. should be used to increase the yield of Lymph Nodes. Comparative studies between

these visual enhancement techniques and GEWF solution can be done to determine which is more efficient in increasing the yield of Lymph Nodes, and improving the staging of cancer. Also, doing the study over a longer time span, will allow for more accurate results, therefore assisting us in the determination of whether the utilization of GEWF solution is necessary.

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