Paper

Assessment of the impact of the concentration of formalin on receptor studies in surgical specimens of breast carcinoma: a pilot study

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Abstract

Introduction: Assessment of oestrogen, progesterone and HER2 receptor status are routinely performed on specimens fixed in 10% formalin.

Objective: To assess the impact of the concentration of formalin on the oestrogen, progesterone & HER2 receptor status in surgical specimens excised for carcinoma of breast.

Method: A descriptive, cross sectional study was done on excision biopsy and mastectomy specimens of breast cancer over a period of 18 months. A 1x1cm piece of tumour tissue was fixed in 50% diluted formalin and the remainder in 10% formalin. Following routine tissue processing, staining for ER, PR and HER2 receptor status was done. Allred scoring system was used to interpret ER and PR receptor status and CAP (College of American Pathologists) guidelines were used to interpret HER2 receptor status.

Results: In view of the small sample size (n=20), binomial sign test was selected to assess the differences between the two groups (10% formalin vs 50% diluted formalin). There were statistically significant differences in the results with a marked downgrading of the total score of ER and PR and HER 2 receptor studies with 50% diluted formalin.

Conclusion: It is vital for the specimen to be adequately fixed in 10% buffered formalin before commencing on receptor studies.

Key words: Breast carcinoma, ER, PR , HER2 receptor status, tissue fixatives.

Introduction

A crucial development in the evaluation of breast cancer was the identification of receptors in certain types of breast cancer. These receptors are found on the surface of breast cancer cells, and their cytoplasm or nucleus (1). These receptors have been identified as oestrogen,

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progesterone and HER2 which belongs to the family of epidermal growth factors.

Some breast cancers require oestrogen to continue growth. These tumours can be identified by the presence of oestrogen receptors (ER) and progesterone receptors (PR) on the surface. This type of tumours are treated with drugs such as tamoxifen to block the action of oestrogen (2,3). Progesterone receptors (PR) are usually found along with oestrogen receptors.

Tumours that are positive for ER and PR receptors generally have a better prognosis (4). Between 25% and 30% of breast cancers have an amplification of HER2 gene or overexpression of its protein product. This receptor is normally stimulated by a growth factor which causes the cell to divide. In the absence of the growth factor the cell will stop dividing. Overexpression of HER2 receptors in breast cancer is associated with an increased disease recurrence and a worse prognosis. The monoclonal antibody trastuzumab (Herceptin) in combination with conventional chemotherapy is given for HER2 positive tumours. The tumours with none of these receptors are known as triple negative (5).

Routine histological assessment will provide morphological features of the tumour. The assessment of receptors is done by immunohistochemical methods (6). Formalin fixed, paraffin embedded tissue blocks are used to determine the receptor status of tumours. The principal of immunohistochemistry lies in the formation of immune complexes by an antigen-antibody reaction. A chromogen that binds to the complexes is used to bring out a specific colour which can be readily detected under the light microscope (7). Widely used immunohistochemical stains for breast carcinoma include monoclonal mouse anti-human oestrogen receptor, monoclonal mouse anti-human progesterone receptor, and polyclonal rabbit anti-human HER2 oncoprotein.

The most effective treatment regime for breast carcinoma is decided by the oncologist based on the morphological features and the receptor status of the tumour. Prognosis is a prediction of outcome and the probability of disease-free survival. It is the duty of the reporting pathologist to include the diagnosis and prognostic information in the final histopathological report. With the correct treatment plan, the disease free interval can be prolonged. This will increase the 10 year survival rate of the patients.

The concentration of formalin varies due to many reasons, out of which the most common being over dilution resulting in an incorrect dilution. This problem is encountered in certain institutions. Hence it is vital to maintain proper standards in laboratory procedures to ensure the accuracy of the results.

Objective

To assess the impact of the concentration of formalin on oestrogen, progesterone and HER2 receptor studies of surgical specimens excised for carcinoma of breast.

Methodology

A descriptive cross sectional study was done at the Department of Pathology, Faculty of Medical Sciences, University of Sri Jayewardenepura and Histopathology Laboratory, Lanka Hospital, Narahenpita, Colombo 05. Excision biopsy specimens and mastectomy specimens of breast carcinoma that have been excised for carcinoma of breast, over a period of 18 months from June 2011 to December 2012, were included in the study. After careful macroscopic examination specimens which were received in the fresh state, a 1x1cm piece of tissue was taken from the tumour and fixed in 50% diluted formalin (10% buffered formalin diluted by 50%). The remaining tissue was fixed in formalin with the optimum concentration (10% buffered formalin).

Following adequate fixation, tissues were submitted for processing. After reporting the histological features of the tumour, further sections were prepared from both groups for receptor studies. Results of the 02 groups were independently reviewed by the investigators. Routine histology slides were assessed for tumour type and grade. Results of the receptor studies were evaluated in keeping with the standard reporting guidelines (8,9). The HER 2 receptor status was assessed according to the College of American Pathologists guidelines (10).

Results

Table 1: Histological type, grade and receptor status of the two groups

Patient identification no.	Tumour type & Grade	Group 1 (standard 10% formalin)			Group 2 (50% diluted formalin)		
		ER	PR	HER2	ER	PR	HER2
1. 001	Invasive duct carcinoma-Nottingham grade I	Total score 7/8	Total score 6/8	1+	Total score 3/8	Total score 3/8	1+

2. 002	Invasive duct carcinoma Nottingham grade II	2/8	2/8	3+	2/8	2/8	1+
3. 003	Invasive duct carcinoma Nottingham grade II	6/8	4/8	1+	3/8	2/8	0
4. 004	Invasive duct carcinoma Nottingham grade II	6/8	5/8	0	4/8	4/8	0
5. 005	Invasive duct carcinoma Nottingham grade III	2/8	2/8	3+	0/8	0/8	2+
6. 006	Invasive duct carcinoma Nottingham grade I	7/8	6/8	1+	5/8	4/8	0
7. 007	Invasive lobular carcinoma Nottingham grade II	5/8	4/8	1+	3/8	3/8	0
8. 008	Invasive duct carcinoma Nottingham grade III	2/8	2/8	0	2/8	2/8	0
9. 009	Invasive duct carcinoma Nottingham grade II	2/8	2/8	3+	0/8	0/8	2+

10. 0010	Invasive duct carcinoma Nottingham grade II	7/8	6/8	0	5/8	5/8	0
11. 0011	Invasive duct carcinoma Nottingham grade III	0/8	0/8	3+	0/8	0/8	2+
12. 0012	Invasive lobular carcinoma Nottingham grade II	0/8	0/8	0	0/8	0/8	0
13. 0013	Invasive duct carcinoma Nottingham grade II	5/8	4/8	2+	2/8	2/8	1+
14. 0014	Invasive duct carcinoma Nottingham grade III	2/8	2/8	3+	0/8	0/8	2+
15. 0015	Invasive duct carcinoma Nottingham grade I	8/8	7/8	0	6/8	6/8	0
16. 0016	Invasive duct carcinoma Nottingham grade II	5/8	2/8	1+	2/8	0/8	0
17. 0017	Invasive duct carcinoma Nottingham grade II	6/8	7/8	2+	4/8	4/8	1+

18. 0018	Invasive duct carcinoma Nottingham grade II	5/8	6/8	3+	3/8	2/8	2+
19. 0019	Invasive duct carcinoma Nottingham grade III	2/8	2/8	1+	0/8	0/8	0
20. 0020	Invasive duct carcinoma Nottingham grade II	7/8	8/8	1+	6/8	6/8	0

There was a significant downgrading of the scores of ER, PR, and HER2 studies with 50% diluted formalin. 80% of cases showed a downgrading of the score of oestrogen receptor studies with 50% diluted formalin. Downgrading of the score of progesterone receptor studies with 50% diluted formalin was similar to the results of the oestrogen receptor study. There was downgrading of the HER 2 score in 70 % of cases when 50% diluted formalin was used.

According to the binomial sign test analysis there is a significant difference in the p values of the two groups (10% formalin versus 50% diluted formalin) for ER, PR and HER2 receptors

Discussion

There were statistically significant differences (p values) in the results of the two groups. There was a marked downgrading of the total score of ER and PR receptor studies when formalin with the optimum concentration was not used. The results of HER2 studies also revealed similar changes.

It is extremely important for the specimen to be fixed adequately in 10% buffered formalin before commencing receptor studies. Accurate information with regard to the receptor studies, helps the oncologist to decide on the most suitable drug regime for patients with breast carcinoma. Effective drug therapy will reduce the recurrence rate and prolong the disease free interval (11,12).

Surgical specimens that have been excised for breast carcinoma reach the histopathology

laboratories from the operating theatres. The tissue fixative (10% buffered formalin) is provided by the laboratory and is added to the specimen containers in the theatre complex. Over dilution of formalin can happen in the laboratories or in the operating theatres.

It is vital for laboratory as well as theatre staff to be aware of this problem which interferes with proper fixation of surgical specimens. Working together as a team and close communication between the laboratory and theatre staff may help to overcome most of the problems.

Suggestions to overcome the deficiencies:

1. Regular supervision of laboratory and theatre staff
2. Adherence to laboratory protocols and guidelines
3. Active participation in external and internal quality control programs
4. Enrolment for laboratory accreditation
5. Regular consultative meetings with all categories of laboratory and theatre staff
6. Improvement of storage facilities in theatre complexes and laboratories
7. Maintenance of an inventory
8. Regular audit checks
9. Continuous medical education
10. Regular checking of purchase orders

Conclusion

The results of the study indicated that the poor quality of formalin interfered with the accurate interpretation of ER, PR and HER2 receptor studies. Hence it is vital to check the quality of formalin at regular intervals. This task cannot be achieved without the help of different categories of staff involved in the process of purchasing chemicals, handling and dispatch. Administrative staff should be informed about the seriousness of this problem.

It is also advisable to seek advice from the heads of laboratories before purchasing chemicals. The problems with regard to the quality of chemicals can be addressed in such instances. Measures should be taken to improve the supervision of hospital stores where the chemicals are kept. Strict measures should be adopted to avoid malpractice during dispatch of formalin to the laboratories. In laboratories the preparation of chemicals should be carried out under the supervision of senior technicians. Regular monitoring of laboratory work by administrative staff, senior technicians and laboratory consultants will ensure the smooth functioning of routine laboratory work.

In addition, the regular participation in internal and external quality assurance programs will help to identify the strengths and weaknesses of each institution and thereby improve the quality of work.

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