Original Article



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A faster technique for intraoperative assessment of resection margins in breast cancer

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ABSTRACT

Objective: Breast cancer is considered the most common cancer among females. Breast-conserving therapy (BCT) is the preferred treatment option for females presented by early breast cancer. BCT is equivalent to modified radical mastectomy as regarding the overall survival. Intraoperative assessment of the lumpectomy margins is required, the best methods are frozen section (FS) and imprint cytology (IC).

Methods: This study was conducted on 40 female patients admitted to the Surgical Oncology Unit, Alexandria Main University Hospital in 2017, who were eligible for BCT. Excised specimens of breast conservation surgery were sent to the pathologist for both IC and FS to assess safety margins.

The study compared results of IC for the 259 margins with the results of paraffin section for the same number of margins. **Results:** Sensitivity of IC was 91.35%, and its specificity was 95%. The overall accuracy rate for this method was 94.21%. The sensitivity of FS was 96.91%, and its specificity was 94%. The overall accuracy rate for this method was 94.59%. **Conclusion:** Breast conservation therapy must be done in the presence of intraoperative safety margin assessment to improve survival and prevent recurrence. Imprint cytology is a fast and accurate method for intraoperative margin assessment in breast cancer.

Key words: Breast cancer, frozen section, imprint cytology, margin assessment

Introduction

Breast cancer is the commonest cancer among females and is responsible for most of the cancer-related deaths in females. Breast cancer represents 29% of all newly diagnosed cancers in females [1]. Breast conservation therapy (BCT), including wide local excision and sentinel lymph node biopsy or axillary lymph clearance followed by radiotherapy, is the preferable treatment for early-stage breast cancer. The overall survival is the same for BCT and mastectomy in females presented by early breast cancer [2,3].

The goal of BCT is complete tumor excision with adequate safety margins and maintaining acceptable cosmetic appearance. The positive margin at initial lumpectomy was reported to range between 15% and 47% [4].

Intra-operative margin assessment methods are created to provide information on margin status while

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the patient is still on the operating table. The major advantage of intraoperative margin status evaluation is to allow immediate re-excision during the same operation. Frozen section examination and imprint cytology are considered the best techniques for intraoperative evaluation of the lumpectomy margins, despite this, both of methods have some disadvantages [5,6].

The limitations of the frozen section are a long time needed and its cost. So, the amount of tissues which is examined is limited, and the false negative rate is encountered in 19% of patients [7]. Fat around the tumor may make it difficult to obtain good sections, so relying on FS alone may give a false interpretation of margin (closer than the actual distance). Moreover, Frozen section has an important advantage of helping the surgeon to obtain sufficient width of negative surgical margin.

Imprint cytology does not give any information about the width of the margin; it only shows tumor cells on the margins of the lumpectomy specimens [8]. There was a higher re-excision rate in studied depended upon paraffin (PS) section alone for margin assessment compared to intraoperative margin evaluation. The rates of re-excision ranged from 35% to 59% after margins assessed by paraffin sections alone [8,9]. Camp et al. reported that the rates of re-excision in patients by intraoperative frozen section and PS were 6.3% and 36.5%, respectively, and there was a significant difference [10].

Imprint cytology is an easy, fast and its cost is less than the frozen section. Survey of all the surface area of the resected biopsy is always possible by IC whereas this survey is difficult with non-cytologic techniques [11], but it has higher false negative rates when compared to the frozen section. The pitfalls of IC include errors of interpretation related to the irregularity of the specimen surface, dryness and the use of diathermy [12,13,14]. Moreover, training for cytopathology and certification is necessary for proper interpretation of the results [13]. Again, it is difficult to differentiate between invasive or in situ components of the tumor by cytological examination, while it can be assessed by frozen section.

Purpose: The aim of the present study was the study of intraoperative imprint cytology method in relation to frozen section method for margin evaluation in breast conservation surgery. The evaluation by hematoxylineosin (H&E)-stained paraffin-embedded sections was taken as a standard for comparing both methods.

Materials and Methods

This study was conducted on 40 female patients admitted to the Surgical Oncology Unit, Alexandria Main University Hospital, who were eligible for breast conservation surgery in 2017.

Exclusion criteria: Presence of any absolute or relative contraindication to breast conservation.

During the operation, the breast mass was excised with 1 cm macroscopic margin. Then surgical specimens were immediately marked with orienting sutures for intraoperative pathologic examination.

Excised specimens of breast conservation surgery were sent to the pathologist for both imprint cytology and frozen section to assess safety margin. All margins of the resected specimens were assessed microscopically by imprint cytology and frozen section. Frozen section was performed using the cryostat, which is essentially a microtome inside a freezer.

All excised specimens were processed for permanent section evaluation of hematoxylin-eosin (H&E)– stained paraffin-embedded sections that were taken as a standard for comparing results of both imprint cytology and frozen section. All patients underwent axillary lymph node dissection by a separate incision or sentinel lymph node biopsy (minimum two nodes) and frozen section examination. If the tumor was identified in the sentinel node, an axillary lymph node dissection was done during the initial operation.

Results

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp) Qualitative data were described using number and percent.

Table 1 shows the distribution of the studied cases according to demographic data. Table 2 shows the distribution of the studied patients according to the clinical features of the tumors. Table 3 shows the histopathological criteria of the tumors in the studied patients. Eight cases (20%) received neoadjuvant chemotherapy for downstaging of the tumor.

Postoperative paraffin section (PS) histopathology:

The study compared results of FS for the 259 margins with the results of paraffin section for the same

Table 1. Distribution of the studied cases according to demographic data (n= 40).

	No.	%				
Age (years)						
<40	5	12.5				
40 - 50	17	42.5				
>50 - 60	16	40.0				
>60	2	5.0				
Min. – Max.	26.0	- 65.0				
Mean ± SD.	49.2	2 ± 8.1				
Median	5	50.0				
Menopause						
Pre-menopause	12	30.0				
Post menopause	28	70.0				
Family History						
No	34	85.0				
Yes	6	15.0				

Table 2. Distribution of the studied cases according to different parameters (n=40).

	No.	%
Lump		
Non-palpable	0	0.0
Palpable	40	100.0
Quadrant		
Upper outer	29	72.5
Lower outer	4	10.0
lower inner	1	2.5
Upper inner	4	10.0
Central	2	5.0
Staging		
T 1	19	47.5
T 2	21	52.5
Axillary LNs		
No	19	47.5
Yes	21	52.5

Table 3. Distribution of the studied cases according to histopathology (n= 40).

3 , ()							
Histopathology	No.	%					
Pathological Type							
Invasive ductal carcinoma	35	87.5					
Invasive lobular carcinoma	2	5.0					
Mixed	3	7.5					
DCIS							
No	20	50.0					
Yes	20	50.0					

number of margins. There were 57 true positive margins (22.01%) that were positive on both FS Figures (1,2) and PS. There were 188 true negative margins (72.59%) that were negative on both FS and PS. There were two false negative margins (.77%) that were negative on FS and positive on PS. There were 12 false positive margins (4.63%) that were positive on FS and negative on PS. The sensitivity of FS was 96.91%, and its specificity was 94%. The positive predictive value was 82.61%, and the negative predictive value was 98.95%. The overall accuracy rate for this method was 94.59% tables 5 and 6.

The study compared results of IC for the 259 margins with the results of paraffin section (PS) for the same number of margins. There were 54 true positive margins (20.85%) that were positive on both IC Figures (3-5) and PS. There were 190 true negative margins (73.36%) that were negative on both IC and PS. There were five false negative margins (1.93%) that



Figure 1. Positive margin by frozen section (H&E x400).



Figure 2. Positive margin by frozen section (H&E x400).

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Table 4. Relation paraffin and imprint cytology (n=259)

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Paraffin											
	Negative (n=200) Positive (n=		e (n=59)								
	No.	%	No.	%	X ²	р	Sensitivity	Specificity	PPV	NPV	Accuracy
Imprint cytology											
Negative	190	73.36	5	1.93	164 710*	<0.001*	01 52	05	01 20	07.44	04.21
Positive	10	3.81	54	20.85	104.710	<0.001	91.55	90	04.30	97.44	94.21
Kappa (Agreement)	0.787	7*(Substan	tial agree	ment)							

Table 5. Relation paraffin and frozen section (n=259).											
		Para	affin								
	Negativ	e (n=200)	Positive	e (n=59)							
	No.	%	No.	%	X ²	р	Sensitivity	Specificity	PPV	NPV	Accuracy
Frozen section											
Negative	188	72.59	2	.77	200 200*	~0.001*	06.01	04	82.61	08.05	04 50
Positive	12	4.63	57	22.01	200.390	<0.001	90.91	94	02.01	90.90	94.59
Kappa (Agreement)	0.862*	(Almost pe	erfect agre	ement)							

Table 6. Relation between frozen section and imprint cytology.									
	Sensitivity	Specificity	ЪРV	NPV	Accuracy				
Imprint cytology	91.53%	95%	84.38%	97.44%	94.21%				
Frozen section	96.91%	94%	82.61%	98.95%	94.59%				

were negative on IC and positive on PS. There were ten false positive margins (3.81%) that were positive on IC and negative on PS. The sensitivity of IC was 91.53%, and its specificity was 95%. The positive predictive value was 84.38%, and the negative predictive value was 97.44%. The overall accuracy rate for this method was 94.21% tables 4 and 6.

Discussion

Breast cancer is one of the commonest cancers worldwide among women. The main line of treatment of non-metastatic breast cancer is surgery whether it is breast-conserving surgery (BCS) or modified radical mastectomy (MRM).

Intraoperative margin assessment the advantage of immediate re-excision that can be done during the same operation [5,13]. The best methods for intraoperative margin assessment are frozen section analysis and imprint cytology, but both methods have some disadvantages.

There were many studies which assessed the use of frozen section for margin assessment in breast conservative surgery. Cendan et al. [15] did his study in 2005 at the USA on 97 cases of breast cancer in which the age ranged from 48 to 71 years. Olson et al. [16] did his study in 2007 on 290 patients with breast cancer, and the age ranged from 27 to 89 years. Weber et al. [17] also did a comparative study between frozen section and paraffin section in 2008 at Switzerland, and it was done on 80 patients, and the age ranged from 34 to 86 years. The current study was done on 40 patients with breast cancer in which the patients' age range from 26 to 65 years.

Data from the literature reported that frozen section might give a false positive margin ranging between 0% and 0.4%, and a rate of false negative results between from 0.5% and 3.4% [18]. The technical problems mentioned above have never occurred among 672 cases in a study made by Bianchi et al. [7]. Cendan et al. had no false positive cases in his study and had 22 false negative cases most of them were DCIS, but Olson et al. had 27 false positive cases and 17 false negative cases. There were 12 false positive cases (4.63%) and two false negative cases (77%) in the results of frozen section in comparison to paraffin section at the current study.

Frozen section examination was proved to be a reli-



Figure 3. Positive margin imprint cytology (H&E x100).



Figure 4. Positive margin imprint cytology (H&E x400).



Figure 5. Positive margin imprint cytology (H&E x400).

able and accurate method [7,19]. It has a sensitivity of 91.7% to 97.9%, and a specificity of 89.5% to 100% [7,16,17]. Sensitivity and specificity of the frozen section were discussed in many studies. Cendan et al. reported that the Sensitivity was 58.1 % and the specificity was 100.0 % while Olson et al. reported 73.1 % Sensitivity and 99.6 % specificity. Weber et al. reported 80.0 % Sensitivity and 87.5 % specificity. The sensitivity of frozen section of the current study was 96.91%, and its specificity was 94%.

Imprint cytology has been studied by many authors for intraoperative assessment of resection margins [20,21]. It has a sensitivity between 80% and 100%, and a specificity between 85% and 100% [20,21]. It is an easy, fast and its cost is less than the frozen section. The whole surface area of the resected specimen margin can be examined by imprint cytology, but it has higher false negative rates than the frozen section. IC has some disadvantages, such as interpretation errors related to the irregularity of the specimen surface, dryness and use of diathermy [12,14]. Furthermore, IC needs more training and certification to obtain successful results. It is difficult to differentiate between invasive and in situ component of the tumor by IC, while it can be done by frozen section [22].

Many studies assessed the use of imprint cytology for margin assessment in breast conservative surgery. Cox et al. [23] did his study in 1997 on 104 cases in which the age ranged from 30 to 82 years. Klimberg et al. [6] did his study in 1998 on 428 cases in which the age ranged from 22 to 86 years. Valdes et al. [24] had 61 cases in his study in 2007.

Few studies were done to compare frozen section and imprint cytology at the same study with comparing the results with paraffin section as gold standard.

Tribe et al. [25] did his study on 311 cases in which he used both frozen section pathology and imprint cytology methods for intraoperative margin assessment. He had 0% false positive and 1.6% false negative in frozen section results. He had 0.65% false positive and 5.15% false negative in imprint cytology results.

Sakai and Lanslanti et al. [26] did his study on 196 cases, and he used both frozen section pathology and imprint cytology methods for intraoperative margin assessment. He had 0% false positive and 0.1% false negative in frozen section results. He had 0% false positive and 0% false negative in imprint cytology results.

Esteban et al. [27] did his study on 140 cases in which he used both frozen section pathology and imprint cytology methods for intraoperative margin assessment. He had 1.1% false positive and 0.7% false negative in frozen section results. He had 1% false positive and 2% false negative in imprint cytology results.

Ku et al. [12] and Cox et al. [14] did his study on 90 cases in which he used both frozen section pathology and imprint cytology methods for intraoperative margin assessment. He had 0% false positive and 4.4% false negative in frozen section results. He had 2.2% false positive and 0% false negative in imprint cytology results.

The current study proved that both frozen section pathology and imprint cytology methods are the good methods for intraoperative margin assessment that can prevent local recurrence. The current study also proved that both methods are almost equal in assessing safety margin.

Tumor must be excised with a negative safety margin to improve survival and prevent recurrence. Both frozen section and imprint cytology can be used safely to assess the margins intraoperatively. Sensitivity and specificity of both methods are excellent and almost equal. Coupling frozen sections and imprint cytology in such cases may increase diagnostic accuracy and avoid false results. Imprint cytology is an easier and faster method for intraoperative margin assessment in breast cancer.

BCT should not be done without intraoperative margin assessment that can be done using either FS or IC.

Conflict of interest statement

The authors have no conflicts of interest to declare. **References**

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