Histopathological Study of Breast Cancer After Ultrasound Guided Cryotherapy Ablation

Dong-Won Ryu
Department of General Surgery, Kosin University College of Medicine, Busan, Korea

Abstract

Introduction

Widespread use of screening mammography and breast cancer awareness is detecting more women at younger ages with earlier stages tumors. Less invasive procedures have been increasingly used for the treatment of breast cancer due to a lack of benefit from more radical forms of therapy. Cryotherapy has been used for ablation of both malignant and benign tumors for a long time. In 1850 treatment of a malignant tumor by cooling to low temperatures was reported for the first time. In 1963 cryosurgery underwent a renaissance and it has since been used for the treatment of malignant prostate and liver tumors. However, the practice is not new and cryotherapy has been used for more than 30 years in the treatment of small as well as advanced and unresectable breast cancer. Cryoablation destroys tissue through localized freezing. Maximum tissue destruction is achieved through multiple freeze-thaw cycles, which are determined by the size of the tumor. In contrast, fewer studies have been published in breast cancer in humans and few of them have focused on the microscopic lesions of breast cancer and normal breast tissue after cryotherapy. In this study, we analysed the histopathological aspects of tumor in 6 patients who underwent tumorectomy before cryotherapy.
Material and methods

Between April 2007 and October 2007, 6 patients with ultrasound (US)-visible primary invasive breast cancer were enrolled in the study approved by the local medical ethics committee. All participating patients signed written, informed consent. The clinical characteristics of the patients are listed in Table 1. The patients were between 50 and 82 years (mean age: 63 years). The diagnosis of invasive breast cancer was made on a large-core needle biopsy before cryotherapy. The mean size of the tumor was 4.7 cm on US. There were 6 invasive ductal carcinomas of varied histological grade. They all underwent ultrasound-guided cryoablation after surgical resection. Postoperative radiotherapy and hormonal therapy were later performed according to the general guidelines for breast cancer therapy.

Table 1. Summary of clinical, imaging and pathological data of patients before cryotherapy

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)</th>
<th>Tumor Type</th>
<th>Histologic Grade</th>
<th>Tumor Localization</th>
<th>Clinical Size (cm)</th>
<th>Ultrasound Size (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65</td>
<td>IDC</td>
<td>G2</td>
<td>Rt upper</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>IDC</td>
<td>G3</td>
<td>Lt upper</td>
<td>6.0</td>
<td>5.5</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>IDC</td>
<td>G3</td>
<td>Rt lateral</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
<td>IDC</td>
<td>G2</td>
<td>Rt lower lateral</td>
<td>6.0</td>
<td>6.0</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>IDC</td>
<td>G3</td>
<td>Lt lower lateral</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>6</td>
<td>80</td>
<td>IDC</td>
<td>G2</td>
<td>Lt upper</td>
<td>6.0</td>
<td>6.0</td>
</tr>
</tbody>
</table>

IDC, invasive ductal carcinoma; Histologic Grade, Elston & Ellis Classification of Histologic Grade

Cryoablative procedure

Cryotherapy was done at resected breast cancer specimen containing surgical safe margin. Cryotherapy was performed using a CRYO-HIT TM System-3 (Galil Medical, Yokneam, Israel). It is a gas system using argon as the cooling agent, which was designed to create probe temperatures of −180°C. One cryotherapy needle was inserted in the tumor under US guidance. The tip of the cryoprobe was placed via the center of the lesion at the opposite margin of the tumor (Fig. 1).

Histological analysis

Tumorectomy specimens were fixed in 4% formaldehyde. They were then sliced to grossly determine the dimensions of the region exposed to cryosurgery and photographed. The cryoablation region and surgical margins were sampled, embedded in paraffin, and sections stained with Hematoxylin-Eosin (HE). Tumor destruction or residual invasive and/or in situ carcinoma was documented. Staging of the residual tumor was based on TNM/AJCC staging system (6th ed.)

Results

In 2 cases, the targeted tumor area was completely included in the cryozone. The cryozone or “iceball” is a well-defined yellow round lesion with a central hemorrhagic core. In this case, the tumor, a white stellate mass with irregular borders is found adjacent to the cryozone. Residual tumor in the cryozone is illustrated in Figure 2.
Fig. 2. Post cryotherapy histology. Hyaline necrosis with hemorrhage in the center of the cryo lesion. Tumor area was replaced by dense fibrosis partly admixed with coagulative necrosis, usually without recognizable tumor parts. (H&E stain, x40)

**Tumor response**

The pathological evaluation of the tumor after cryotherapy is summarized in Table 2. The tumor response after cryotherapy was very variable. In 2 cases, there was no evidence of residual invasive carcinoma (IC) or ductal carcinoma in situ (DCIS). In these cases, the tumor area was replaced by dense fibrosis partly admixed with coagulative necrosis, usually without recognizable tumor parts. In 4 cases, there was evidence of residual IC of various sizes with or without DCIS (inside or outside the cryozone).

**Table 2. Summary of histopathological evaluation of tumors after cryotherapy**

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Residual invasive carcinoma type</th>
<th>Size (cm) Tumor area</th>
<th>Histology in Tumor area</th>
<th>DCIS inside cryozone</th>
<th>DCIS outside cryozone</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>2</td>
<td>No tumoral necrosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Yes</td>
<td>3.5</td>
<td>No tumoral necrosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>No</td>
<td>0</td>
<td>Fibrinoid necrosis</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Yes</td>
<td>3.5</td>
<td>No tumoral necrosis</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>No</td>
<td>0</td>
<td>Fibrosis and fibrinoid necrosis</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Yes</td>
<td>3.5</td>
<td>No tumoral necrosis</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

DCIS, ductal carcinoma in situ.

**Complications of cryotherapy**

Skin ulceration and/or necrosis were seen in five patients. The cryozone was in contact with the skin. No other complications were reported.

**Discussion**

The present study demonstrates that cryotherapy is a feasible procedure in the treatment of breast cancer without severe complications. In this study, cryotherapy allowed variable and unpredictable tumor destruction. Only two patients (33%) had a complete response without remnants of viable tumor cells. Four patients (66%) had partial response with some degree of tumor downstaging. In previous studies of breast cancer cryoablation, the technique has been reported as being more successful in destroying invasive carcinoma (IC) rather than ductal carcinoma in situ (DCIS) as well as destroying smaller (<1.5 cm) tumors. In their latest study in 2005, Pfleiderer et al. studied cryoablation under ultrasound guidance on 30 breast tumors. The tumor diameter was 15 cm or smaller (mean: 12 mm). The tumors were resected within 6 weeks of cryoablation. In five patients, there was remnant of DCIS beyond the margin of the cryozone. In 24 patients, no viable tumor cells were found. No severe side effects were observed. Cryoablation induces tissue destruction through at least three important mechanisms: intracellular ice formation and osmotic injury, both direct effects; and ischemia, an indirect mechanism. A possible theory might be a greater sensitivity of IC to hypoxia-ischemia, which as mentioned above is the dominant injury mechanism of cryotherapy. Hypoxia-inducible factor-1 (HIF-1) activates the transcription of many genes controlling glucose and high-energy phosphate metabolism, growth factors, erythropoiesis, heme metabolism, iron transport, vasomotor regulation, and nitric oxide synthesis and, thus, may increase the survival of tumor cells under hypoxic conditions. Also, as in chemo or radiotherapy, the stage of the cell cycle can also influence cryosensitivity. Besides these biological factors, the location of DCIS in the tumor might also explain to some extent its apparent greater resistance to cryosurgery. Indeed, in many cases the DCIS is more often located in the peripheral area, which is
further away from the cryoprobe and thus less sensitive to cryoinjury.\textsuperscript{11, 12}

In cases where the procedure does not destroy the whole tumor (for example in tumors >1.5 cm), cryotherapy (with sublethal temperature) might behave similarly to chemotherapy, destroying susceptible clones and allowing cryoresistant clones that produce stress-induced protective (cold shock or HIF-1 like) proteins to survive.\textsuperscript{13} Besides tumoral destruction due to direct and indirect damage, cryoablation might also have additional antitumor effects. One of the principal advantages of cryoablation over conventional treatment and other ablative techniques is that cryosurgical destruction of tumor could elicit an antitumor response and improve localized or secondary destruction of tumor cells (cryoimmunology).\textsuperscript{14, 15} Because nearly all ablation studies are retrospective and have been coupled with postprocedure resection in order to evaluate the efficiency of cryotherapy, the natural history of the primary tumor after cryoablation, with or without tumoral response, is not known and can be only guessed from the tumor response to cryotherapy on tumorectomy specimens.\textsuperscript{16} Although cryotherapy does not achieve complete tumor ablation in every case, even partial tumor sterilization might still be satisfactory because adjuvant radiotherapy might destroy the residual tumor cells.\textsuperscript{17} In the literature, there is only one report of a cryotherapy treatment not followed by surgical excision. It could have been of interest to correlate local destruction of the tumor by the cryotherapy with imaging studies and with precise temperature measurement within the tumor or around it using thermosensors. In cryotherapy and other ablative techniques used for small tumors, ablated tissues remain in the breast for resorption over time. Hence, the ability to pathologically examine the specimen is compromised.\textsuperscript{18} Therefore, histopathological and biological information regarding the tumor must be obtained in advance using tissue biopsy and imaging techniques which usually give enough morphological and biological informations about the tumor. A better knowledge and definition of the pathological response of the tumor to cryotherapy in lumpectomy specimens will also provide a base whether a post cryotherapy biopsy or lumpectomy is necessary in order to evaluate residual or recurrent disease.

**Conclusion**

Cryotherapy allows variable tumoral destruction which is not influenced by tumor size or other histological parameter. Cryotherapy of breast cancer is an attractive minimally invasive technique which might be used as an adjuvant or neoadjuvant treatment or to guide lumpectomy; however, as with all ablative techniques, prospective randomised trials are needed to determine its long-term effectiveness on breast cancer.

**Acknowledgment**

The present has been supported by Korea Breast Cancer Foundation

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