Immunohistochemical changes of adenomyosis after heat therapy: comparison of radiofrequency myolysis and endoablation

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Summary

Purpose: To check the pathologic changes of focal adenomyosis after heat therapy using radiofrequency and to evaluate which approach – endometrial ablation or direct heat therapy – is better for adenomyosis. To evaluate whether the timing of the procedure and the menstrual cycle are related to pathologic outcomes after heat therapy. *Methods:* This study included nine women who underwent total hysterectomy for adenomyosis (diameter, ≥ 6 cm). Six fresh uteri were excised in the midline and subjected to radiofrequency heat therapy at the center of the adenomyomas (direct heat therapy) and three uteri were subjected to endometrial ablation. Thereafter, 1 cm^3 myometrial tissue was obtained at 1 cm, 2 cm, and 3 cm away from the endometrium. Tissue sections were stained with hematoxylin and eosin. Immunohistochemical analysis using antibodies against cytokerain-19 (CK-19), actin, and estrogen receptor/progesterone receptor (ER/PR) was performed to evaluate CK-19 (endometrial epithelium marker), actin (myometrial marker) and ER/PR (checking the state of the menstrual cycle), respectively. *Results:* After endometrial ablation, cauterized tissues were not noted 2 cm away from the endometrium. All tissues between the endometruim and center of adenomyosis were cauterized after direct heat therapy. During the uterine proliferative phase, unlike the secretory phase, subendometrial layers were cauterized 10 min after direct cauterization. *Conclusion:* Direct heat therapy is more effective than endometrial ablation in adenomyosis, and heat is conducted effectively when the patients are in the proliferative phase.

Key words: Radiofrequency; Adenomyosis; Heat therapy.

Introduction

Adenomyosis uteri is a common gynecological disorder with unclear etiology leading to menorrhagia, dysmenorrhea, and infertility [1]. Classically, various surgical and medical treatments have been prescribed for managing uterine adenomyosis; however, the most effective treatment for symptomatic adenomyosis is still total hysterectomy [2].

To avoid total hysterectomy, less aggressive therapeutic options have been introduced. These include surgical excision of adenomyomas, uterine artery embolization, and heat therapy [3]. Radiofrequency myolysis, which is indicated as a topical heat therapy for uterine leiomyomas, results in 73% volume reduction and 90% symptom-improvement rates after an 18-month treatment regimen [4]. Heat therapy for adenomyosis is an endometrial ablation therapy because histopathologically, adenomyosis is caused by endometrial invasion into the myometrium [2, 5-7]. Although direct heat therapy for adenomyosis nodules has been applied using MRgFUS, this approach is still uncommon.

Although following radiofrequency myolysis, myomas show inflammatory reactions with hyaline degeneration [8], the histopathological changes following heat therapy in adenomyosis patients are still unknown. An adenomyosis nodule is composed of ectopic endometrium and hypertrophied myometrium [9]. Adenomyosis tissue reaction to heat may differ from leiomyoma reactions but the precise histopathological reactions have not been studied.

In this study, we used immunohistochemical analysis to study histopathological changes of fresh adenomyosis tissues after heat therapy. Heat-therapy-induced histopathological reactions differed with menstrual cycle, the depth of heat conduction, or the direction of heat application. Heat application was applied for complete endometrial ablation or for direct heat therapy into the adenomyotic lesions.

Materials and Methods

Patient selection

Nine patients, who underwent total hysterectomy due to previous focal uterine adenomyosis lesions of over 6 cm in diameter were enrolled in this study. Six patients were in the proliferative phase and three in the secretory phase (Figure 1). Fresh uteri were used for endometrial ablation and direct heat therapy after total hysterectomy. The study protocol was reviewed and approved by the Ethics and Research Committee of the Catholic University Medical College of Korea.

Methods

Endometrial ablation was done on three uteri in the proliferative phase (group A). Direct heat therapy on adenomyotic nodules was done on the remaining three uteri in the proliferative phase (group B) and three uteri in the secretory phase (group C). Endometrial ablation was done using a radiofrequency generator for 10 min at 80 W, a commonly used setting

Revised manuscript accepted for publication April 11, 2011

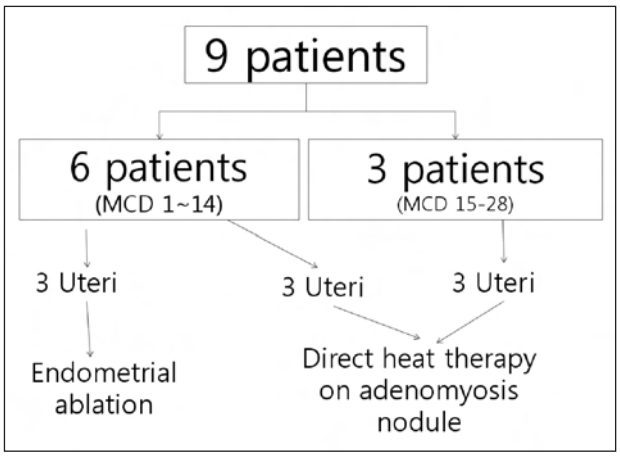

Figure 1. — Study design.

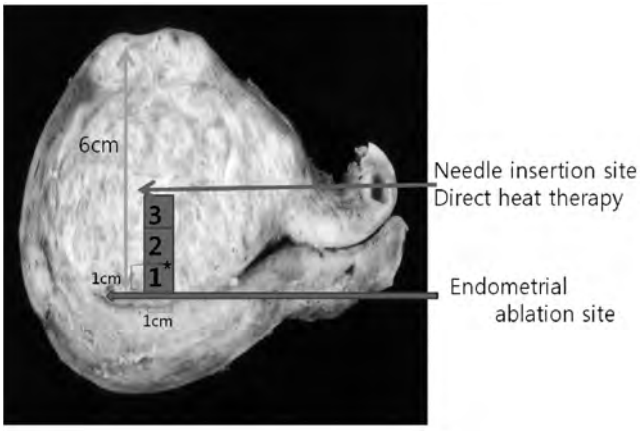

Figure 2. — The photograph shows the sites of heat therapy site and biopsy. *: biopsy site, 1: point 1, 2: point 2, 3: point 3.

for radiofrequency endometrial ablation. Direct heat therapy on adenomyotic nodules was performed as follows: a needle was inserted parallel to but 3 cm away from the endometrium (at the center of the adenomyotic nodule) and heated for 10 min at 90 W, which is a usual setting for leiomyoma ablation (Figure 2). After heat therapy, the myometrium was biopsied using 1 cm^3 of tissue sections taken at 1 cm (point 1, including endometrium), 2 cm (point 2), and 3 cm (point 3) away from the endometrium. Heat conduction direction was from point 1 to point 3 during endometrial ablation, and from point 3 to point 1 during direct heat therapy.

Staining

Hematoxylin-eosin staining and immunohistochemical analysis using cytokeratin-19 (CK-19) and actin antibodies were performed on the biopsied specimens.

Four-micrometer-thick paraffin sections were heated for 1 h at 58°C. For deparaffinization, tissue sections were treated with xylene and rehydrated in graded ethanol. For antigen retrieval, the sections were treated with 0.01 M citric acid buffer (pH 6.0) (DakoCytomation, Carpinteria, CA, USA) and boiled in a microwave three times for 5 min. Sections were then treated with 3% hydrogen peroxide in methanol for 10 min at room temperature to quench endogenous peroxidase activity. After washing the excess solution off the slides, slides were incubated with the primary antibody for 1 h at room temperature and rinsed with the wash buffer. After 10 min, slides were incubated with Dako Envision/HRP-conjugated antibodies (secondary antibody and dextran polymerase) for 30 min and finally rinsed. The primary antibodies used were anti-mouse anti-SMA antibody against actin (Neomark) and RCK 108 (Biogenex, San Ramon, USA) against CK-19. For checking estrogen receptor/progesterone receptor (ER/PR), an immunohistochemistry kit (Abbott, Germany) was used. After incubation with 20% sheep serum for 15 min, sections were incubated with primary sheep monoclonal antibody (ERICA and PRICAm Abbott, Germany) at 4°C overnight. Sections were incubated with sheep anti-rat IgG for 2 h and washed in phosphate-buffered saline (PBS). To visualize immunohistochemical reactions, sections were incubated with the peroxidase-anti-peroxidase complex for 2 h and diaminobenzidine tetrahydrochloride and $H2O_2$ were added and incubated for 12 min until color development. Counterstaining was in hematoxylin for 5 min.

The specimens from all patients were examined by two histologists who were blinded to the details of the study. The sections were evaluated for actin and CK-19 expression based on the percentage of stained cells: (–) trace (0–25% of cells stained), (+) weak positive (26-50% of cells stained), (++) moderate (51-75% of cells stained), and (+++) strong (76-100% of cells stained).

Results

Results of immunohistochemical analysis

After endometrial ablation in group A, ectopic endometrial tissue at point 1 (1 cm away from the endometrium) showed trace antibody reactivity to CK-19 and actin, whereas this reactivity increased from moderate to strong positive staining at points 2 and 3 (2 cm and 3 cm away from the endometrium, respectively). In group B, all ectopic endometrial tissues at points 1, 2, and 3 were weakly positive for CK-19 and actin after direct heat therapy. These results suggest that endometrial ablation could not effectively cauterize the adenomyotic lesions located deep in the myometrium 2 cm peripheral to the endometrium (Table 1).

To compare the results after direct heat therapy during the menstrual phase, group B and group C were examined.

Table 1. — *Comparison of immunohistochemical findings for CK-19 and actin after endometrial ablation and direct heat therapy of adenomyosis*.

*		Endometrial ablation (n = 3)	Adenomyosis direct heat therapy (n = 3)
1 cm	CK-19	±	±
	Actin	±	+
2 cm	CK-19	++	±
	Actin	+++	+
3 cm	CK-19	+++	±
	Actin	+++	±

*: distance from endometrium.
±: trace, +: weakly positive, ++: moderately positive, +++: strongly positive.

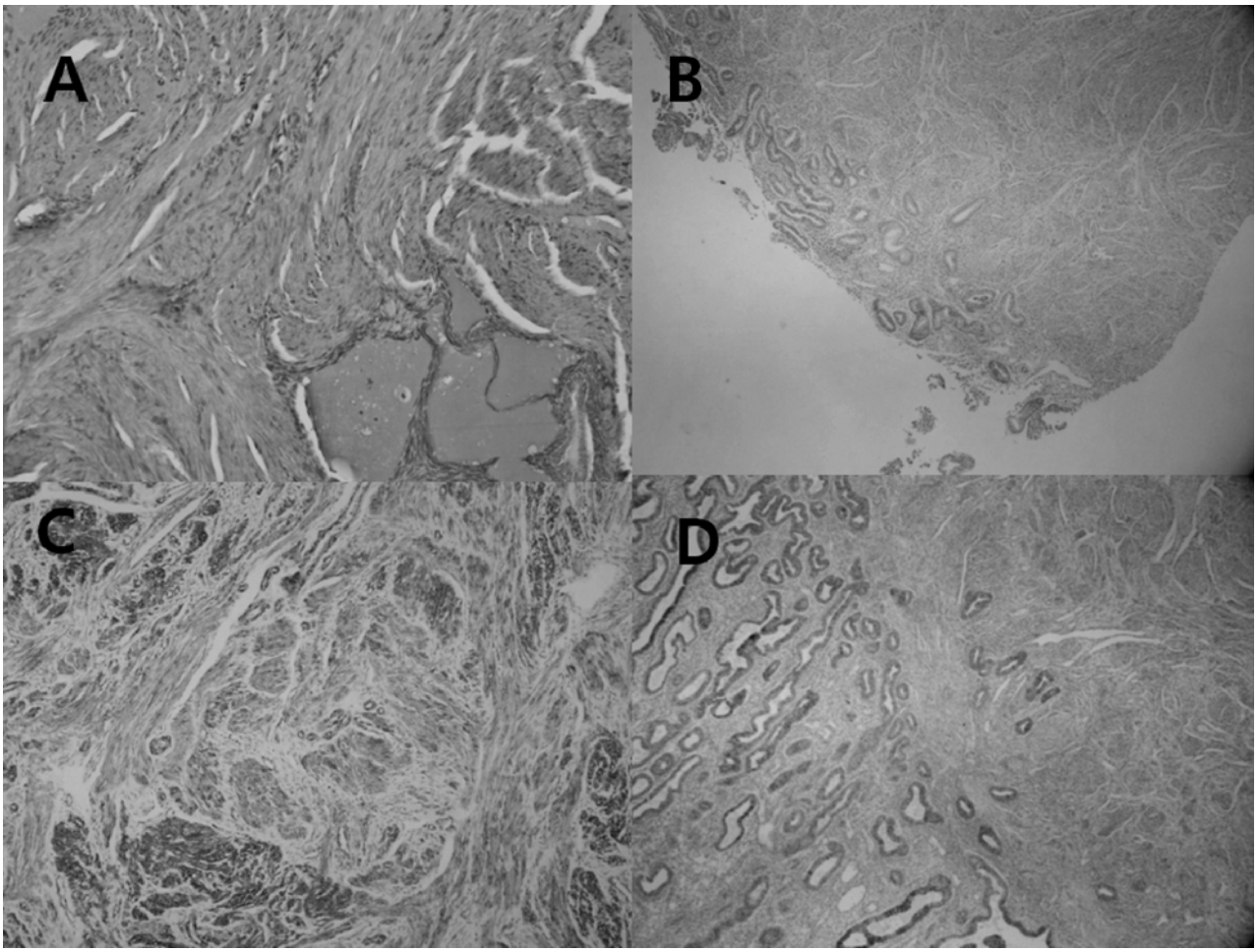

Figure 3. — Comparison of actin (A, C) and CK-19 (B, D) immunohistochemical findings following radiofrequency ablation during the proliferative (A, B) or secretory phase (C, D).
Actin antibody weekly stained in the proliferative phase (A) and moderately stained the myometrium in the secretory phase (C). CK-19 staining showed trace reactivity in the proliferative endometrium (B) and was moderately positive in the secretory endometrium (D) (objective power: 400×).

Uteri in group B, which were in the proliferative phase, showed trace to weak CK-19 and actin immunoreactivity at points 1, 2, and 3. In group C, point 2 and 3 showed trace to weak CK-19 and actin immunoreactivity, but CK-19 and actin were moderately positive at point 1 (Figure 3). This finding suggests that cauterization after direct heat therapy was more effective in the proliferative phase.

Discussion and Conclusions

Adenomyosis is a common benign gynecological disorder characterized by presence of endometrial islets within the myometrium, typically situated at least 2.5 mm below the endometrium-myometrium (EM) junction [9]. The posterior uterine wall is more often affected than the anterior wall [10, 11].

Uterine extirpation had been the only therapy completely resolving uterine adenomyosis symptoms [2]. However, other options allowing uterine preservation in uterine adenomyosis patients comprise medical treatment, surgical resection, uterine artery embolization, and hysteroscopic procedures [3].

Cytoreductive surgery results in an increased sensitivity to hormonal treatment due to improved blood supply to the adenomyotic tissues and leads to improved immunity [12]. In contrast to leiomyoma, adenomyotic lesions are not well-demarcated or segregated from the adjacent myometrium. Removal of adjacent healthy myometrium may increase the risk of bleeding and negatively affect the uterine tensile strength during pregnancy and labor. Intra- and perioperative complications after adenomyomectomy such as pyrexia, infections, adhesions, and uterine rupture can develop after surgery [13, 14]. In leiomyoma patients, decreased ovarian function [15-17] and gestational complications [18] have been reported following uterine artery embolization while operational safety in adenomyosis patients was not guaranteed.

Endometrial ablation has been used for treatment of adenomyosis symptoms such as menorrhagia [2, 3]. It is believed that adenomyosis results from the abnormal

ingrowth and invagination of basal endometrium into subendometrial myometrium and an intact endometrium-myometrium interface may be important in preventing adenomyosis [19]. Wood *et al*. reported a symptom-relief rate of 55-67% after endometrial resection from 1993 to 1998 [2, 5, 6], but McCausland *et al*. reported no symptom improvement in seven adenomyosis patients treated by endometrial resection. The authors insisted that adenomyotic lesions located more than 2 mm from the endometrial layer have a poor chance of improvement after ablation therapy [20, 21].

In this report, adenomyotic lesions below 1 cm (point 1), 2 cm (point 2), and 3 cm (point 3) from the endometrium were examined. After endometrial ablation, ectopic endometrial tissues at points 2 and 3 were intact by immunohistochemical staining, indicating that these lesions were not cauterized and endometrial ablation was ineffective in managing uterine adenomyosis. Using direct radiofrequency heat therapy, the ablation needle was inserted up to 3 cm deeper than the endometrium. Immunohistochemical results in these lesions indicated that ectopic endometrial tissues at points 1, 2, and 3 were completely cauterized. Heat therapy is uncommonly used in adenomyosis. Rabinovici *et al*. reported successful gestation in patients with previous focal adenomyosis and treatment using heat therapy by MRgFUS [22]. Significant symptom improvements (26%) and 12.7% volume reduction were reported six months after heat therapy [23, 24].

The most important point in managing adenomyosis using heat therapy is the consideration that adenomyosis pathologically differs from leiomyoma. Adenomyotic nodules are a mixture of normal smooth muscle cells and ectopic glandular and stromal endometrial tissue, with hypertrophied smooth muscle cells around the ectopic endometrium. In these results, histopathology confirmed varying extent of tissue cauterization due to the menstrual cycle. In the proliferative phase, heat cauterized all the ectopic endometrial tissue from point 3 to point 1. However, in the secretory phase, point 1 was not cauterized. Heat conduction may have been affected due to prominent endometrial glandular secretions and prominent stromal vessels during the secretory phase. The conductive heat radiation was thought to be more effective in the living donors because of the blood flow. Therefore, much more time was required to cauterize adenomyotic lesions than leiomyomas. This possibility should be considered when performing heat therapy in uterine adenomyosis. The limitations of our study are the limited number of cases and conduct of the study in situ. Heat therapy could be a better therapeutic option considering preservation of the uterus in adenomyosis patients. Further heat-therapy studies are required to allow determination of the in vivo histopathological changes, patients' symptom improvement, and pregnancy potential in adenomyosis patients.

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