Original Article

Histologic Characteristics of Vaginal Cuff Tissue From Patients With Vaginal Cuff Dehiscence

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ABSTRACT **Study Objective:** To describe the histologic characteristics of vaginal tissue in patients with vaginal cuff dehiscence (VCD) after robotic hysterectomy and to compare this group with patients without dehiscence.
Design: Retrospective analysis (Canadian Task Force classification II-3).
Setting: Academic center.
Patients: Seven patients with VCD and 6 patients without VCD.
Interventions: Vaginal cuff tissue was obtained from all patients and was stained using hematoxylin-eosin and evaluated for acute and chronic inflammation markers including neutrophils, lymphocytes, and plasma cells. Immunohistochemical staining was performed and evaluated using the semiquantitative method for collagen types I and III, smooth muscle actin, and SM22α (myofibroblast) content. Grading was performed by 4 blinded investigators. The Mann-Whitney test was used to evaluate the 2 groups, and correlation coefficients for interobserver variability.
Measurements and Main Results: The VCD group, compared with the non-VCD group, demonstrated significantly greater numbers of neutrophils (1.71 vs 1.0; p = .04), lymphocytes (2.85 vs 1.33; p = .002), and plasma cells (2.2 vs 1.0; p = .001). There was no statistical difference between the groups in amounts of collagen I (1.71 vs 1.27; p = .09) and collagen III (1.66 vs 1.38; p = .37), smooth muscle actin (1.23 vs 1.33; p = .65), and SM22α (1.85 vs 1.27; p = .09). Interobserver variability was low (κ = 0.86; p = .76).
Conclusion: Compared with the control group, patients with VCD demonstrated significantly higher levels of acute and chronic inflammatory cells. This finding suggests that a prolonged inflammatory phase may be delaying normal progression to reparation in patients with dehiscence. Journal of Minimally Invasive Gynecology (2014) 21, 442–446 © 2014 AAGL. All rights reserved.

Keywords: Histologic characteristics; Robotics; Vaginal cuff dehiscence

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Vaginal cuff dehiscence, a rare complication of gynecologic surgical procedures, can be devastating, in particular when associated with bowel evisceration. A review of medical records from 1970 to 2001 at the Mayo Clinic (Rochester, MN) revealed a low incidence (0.03%) of vaginal cuff dehiscence after pelvic surgical procedures performed via both abdominal and vaginal approaches [1]. In contrast, total laparoscopic hysterectomy and robotic hysterectomy were associated with a higher incidence of vaginal cuff dehiscence, which was as high as 4.9% in a single year and 4.1%, respectively, at separate large tertiary care centers [1,2], When compared with abdominal, vaginal, and laparoscopically assisted vaginal hysterectomy, total laparoscopic hysterectomy was

associated with a higher incidence of dehiscence (risk ratio 2.0, 6.9, and 1.6, respectively) [3]. An increasing number of laparoscopic and robotic hysterectomies are being performed in the United States. For laparoscopic hysterectomy, one study showed an increase from 0.3% in 1990 to 11.8% in 2003 [4]. A more recent study showed that laparoscopic hysterectomy rates increased from 24.3% in 2007 to 30.5% of all hysterectomies in 2010 in 441 hospitals across the United States. During the same period, the use of robotic hysterectomy also increased, from 0.5% to 9.5% [5]. With 10% to 30% of hysterectomies being performed via the laparoscopic and robotic routes, there would be an estimated 3000 vaginal cuff dehiscence (VCD) cases per year.

To provide better insight into the healing process in patients with dehiscence after robotic hysterectomy, we sought to describe the histologic findings of vaginal cuff tissue by evaluating it for inflammatory and healing markers. Specimens were compared with those from a group without VCD to determine whether there was any difference.

Materials and Methods

Twenty-one patients had VCD after robotic hysterectomy performed from 2006 to 2009 [2]. All of the original robotically assisted hysterectomies were performed using monopolar scissors or a spatula for colpotomy. Further, no adhesion barrier materials were used. During repair of dehiscence, distal portions of the anterior and posterior cuff were excised using a cold knife or scissors. Seven of these vaginal cuff tissue samples in paraffin-embedded blocks were identified from the Pathology archives and were used for analysis. For a comparison group, archived vaginal tissue blocks from 6 patients who underwent robotically assisted upper vaginectomy after previous hysterectomy and did not have VCD were identified. Within the comparison group (i.e., non-VCD group), 5 hysterectomies were performed via the abdominal route and 1 via the laparoscopically assisted vaginal route. Indications for surgery in the comparison group included ovarian remnant syndrome and pelvic pain. Patients with prolapse, autoimmune conditions, and/or endometriosis were excluded from the non-VCD group. Demographic data recorded included age, body mass index, menopausal status, hormone use, smoking status, and time to VCD. Approval from the Mayo Clinic Institutional Review Board was obtained for the study.

Tissue blocks were cut into 5-μm sections, stained with hematoxylin-eosin, and evaluated for markers of acute and chronic inflammation including neutrophils, lymphocytes, and plasma cells. To evaluate for healing, immunohistochemical staining was performed on additional tissue slides for collagen types I and III, smooth muscle actin, and SM22α for muscle and fibroblast content.

Immunohistochemistry

Paraffin-embedded tissue blocks were cut into 5-μm sections, dewaxed in xylene, and rehydrated through graded ethanol solutions. After washing with Tris-buffered saline solution–Tween 20 (TBST), endogenous peroxidase was blocked using 3% H_2O_2 in TBST, and nonspecific binding was blocked using 1% bovine serum albumin and 5% normal secondary antibody host serum in TBST at room temperature for 1 hour. After rinsing with TBST, the slides were incubated with rabbit anti-collagen I (1:100), mouse anti-collagen III (1:20), goat anti- SM22· (1:20), or rabbit anti-SMA (1:20) (Abcam, Cambridge, MA) primary antibody overnight at 4·C. Nonspecific IgG was used as a negative control. After rinsing with TBST, slides were incubated with a secondary antibody, goat anti-rabbit biotin conjugate, horse anti-mouse biotin conjugate, or rabbit anti-goat biotin conjugate (1:50) (Vector Laboratories, Inc., Burlingame, CA). The slides were incubated using the Vectastain ABC Kit (Vector Laboratories) reagent for 30 minutes at room temperature. Slides were then counterstained with 25% hematoxylin (Fisher Fair Lawn, Fair Lawn, NJ).

Analysis and Grading

Using a semiquantitative method, the numbers of inflammatory cells and the level of staining for collagen types I and III, SMA, and SM22α were scored by 4 independent blinded reviewers (I.T.O., D.L.H., Y.W.). Individual sections from each of the 13 specimens were examined under a light microscope at low and high power for semiquantitative assessment. For number of neutrophils, the following grading criteria were used: mild, <1 per high-power field (HPF); moderate, 3 to 5 per HPF; and severe, >5 per HPF. For markers of chronic inflammation (lymphocytes and plasma cells), grading criteria were as follows: mild, scattered/individual; moderate, 5 to 10 per HPF; and severe, >10 per HPF. Staining intensity for collagen types I and III, smooth muscle actin, and fibroblasts was similarly evaluated as mild, moderate, or severe. For analytic purposes, the severity scale was assigned a numerical score: 1, mild; 2, moderate; and 3, severe.

Statistical Analysis

Demographic data were analyzed using the Student t-test. The Mann-Whitney test was used to compare the results between the groups (SPSS version 13.0; SPSS, Inc., Chicago, IL). Interobserver variability was calculated using a kappa correlation. Statistical significance was set at $p < .05$.

Results

Patients in the VCD and non-VCD groups were similar in age, body mass index, menopausal status, smoking status, and history of receiving hormone replacement therapy. All patients in the study group had vaginal cuff dehiscence after 6 weeks (range, 42–85 days) (Table 1). None of the patients in the VCD group had clinical evidence of hematoma, postoperative vaginal cuff cellulitis, or abscess.