Video Article

Intraoperative Detection of Subtle Endometriosis: A Novel Paradigm for Detection and Treatment of Pelvic Pain Associated with the Loss of Peritoneal Integrity

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URL: http://www.jove.com/video/4313
DOI: doi:10.3791/4313

Keywords: Medicine, Issue 70, Anatomy, Physiology, Endocrinology, Obstetrics, Gynecology, Surgery, endometriosis, pelvic pain, dysmenorrhea, diagnostics, laparoscopy, peritoneum, scanning electron microscopy, SEM

Date Published: 12/21/2012

Citation: Lessey, B.A., Higdon, H.L., Miller, S.E., Price, T.A. Intraoperative Detection of Subtle Endometriosis: A Novel Paradigm for Detection and Treatment of Pelvic Pain Associated with the Loss of Peritoneal Integrity. *J. Vis. Exp.* (70), e4313, doi:10.3791/4313 (2012).

Abstract

Endometriosis is a common disease affecting 40 to 70% of reproductive-aged women with chronic pelvic pain (CPP) and/or infertility. The purpose of this study was to demonstrate the use of a blue dye (methylene blue) to stain peritoneal surfaces during laparoscopy (L/S) to detect the loss of peritoneal integrity in patients with pelvic pain and suspected endometriosis. Forty women with CPP and 5 women without pain were evaluated in this pilot study. During L/S, concentrated dye was sprayed onto peritoneal surfaces, then aspirated and rinsed with Lactated Ringers solution. Areas of localized dye uptake were evaluated for the presence of visible endometriotic lesions. Areas of intense peritoneal staining were resected and some fixed in 2.5% buffered gluteraldehyde and examined by scanning (SEM) electron microscopy. Blue dye uptake was more common in women with endometriosis and chronic pelvic pain than controls (85% vs. 40%). Resection of the blue stained areas revealed endometriosis by SEM and loss of peritoneal cell-cell contact compared to normal, non-staining peritoneum. Affected peritoneum was associated with visible endometriotic implants in most but not all patients. Subjective pain relief was reported in 80% of subjects. Based on scanning electron microscopy, we conclude that endometrial cells extend well beyond visible implants of endometriosis and appear to disrupt the underlying mesothelium. Subtle lesions of endometriosis could therefore cause pelvic pain by disruption of peritoneal integrity, allowing menstrual or ovulatory blood and associated pain factors access to underlying sensory nerves. Complete resection of affected peritoneum may provide a better long-term treatment for endometriosis and CPP. This simple technique appears to improve detection of subtle or near invisible endometriosis in women with CPP and minimal visual findings at L/S and may serve to elevate diagnostic accuracy for endometriosis at laparoscopy.

Video Link

The video component of this article can be found at http://www.jove.com/video/4313/

Protocol

1. Patient Selection

1. Women with chronic pelvic pain undergo a history and physical exam.
2. Localization of pain, timing with regards to the menstrual cycle, associated symptoms including irritable bowel complaints or bladder complaints are noted and recorded.
3. Women undergoing laparoscopy are consented for possible resection of endometriosis and use of their tissues for research.

2. Laparoscopic Techniques

1. Laparoscopy is performed using standard techniques through a 5 mm umbilical port and additional 5 mm ports in the lower abdomen.
2. Careful inspection of the pelvic is performed to correlate any findings of endometriosis with the pre-operative localization of pain, as indicated by the patient.
3. Methylene blue dye is mixed 1:200 with sterile saline and injected onto pelvic surfaces using an aspiration needle.
4. Suction irrigation is performed to remove the excess dye and rinse the peritoneal surfaces.
5. The pelvis is inspected and the areas of blue dye uptake are recorded.
6. When possible, resection of the affected areas of peritoneum is performed.

3. Scanning Electron Microscopy

1. Peritoneal sections are sutured to a sterile telfa board to flatten the specimen and maintain orientation (intraperitoneal side up) and placed in gluteraldehyde fixative (Sigma, St. Louis, MO) for SEM.
2. Samples were oriented and post-fixed in 1% buffered osmium tetroxide for 1 hr and dehydrated with sequential ethanol concentrations.
3. Samples were dried using liquid carbon dioxide and mounted onto aluminum scanning electron microscope stubs with colloidal silver paste and sputter coated with gold:platinum alloy to a thickness of 20 nM.
4. Scanning electron microscopy was performed using a JEOL Model JSM-6400 Scanning microscopy and photomicrographs obtained from 12 random areas.

Representative Results

An example of subtle endometriosis that was nearly invisible is shown in **Figure 1A.** Prior to application of blue dye an irregular area of stippled peritoneum was appreciated aided by reflection of the laparoscopic light. In **Figure 1B**, the appearance of this same area after staining shows the same irregular pattern over the bladder (**Figure 1B**). Once this portion of peritoneum was resected (**Figure 1C**) it was subjected to SEM which showed overlying endometrial cells and disruption in the cell-cell contacts of the underlying mesothelium. Normal peritoneum from a non-blue stained area shows intact cell-cell contacts and no endometriotic cells present (**Figure 1D**). In another woman with CPP and a diagnosis of interstitial cystitis (IC), no obvious endometriosis was seen prior to application of blue dye (**Figure 1E).** With application of dye, the bladder showed obvious blue staining (**Figure 1F**). Her symptoms of CPP and IC improved dramatically for over 6 months with resection of these areas of blue stained peritoneum, despite the fact that no endometriosis was seen visually. Endometriosis was seen on pathology report from the left uterosacral biopsy, however.

As shown in **Table 1**, of 40 patients with CPP, we found MB staining in 34 (85%) compared to 2/5 (40%) of women without CPP. The age was similar in cases vs. controls and by stage of endometriosis found. Blue dye uptake was seen in most of the samples with endometriosis and only 2/5 of women without visible endometriosis. Endometriosis was highly associated with the localization of the blue dye. Subjectively, pain relief was reported to be improved in a majority patients (32/40; 80%), with the exception being women without visible endometriosis.

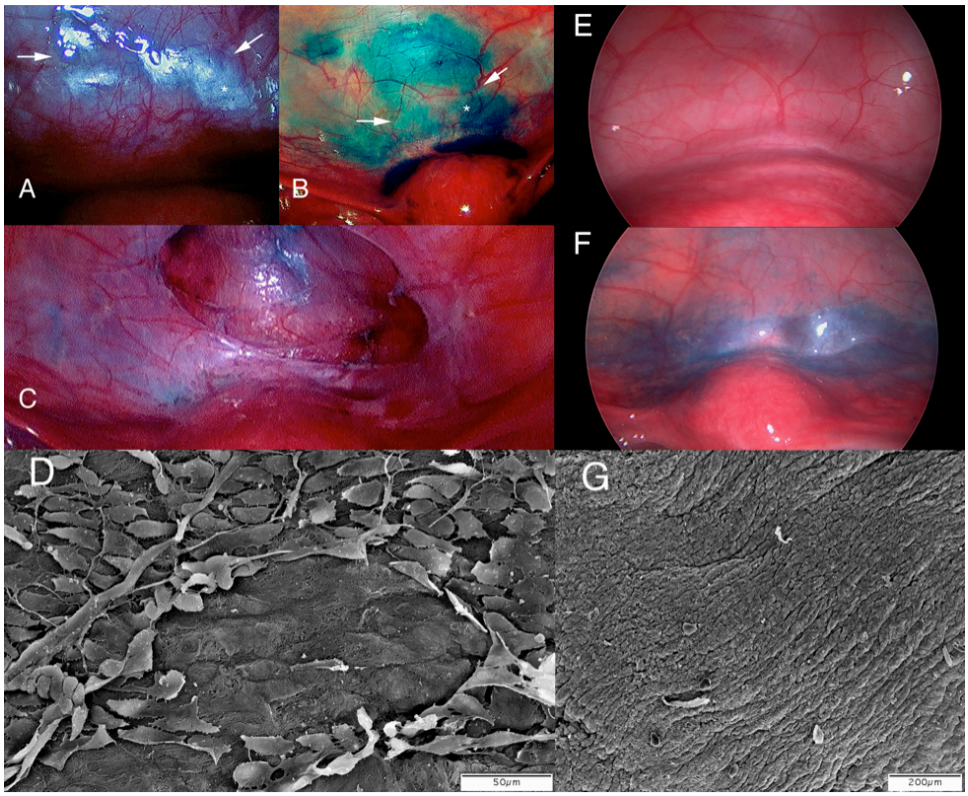

Figure 1. Examples of laparoscopic findings before application of blue dye are shown in (A) and (E) in two women with CPP, both with bladder complaints that worsened at the time of menses. In (A), just reflection of laparoscopic light showed areas suspicious for clear lesions of endometriosis (arrows). Blue dye applied to these areas stained distinct areas of over the bladder (B (arrows), and F). Resection of the affected peritoneum was examined by scanning electron microscopy (SEM). In the sample resected from the bladder (C), a single layer of endometriotic cells can be seen on top of the peritoneum by scanning electron microscopy (SEM) (D). The mesothelial cells of the peritoneum have gaps in their cell-cell contacts exposing underlying extracellular matrix. In a case of normal peritoneum without blue dye staining, these cells are tightly connected as shown in (G). Size bars for SEM are included bottom right (D and G). Click here to view larger figure.

Parameter	Cases (n = 40)	Controls (N = 5)	Significance*(p < 0.05)
Age (mean ± SD)	30.17 ± 4.65	32.0 ± 2.55	p = 0.39
Age by Stage O (n = 6) I-II (n = 29) III- IV (n = 5)	33.16 ± 3.82 29.2 ± 4.85 32.0 ± 3.03	N/A	
MB Uptake O (n = 6) I-II (n = 29) III-IV (n = 5)	(34/40) 85% (2/6) 33.3% (28/29) 96.5% (4/5) 80%	(2/5) 40%	p = 0.2
MB associated w/ endometriosis O (n = 6) I-II (n = 29) III-IV (n = 5)	(0/6) 0% (28/29) 96.5% (5/5) 100%	N/A	
Subjective Pain Relief	(32/40) 80%	N/A	
Pain Relief by Stage (%) O (n = 6) I-II (n = 29) III-IV (n = 5)	(1/6) 16.7% (27/29) 93.1% (4/5) 80%	N/A	

Table 1. Experience with blue dye staining in women with chronic pelvic pain.

- Statistical comparisons were performed using Pearson chi square test.

Discussion

Chronic pelvic pain in women is a costly and poorly understood problem [1]. Dysmenorrhea is the most common form of pelvic pain affecting 60% of women [2] and 72% of adolescents [3]. Endometriosis is an inflammatory condition that affects 5% of normal women but is present in up to 70% of women with pelvic pain. While endometriosis contributes to chronic pelvic pain (CPP), a mechanism by which lesions cause pain remains uncertain [4]. Paradoxically, the severity of pain does not correlate with the severity of endometriosis [5,6]. Chronic pain symptoms including associated irritable bowel syndrome (IBS) and IC may go undiagnosed for years [7,8]. The estimated cost of endometriosis is over $22 billion in the US alone [9], though true accounting is difficult to assess since endometriosis is associated with other chronic illnesses including migraine headache, chronic fatigue syndrome, IBS and IC [10,11]. Establishment of a diagnosis for endometriosis requires laparoscopy, but this disease is often diagnosed late [12]. Disagreement over what constitutes endometriosis and a lack of specialized training contributes to delayed detection [13]. Once visualized, not all suspected lesions can be verified by a pathologist [14] and in many cases of CPP, endometriosis is not appreciated [15], perhaps due to subtle or invisible forms of the disease [16-19]. Clearly a new paradigm is needed to address this gap between clinical symptoms of pain and our diagnostic approach for this significant disease.

While neural pathways involved in the etiology of pelvic pain associated with endometriosis are increasingly being studied[20-25] surprising little is known about how mild endometriosis contributes to localized chronic pelvic pain (CPP). Surgical and medical management often fails to address the pain associated with this disorder [4] and recurrence is common. Based on studies presented in this study, we hypothesize that localized, repetitive menstrual and premenstrual pelvic pain associated with endometriosis is likely due to a disruption in peritoneal integrity, secondary to overlying endometriosis. Inflammatory cytokines and blood within the peritoneal cavity of endometriosis patients would have direct access to the underlying innervated pelvic tissues at points of peritoneal disruption. While substance P has been measured and not found to be different in endometriosis patients versus controls [26], TNF-α and glycodelin are two candidates that have been associated with pain in endometriosis patients [27]. While nerve fibers have been described in endometrium and endometriotic lesions [25,28-30], these findings do not account for localized pain associated with subtle or minimal disease.

Reports for the use of methylene blue date back to 1994, when Manhes reported its utility in identifying subtle forms of endometriosis [31]. Methylene blue dye is not FDA approved for the detection of endometriosis and therefore, we recognize that is represents an off-label usage

of this product. We have used methylene blue staining of the peritoneum to help identify subtle or otherwise invisible endometriosis (Lewis and Lessey Abstract AAGL/AGES Brisbane, Australia 2008). Recently, Rauh-Hain and Laufer reported a similar use of indigo carmine to detect subtle forms of this disease [32]. In our experience, indigo carmine dye does not perform the same and in some patients does not stain areas of endometriosis that MB recognizes. The basis of dye uptake may be associated with disruption of epithelial surfaces, as shown in the rat bladder model by Morrison [22]. Here we report for the first time evidence that peritoneal uptake of dye is associated with mesothelial disruption and associated subtle or invisible endometriosis by scanning electron microscopy. In addition, we note that the stained peritoneal surfaces appeared to correlate with the pre-assessment localization of pain in women with CPP. For example, if a patient states that her pain is characteristically on the right, that is the side we typically see blue staining. Further, we found significant improvement in the post-operative reports of pain in a majority of patients in whom these peritoneal lesions were resected or ablated. We believe this simple technique will allow better diagnostic accuracy for the identification of subtle endometriosis while providing a more complete treatment of the endometriosis associated with CPP. The paradoxical lack of association between the stage of endometriosis and the perception of pain may finally be understood in terms of peritoneal defects that extend much farther than the visible lesion of endometriosis.

Surgical management often results in symptomatic improvement [33,34] but 40 to 50% of women who undergo treatment have incomplete relief or rapid recurrence of their symptoms [35,36]. Invisible endometriosis or subclinical forms of the disease have been described [16-19,37]. Therefore, very subtle or single cell layer endometriosis may not be easily identified, leading to misdiagnosis of CPP patients and lost opportunities to alleviate pain.

Peritoneum is an under-studied area of human biology with only a few papers that describe the biology of peritoneal healing [38]. Models to study peritoneal-endometrial interaction have shown promise [39-43]. As previously shown by Witz [42], we found that endometrial cells are present on peritoneal surfaces associated with peritoneal cell-cell disruption. Using explants of human peritoneum and isolated endometrial epithelial and stromal cells Witz and colleagues have demonstrated that endometrial cells readily adhere to peritoneal mesothelium [42], and invade through the mesothelium in 18 to 24 hr [44]. *In vivo*, we found that endometrial cells adhere to the outer surface of peritoneum without apparently invading. Thus, non-invading surface endometriosis may be a stable and persistent form of endometriosis. Surface "red" lesions may be similar and have been recognized as a more biologically active form of endometriosis [45].

Koks described attachment and spreading of menstrual cells only to the areas of peritoneum that were damaged or absent [46], but further work by this group suggested soluble factors caused mesothelial cell separation, similar to what we observed [47], including "retraction, shrinking and gap formation". While their study and the later study by Witz [40] concluded that disruption allowed for sites of adhesion and invasion, our findings of gaps in the mesothelium of patients with endometriosis associated with MB absorption suggests that endometriotic lesions spread far from the initial lesion and disrupt the surface peritoneum without invading. Known disruptors of cell-cell contact expressed by endometrium or endometriosis include TNF-α [48]and Ephrin A1 [49,50].

Resection or ablation of the MB stained peritoneum is not yet the standard of care for endometriosis and CPP. If such areas of peritoneum are in fact coated with microlayers of endometriotic cells, then the use of dye techniques to identify affected areas could improve the diagnostic accuracy for endometriosis and allow for a more complete resection of otherwise invisible implants. In this preliminary study of CPP before and after surgery, we incidentally found that most patients reported improvement in pain symptoms. Hurd suggested three criteria be used to prove the association between pain and endometriosis: 1) that the pain be cyclic, 2) that endometriosis be diagnosed at laparoscopy and 3) that medical or surgical therapy improves the pain [51]. While further validation is needed to confirm our findings, the association of minimal disease, CPP and peritoneal defects fit the Hurd criteria and offers a new way of thinking about the evaluation and treatment of pelvic pain associated with endometriosis. This study was limited by the lack of a formal pain survey. Future studies are now being planned to use validated pelvic pain surveys and visual analog scales (VAS) before and after surgery to quantify the long-term resolution of pain in these women with subtle endometriosis and CPP treated with resection of blue-staining areas associated with endometriosis.

Disclosures

No conflicts of interest declared.

Acknowledgements

We would like to acknowledge the excellent technical help of Angela Houwing and help with data collection from Greta Bushnell.

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