Published in final edited form as:

Acta Obstet Gynecol Scand. 2009 ; 88(9): 968–975. doi:10.1080/00016340903176826.

Endometriosis-associated nerve fibers and pain

Melissa G. Medina¹ and Dan I. Lebovic²

¹University of Michigan, Ann Arbor, MI, USA

²Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility, University of Wisconsin, Madison, WI, USA

Abstract

The assessment and diagnosis of endometriosis remain elusive targets. Patient and medical-related factors add to delays in the detection and treatment. Recently, investigators have revealed specific nerve fibers present in endometriotic tissue, with existing parallels between density and pain severity. The aim of this review is to compile a comprehensive review of existing literature on endometriosis-related nerve fiber detection, and the effects of medical therapy on these neural fibers. We performed a systematic literature-based review using Medline and PubMed of nerve fibers detected in eutopic endometrium, endometriotic lesions, and the peritoneum. Various arrangements of significant medical terms and phrases consisting of endometriosis, pelvic pain, nerve fiber detection/density in endometriosis, and diagnoses methodology, including treatment and detection were applied in the search. Subsequent references used were cross-matched with existing sources to compile all additional similar reports. Similar nerve fibers were detected within lesions, endometrium, and myometrium, though at varying degrees of density. Hormonal therapy is widely used to treat endometriosis and was shown to be related to a reduction in fiber density. A direct result of specific nerve fiber detection within eutopic endometrial layers points to the use of a minimally invasive endometrial biopsy technique in reducing delay in diagnosis and subsequent possible preservation of fertility.

Keywords

Endometriosis; nerve fibers; pelvic pain

Introduction

Endometriosis is a chronic, complex gynecological disease affecting reproductive age women, characterized by endometrial-like tissue found outside of the uterine cavity. Clinical symptoms are manifested as dysmenorrhea, dysuria, dyspareunia (1), and/or subfertility. Up to 10–15% of women are approximated to be affected (2), yet the disease may remain asymptomatic making it a difficult disorder to detect and diagnose. A number of studies

Correspondence: Dan I. Lebovic, Department of Obstetrics and Gynecology, University of Wisconsin, H4/628 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792-3236, USA. Lebovic@wisc.edu.

Declaration of interest: The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

have posited biochemical factors involved in immune responses linked to endometriosis such as cytokine production, gene product expression, and various other immunological components (2–5). More recently, there is accumulating literature describing nerve fiber density differences between women with diagnosed endometriosis and women without endometriosis.

A significant interval of time exists between the onset of symptoms and a definite diagnosis for endometriosis (6). Multiple factors, both on individual patient and medical levels, contribute to the delay; the perceived inability to recognize problematic menstrual episodes, mistaking them for ‘normal’ experiences may add to individual reporting variability as well as misdiagnoses. Ballard et al. described possible medical delays, discussing possible instrumental errors in diagnosis or physician ‘dismissal’ of symptoms (7). A widely used existing method of detection is based on laparoscopic visual assessment followed by histological confirmation; however, this surgical approach poses potential complications and side effects, in addition to physician variability in detecting lesions or ectopic endometrial tissue (7,8). Endometrial biopsies accompanied by immunohistological staining of detected nerve fibers are also being evaluated as a minimally invasive, efficient, and more reliable diagnostic technique (9), aiming to reduce the time delay from symptom onset to definite endometriosis disease diagnosis.

Methods

With the goal of providing a current, expansive review of nerve fibers associated with the detection, mechanism and progression of endometriosis, an electronic database search (Medline, PubMed) was implemented. Various arrangements of significant medical terms and phrases consisting of endometriosis, pelvic pain, nerve fiber detection/density in endometriosis, and diagnoses methodology, including treatment and detection were applied in the search. Subsequent references used were cross-matched with existing sources to compile all additional similar reports. A majority of acquired data in relation to nerve fiber density reports were published primarily by Tokushige, Markham, Russell and Fraser, specifically regarding nerve fiber densities within endometrial layers (see Reference list). Studies and reports were selected according to specific topic relevance, subject pool and number, efficacy and specificity. Due to the wide range of published articles, we specifically included a comprehensive number of studies on endometriosis-associated pain as well as observational and pilot studies in relation to current diagnosis techniques and effective treatment regimens.

Nerve fibers: detection

There have been numerous attempts oriented at distinguishing women with endometriosis from those without endometriosis (10–13). A majority of the ongoing research centers around cytokines, growth factors and more recently, nerve fibers. Biological neuronal markers were used to discover specific types of nerve fibers present within endometrial layers (14,15), such as protein gene product 9.5 (PGP9.5 – specific marker for unmyelinated and myelinated nerve fibers), Neurofilament (NF – specific for myelinated fibers), substance P (SP) and calcitonin gene-related peptide (CGRP) for sensory nerve fibers, vasoactive

intestinal polypeptide (VIP – a sensory A δ , sensory C and cholinergic nerve fibers marker), neuropeptide Y (NPY – marker for A δ and C sensory fibers, and adrenergic nerve fibers). Various functions of these neuronal biological molecules are summarized in Table I.

Tokushige et al. demonstrated that functional layers in the endometrium of women diagnosed with endometriosis stained positively for PGP9.5, but not for NF, a marker for myelination of nerve fibers. These nerve fibers were not detected in the functional layer of women without endometriosis. The endometrial functional layer is that which is shed during the menstrual cycle; whereas the innermost basal layer, lying below the functional layer of endometrium is mostly preserved during menstruation and is known to give rise to the new functional layer, developing through cellular growth and proliferation. These results indicate that multiple, fine, and unmyelinated sensory nerve fibers were present in women diagnosed with endometriosis within the functional layer of eutopic endometrium (16). Not surprisingly, many of the detected PGP9.5 neurons were present near vascularized sites. The functional layer of endometrium showed mostly sensory unmyelinated C nerve fibers responsible for transmitting dull, throbbing, and diffuse pain (16). The functional layer of the endometrium was also positively stained for SP, CGRP, VIP, and NPY, however, the observed densities of PGP9.5, NPY and VIP stained nerve fibers were greater in women with endometriosis compared to those without (17).

Within the basal layer, NPY, CGRP, SP, and VIP were detected in both women with and without endometriosis (17). Researchers also verified several large myelinated and small unmyelinated nerve fibers as well as discovered an increase in density of PGP9.5-stained nerve fibers in the basal layer (15). Additionally, there were many more small unmyelinated nerve fibers detected in the endometrial basal layer in affected women than in women free of endometriosis. NF stains indicated the occurrence of myelinated A δ fibers, which send quick localized pain signals toward the brain and spinal cord, in the deep endometrial basal layer of confirmed endometriosis patients; similar findings were not detected in women without endometriosis. Furthermore, in women without endometriosis, the small unmyelinated fibers were minimally detected and the large myelinated nerve fibers were never detected in the basal layer.

Although the role of these immunoreactive nerve fibers has not been specifically determined, the detection of a number of nerve fibers is not exclusive to endometrium within the uterus (Figure 1). Nerve fibers in the myometrium were detected in both normal and endometriotic women, identified as being a combination of A δ , sensory C, cholinergic and adrenergic fibers, although densities of some of these nerve fibers were significantly higher in women with endometriosis (17). Similar results were obtained with NPY staining, where nerve fiber densities in women without endometriosis were significantly decreased. Broad PGP9.5-stained trunks of nerve fibers were formed by small unmyelinated nerve fibers within the endometrial–myometrial border or basal layer, and were present in women with diagnosed endometriosis, but never in those without (16). SP, a neuropeptide, and CGRP, a vasodilator, are both involved in the inflammatory and pain responses and are present in the myometrium indicating the occurrence of sensory C and A δ fibers; Bulletti et al. described greater contractility at the time of menses in endometriosis cases than controls (18), suggesting a possible role of these SP and CGRP nerve fibers in the generation of pain

related to endometriosis and dysmenorrhea. Moreover, in endometriotic women, nerve fiber densities were significantly greater in the functional and basal layers in comparison with myometrial density levels (16). A majority of nerve fibers have been detected in the myometrium and endometrium of regularly menstruating women without endometriosis, however, studies have shown an increase in nerve fiber density in those women with endometriosis (17) and increased myometrial nerve fiber densities in women reporting pelvic pain (19).

The neuronal markers detected in the various endometrial layers and myometrium are summarized in Table II. Although pain mechanisms associated with endometriosis are not well known, these findings reveal density differences between women as well as the specific combinations of nerve fiber types present in eutopic endometrial layers and myometrium.

Tokushige and his colleagues also recognized the varying densities of nerve fibers associated with each phase of the menstrual cycle. Using endometrial curettage samples from women with endometriosis, they report significantly greater densities of nerve fibers within the functional and basal layers of eutopic endometrium during the secretory stage of the menstrual cycle compared to the menstrual or proliferative stages (16).

Using a number of the same biological markers to stain for specific nerve fibers, researchers have provided evidence of nerve fibers within endometriotic lesions infiltrating the pelvic area, targeting sites such as the intestinal tract, peritoneum, Fallopian tubes, ovaries, or rectovaginal septum. After collecting peritoneal endometriotic tissue and normal peritoneum tissue from women without endometriosis, there was a greater density of nerve fibers stained with PGP9.5 and NF in the tissue samples collected from women with endometriosis compared to those without ($6.7 \pm 3.7/\text{mm}^2$; range 2.0–20.0/ mm^2 ; versus $1.0 \pm 0.8/\text{mm}^2$; range 0–2.7/ mm^2 , respectively) as published by Tokushige et al. (16); only 10% (4 out of 40) NF immunoreactivity (staining for myelinated nerve fibers) was seen within peritoneal endometriotic lesions located near the glandular cell component, indicating that the remaining 36 peritoneal endometriotic lesions depicting NF immunoreactivity were likely unmyelinated fibers and located far from endometriotic glands and transmitting dull and diffuse pain. There was also a moderate number of nerve fiber presence stained with SP, CGRP, and tyrosine hydroxylase (TH), with few acetylcholine immunoreactive nerve fibers present within peritoneal endometriotic lesions. What was in abundance (90–100%), however, was the presence of nerve growth factor Receptor p75 (NGFRp75) and nerve growth factor (NGF) immunoreactive nerve fibers, suggesting a possible mechanism of nerve fiber growth and recruitment involved in the generation of endometriosis-related pain. Subsequent staining showed that SP, CGRP, TH, NGF, and NGFRp75 were co-localized within lesions (14).

It was also seen that in women reporting pain, the distance separating nerve fibers in lesions and endometriotic glands were closer in proximity than in women who did not report any discomfort or pain (20). In women with endosalpingiosis lesions, defined by the existence of tubal-like epithelial tissue found on the peritoneal side of the uterus, Fallopian tubes, or ovaries, the density of nerve fibers stained with PGP9.5 was less (mean $3.8 \pm 0.9/\text{mm}^2$; range 2.7–5.8/ mm^2) in comparison to peritoneal endometriotic lesions (mean $16.3 \pm 10.0/\text{mm}^2$;

range 6.8–53.9/mm²), but still greater than normal peritoneum tissue samples ($2.5 \pm 1.3/\text{mm}^2$) (14).

Endometrial biopsy as a diagnostic technique

Currently there is no justifiably consistent or noninvasive technique in diagnosing endometriosis (9). The standard relies on laparoscopy, occasionally followed by histological confirmation. As a result, diagnosis relies on observer vision of perceived endometrial tissue outside the uterine cavity. This invasive surgical procedure may expose patients to risks associated with surgery (15), in addition to the possibility of ‘missed’ plaques due to plaques being less evident and observer variability; these seemingly undetectable plaques may perhaps continue to develop into larger lesions without being promptly removed.

Although findings of nerve fibers detected as A delta, sensory C, cholinergic, and adrenergic, are present in all women with or without endometriosis, there is substantial evidence indicating the clear presence and greater abundance of these nerve fibers within the endometrial functional and basal layers, as well as within the peritoneum of patients diagnosed with endometriosis (14,17). These findings lead to the potential use of endometrial biopsies as a diagnostic technique to detect endometriosis, in contrast to the variable inconsistency of present methods of diagnosis.

The use of endometrial biopsies utilizing the findings regarding increased detectable nerve fiber density has been investigated. Al-Jefout et al. demonstrated nerve fiber detection within the functional layer of eutopic endometrium of all women with laparoscopically confirmed diagnosis, and no detectable fibers in women without endometriosis, which was consistent throughout their studies. Their findings were then used as the basis to conduct a pilot study assessing the efficiency of using endometrial curettage or endometrial biopsies as a detection method for endometriosis. Using a ‘suction-enhanced Endosampler,’ samples of endometrium were obtained from 37 women, with and without endometriosis, and subsequently stained with pan-neuronal marker 9.5 (PGP9.5). Detected nerve fiber densities of 26.7 ± 55.9 per mm² were returned by the Endosampler biopsies ($n=20$). Results yielded 100% specificity and sensitivity in the detection of present nerve fibers in women with endometriosis and none in those without compared to laparoscopic detection and peritoneal biopsy (9).

Nerve fiber density stained with PGP9.5 in the functional ($11 \pm 5/\text{mm}^2$) and basal ($18 \pm 8/\text{mm}^2$) layers of endometrium, and myometrium ($3 \pm 1/\text{mm}^2$) of women currently not taking hormonal therapy was notably greater than the functional ($0.2 \pm 0.7/\text{mm}^2$), basal ($0.9 \pm 1.3/\text{mm}^2$), and myometrial ($1.5 \pm 0.8/\text{mm}^2$) nerve fiber densities of those under hormone treatment. Given these findings along with the acquisition of a greater patient subject pool to ascertain greater accountability and completeness, this promising technique seems to be both valuable and efficient for the possibility of earlier diagnosis of endometriosis. Another advantage that earlier detection poses can be the future opportunity to preserve the fertility of reproductive-aged women (9), due to the potential of measuring the likelihood of future disease development.

Hormone treatment and effects

A significant amount of research has demonstrated the capability of current hormonal treatments to diminish the reported pain associated with endometriosis symptoms (21,22). Current hormonal therapies utilized presently include aromatase inhibitors, oral contraceptives, progestins, gonadotropin releasing hormone agonists, androgenic mediators, as well as various immuno-regulatory elements having a role in disease development (19).

In addition, there are preliminary indications that women undergoing hormonal therapy may have a reduced number of detectable nerve fibers (15) in peritoneal endometriotic lesions and eutopic endometrium compared to endometriotic women who were not receiving any hormone treatment. Tokushige et al. have previously shown the presence of a greater density of nerve fibers present in women with endometriosis in the endometrium and myometrium (16); in an effort to reveal what effects hormonal treatment has on nerve fiber density, endometrial and myometrial tissues from women with untreated endometriosis and from those undergoing hormonal therapy (oral progestogens or combined oral contraceptives) were obtained; the tissues were subsequently stained with various biochemical markers for nerve fiber identification and localization. PGP9.5-positive staining was not detected within the functional layer of eight women undergoing hormonal therapy. Moreover, total nerve fiber density in hormonally treated women in the functional and basal layers of endometrium as well as the myometrium (functional: $0.4 \pm 0.9/\text{mm}^2$; basal: $0.9 \pm 1.3/\text{mm}^2$; myometrium: $1.5 \pm 0.8/\text{mm}^2$) was significantly decreased compared to the endometrial functional and basal layers, and myometrium of those without hormone treatment ($11 \pm 5/\text{mm}^2$, $18 \pm 8/\text{mm}^2$, $3 \pm 1/\text{mm}^2$, respectively; Table III) (23).

More specifically, in the immunohistological staining of the functional layer of eutopic endometrium from untreated women, the nerve fibers were detected to be VIP, NPY, SP, and CGRP; whereas in hormonally treated women, the presence of SP and CGRP was not seen. Also, in the basal layer, all four markers were present in both treated and untreated women who exhibited a small amount of nerve fiber presence in the functional layer; however, the presence of TH was not detected in women undergoing therapy. Similarly, in myometrium, positive detection for SP, CGRP, VIP, NPY, vesicular acetylcholine transporter (VACHT), TH nerve fibers were found in both women; NGF and NGFRp75 immunoreactivity densities were significantly reduced with hormone therapy administration as well (6,23). Hence, since hormonal treatment tends to reduce the amount of reported pain in patients diagnosed with endometriosis, this may be partly explained by decreased nerve fiber density in women undergoing hormonal treatment.

Regarding peritoneal endometriosis, a number of markers persisted within hormonally treated individuals: NF, TH, VACHT, VIP, NPY, and CGRP. There were more PGP9.5-stained nerve fibers in hormonally treated peritoneum, in comparison to women without peritoneal endometriosis. Nevertheless, more NF immunoreactivity was observed in untreated women and SP was not seen in treated women. With respect to the type of hormone treatment each woman was undergoing (either combined oral contraceptives or progestogens), nerve fiber types and densities stained with NF and PGP9.5 showed no significant association with the treatment mode. The finding of decreased nerve fiber

densities in treated women versus untreated individuals was consistent; however this effect was more significantly seen in eutopic endometrium, compared to peritoneal lesions (24).

Nerve fibers and symptomatology

In a recent pilot study investigating the clinical relevance of nerve fibers in peritoneal lesions, the pain symptoms of endometriosis have been correlated with the density of peritoneal nerve fibers. In women with peritoneal endometriosis, those who reported moderate or severe pelvic pain and/or dysmenorrhea were detected to have more nerve fibers in association with endometriosis (82.6% of patients who reported a 3 on a 10-point scale). Conversely, in the group of patients categorized as having peritoneal endometriosis but who recorded significantly reduced pain symptoms (2), only two women (33%) showed the presence of detectable endometriosis-associated fibers, which were considerably less in density (25).

Yet, in continuation of these findings, questions concerning the difference between types of endometriosis manifested in each patient are still in need of further investigation. Although nerve fiber densities have been correlated with the degree of severity of pain in women with endometriosis, it would be valuable to study women confirmed with endometriosis and differentiate those with infertility/subfertility versus normal fecundity, or even women with proven normal fertility but who have endometriosis; or those experiencing endometriosis-related pain and those who do not report such pain.

Women inclined to have endometriosis may biologically induce neurotrophic factors to be released, which would subsequently lead to the generation of fibers innervating endometriotic implants. It is therefore important to further study the mechanisms of nerve fiber growth and recruitment in women experiencing endometriosis-associated pain. Future research will attempt to explain the factors driving this phenomenon, whether nerve fiber presence is an underlying cause inducing symptoms or if it is the appearance and recurrence of the disease itself that stimulates neurogenesis mechanisms of these detected fibers in diagnosed women with endometriosis.

Future implications and research directions

The results of staining for nerve fibers within endometrial biopsies point to benefits of employing this minimally invasive procedure as a diagnostic technique, leading to benefits such as eliminating anesthetic use and reducing physician variability in detection; paving the way for earlier detection and diagnosis, and resulting in earlier treatment strategies for affected women (9,15). The reliability of using the 'Endosampler' as opposed to current methods of laparoscopy can result in advantages on patient lifestyle changes, including preservation of fertility of women with strong familial predispositions to endometriosis or chronic pelvic pain. In addition to efficiency and its possible use in an outpatient environment, this novel technique offers a more affordable method of diagnosis as well (9).

Future large studies will no doubt address correlations between total peritoneal nerve fiber type and density scores between women with and without diagnosed endometriosis or within patients presenting with other gynecological symptoms. NGF and NGFRp75 expression

levels were shown to be reduced using progestogen and estrogen–progestogen compared to those without endometriosis (23). Continuing research could consider the effect of hormonal therapies on nerve fibers within endometriotic plaques in various locations such as the ovaries, peritoneum, or Fallopian tubes of women with endometriosis-induced pain. A comparison of the effectiveness between current drugs, hormones and other treatment methods might then be used as a surrogate marker for treatment efficacy.

In addition to detecting the type of nerve fibers present in eutopic endometrium, future studies may be directed toward certain molecular products that interact with and/or influence these nerve fibers within eutopic endometrium or endometriotic plaques. Although data are accumulating on NGF recruitment and molecular interactions playing a role in disease recurrence, the exact pathogenesis of the disease and its specific mechanism of recurrence still remain poorly understood. To further investigate a definite mechanism of endometriosis-associated pain, further research is needed to reveal the detailed interplay between functional molecules and identified nerve fibers, and their tandem interactions aiding in the progression of symptoms.

Acknowledgements

The authors thank Sam Creydt (University of Wisconsin, Madison, WI) for his assistance with the manuscript figure. Supported by National Institutes of Health grant no. 5K23HD043952-02 (DIL).

Abbreviations

Ach	acetylcholine
CGRP	calcitonin gene-related peptide
HT	hormone treated
NGF	nerve growth factor
NGFRp75	nerve growth factor receptor p75
NF	neurofilament
NHT	no hormone treatment
NPY	neuropeptide Y
NT	neurotensin
PGP9.5	protein gene product 9.5
SP	substance P
TGF-B1	transforming growth factor B1
TH	tyrosine hydroxylase
VACHT	vesicular acetylcholine transporter
VMAT	vesicular monoamine transporter
VIP	vasoactive intestinal polypeptide

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Figure 1.
Visual representation of nerve fibers present in the endometrium using neuronal markers.

Table I

Biological molecule functions.

Molecule	Function	Nerve fibers detected
Substance P (SP)	Involved in pain generation/mechanism	Sensory A δ and C fibers
Calcitonin gene-related peptide (CGRP)	Involved in inflammatory response; inhibit SP degradation	Sensory A δ and C fibers
Tyrosine hydroxylase (TH)	Present in postganglionic sympathetic neurons	Adrenergic nerve fibers
Vasoactive intestinal polypeptide (VIP)	Present in sensory C nerve fibers	Sensory A δ and C fibers; cholinergic fibers
Neuropeptide Y (NPY)	Present in both sensory and adrenergic nerve fibers; present in both sympathetic and parasympathetic	Sensory A δ and C fibers; adrenergic fibers
Acetylcholine (Ach)	Preganglionic neurons as well as parasympathetic; postganglionic neurons	Cholinergic nerve fiber marker
Transforming growth factor B1 (TGF-B1)	Protein involved in cellular differentiation/proliferation and increased at endometriotic sites	
Vesicular monoamine transporter (VMAT)	Transport of monoamine neurotransmitters within sensory nerve fibers	
Neurotensin (NT)	Leads to release of cytokines associated with inflammation	
Nerve growth factor (NGF)	Upregulated in inflammatory response leading to increased production of CGRP/SP; may increase the number of nerve fibers	

Note: References (15,17,26–29).

Table II

Summary of detected nerve fibers in endometrial layers and myometrium using various neuronal markers.

Marker	Endometrial layer		
	Functionalis	Basalis	Myometrium
NF	○	●	▲
PGP9.5	▲	▲	▲
SP	■	●	■
CGRP	■	●	■
VIP	▲	●	■
NPY	▲	●	■
TH	○	●	■
VACht	○	○	■

Note: NF, neurofilament; PGP9.5, protein gene products 9.5; SP, substance P; CGRP, calcitonin gene-related peptide; VIP, vasoactive intestinal polypeptide; NPY, neuropeptide Y; TH, tyrosine hydroxylase; VACht, vesicular acetylcholine transporter;

○ not detected;

● present in endometriosis women only;

■ present in BOTH women with and without endometriosis;

▲ present in BOTH women – but increased density in those with endometriosis; References (15–17).

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Table III
Summary of hormonal treatment effects on nerve growth factors within the endometrium and myometrium.

Molecule	Functional layer		Basalis layer		Myometrium	
	HT	NHT	HT	NHT	HT	NHT
VIP	●	●	●	●	●	●
NPY	●	●	●	●	●	●
SP	○	●	●	●	●	●
CGRP	○	●	●	●	●	●
VAcHt	-	-	-	-	●	●
TH	-	-	○	●	●	●

Note: HT, hormone treated; NHT, no hormonal treatment;
● present;
○ not present;
- not measured; Reference (23).