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Nerve Bundles and Deep Dyspareunia in Endometriosis

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Abstract

The etiology of deep dyspareunia in endometriosis is unclear. Our objective was to determine whether nerve bundle density in the cul-de-sac/uterosacrals (zone II) is associated with deep dyspareunia in women with endometriosis. We conducted a blinded retrospective immunohistochemistry study (n = 58) at a tertiary referral center (2011-2013). Patients were stringently phenotyped into a study group and 2 control groups. The study group (tender endometriosis, n = 29) consisted of patients with deep dyspareunia, a tender zone II on examination, and an endometriosis lesion in zone II excised at surgery. Control group 1 (nontender endometriosis, n = 17) consisted of patients without deep dyspareunia, a nontender zone II on examination, and an endometriosis lesion in zone II excised at surgery. Control group 2 (tender nonendometriosis, n = 12) consisted of patients with deep dyspareunia, a tender zone II on examination, and a nonendometriosis lesion (eg, normal histology) in zone II excised at surgery. Protein gene product 9.5 (PGP9.5) immunohistochemistry was performed to identify nerve bundles (nerve fibers surrounded by perineurium) in the excised zone II lesion. PGP9.5 nerve bundle density (bundles/high powered field [HPF]) was then scored by a pathologist blinded to the group. We found a significant difference in PGP9.5 nerve bundle density between the 3 groups (analysis of variance, $F_{2,55} = 6.39$, $P = .003$). Mean PGP9.5 nerve bundle density was significantly higher in the study group (1.16 $\pm$ 0.56 bundles/HPF [$\pm$standard deviation]) compared to control group 1 (0.65 $\pm$ 0.36, Tukey test, $P = .005$) and control group 2 (0.72 $\pm$ 0.56, Tukey test, $P = .044$). This study provides evidence that neurogenesis in the cul-de-sac/uterosacrals may be an etiological factor for deep dyspareunia in endometriosis.

Keywords

endometriosis, dyspareunia, nerve, immunohistochemistry, laparoscopy

Introduction

Half of women with endometriosis have deep dyspareunia.[1,2] Deep dyspareunia in endometriosis reduces sexual activity, self-esteem, sexual satisfaction, and quality of life.[3,4] It also negatively affects a woman's sexual response cycle including arousal and orgasm.[5] Male partners of women with endometriosis can also experience depression, anxiety, powerlessness, and grief.[6] In couples with difficulty conceiving, pain with intercourse can also contribute to sexual dysfunction.[7,8]

In this *Journal* in 2011, experts in endometriosis recommended a research focus on deep dyspareunia.[9] The pathophysiology of deep dyspareunia in endometriosis is unclear in many cases. For example, there is only a marginal correlation between amount of disease in endometriosis (classified by stage I-IV) and pain such as deep dyspareunia.[10] Therefore, there must be factors beyond the amount of endometriosis lesions, which result in increased pain and deep dyspareunia.

Location of endometriosis has been shown to be one factor in the etiology of deep dyspareunia. In a recently published laparoscopic classification, the posterior compartment (cul-de-sac and uterosacrals) has been defined as zone II.[11,12] Anatomically, the zone II posterior compartment would be contacted with deep penetration and therefore may be involved in deep dyspareunia. Indeed, a large study showed that zone II posterior compartment endometriosis lesions were statistically associated with deep dyspareunia compared to other sites.[10] However, not all patients with zone II endometriosis have deep

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dyspareunia and in those women who do have deep dyspareunia, there is variability in the severity of symptoms.

Another factor may be the local presence of nerves around endometriosis. A number of studies have documented nerve fibers around endometriosis, which has been the subject of recent reviews.[13-15] Immunohistochemistry for the pan-neuronal marker protein gene product 9.5 (PGP9.5) has been used for many of these studies.[16] There is increasing evidence that these local nerve fibers may be implicated in the pain symptoms of endometriosis.[17-20] Potential mechanisms include an increased sensitivity of the local nerve fibers via peripheral sensitization or else a quantitative increase in local nerve fiber density through local neurogenesis.[13,15]

We hypothesized that an increase in local nerve density in zone II endometriosis leads to a tender posterior compartment on examination and therefore the symptom of deep dyspareunia. To test our hypothesis, our objective was to examine PGP9.5 nerve density in a study group of women with deep dyspareunia due to tender zone II endometriosis in comparison to 2 control groups. Stringent phenotypic criteria were utilized for the study group and control groups, and PGP9.5 nerve density was scored by pathologists blinded to the group. Based on our hypothesis, we expected that PGP9.5 nerve density would be higher in the study group compared to each control group.

Table 1. Phenotypic Groups: Inclusion/Exclusion Criteria for PGP9.5 Nerve Bundle Immunohistochemistry.

	Total Sample (n = 58)		
	Study Group	Control Group 1	Control Group 2
Phenotype	Tender Endometriosis n = 29	Nontender Endometriosis (Infertility) n = 17	Tender Nonendometriosis n = 12
Deep dyspareunia	+	−	+
Infertility	±	+	±
Obliterated cul-de-sac	−	−	−
Zone II tenderness[a]	+	−	+
Zone II lesion excised[b]	+	+	+
Zone II endometriosis[c,d]	+	+	−

Abbreviation: PGP9.5, protein gene product 9.5.
[a]Tenderness in the right/central/left zone II posterior compartment on examination.
[b]For the study group and control group 2, the lesion was excised from the corresponding right/central/left zone II posterior compartment where tenderness was noted on examination. For control group 1, the lesion was excised from the nontender zone II.
[c]Endometriosis was histologically confirmed in the excised lesion for the study group and control group 1. For control group 2, the lesion was negative for endometriosis and showed normal histology (n = 9) or chronic inflammation (n = 3).
[d]The excised lesion meeting the above criteria (in each group) was then utilized for PGP9.5 nerve bundle immunohistochemistry.

Material and Methods

Setting

The setting of the study is an academic tertiary referral center in Vancouver, Canada (Center for Pelvic Pain and Endometriosis). Institutional ethics approval was obtained from the University of British Columbia (H13-02563). Participants were ascertained from the practice of a Reproductive Endocrinology and Infertility (REI) specialist with expertise in laparoscopic excision of endometriosis (CW). Prior to surgery, patients were seen for a gynecologic assessment at which time the severity of deep dyspareunia (on a numeric rating scale 0-10) was recorded. At the gynecologic assessment, endovaginal ultrasound-assisted pelvic examination was performed, where the ultrasound probe was used to visualize and palpate structures for tenderness, such as the uterus, adnexa, uterosacrals, and cul-de-sac. We have previously shown that tenderness-guided endovaginal ultrasound-assisted pelvic examination increases the sensitivity for abnormalities at the time of laparoscopy, without a sacrifice in specificity.[21] At the time of laparoscopic surgery, all visually suspected endometriosis was excised using monopolar electrosurgery and sent to pathology for histological confirmation.

Inclusion Criteria

For this study, we reviewed all surgeries by CW between 2011 and 2013 (n = 477). We focused on cases where laparoscopic excision of suspected endometriosis was performed in the posterior compartment. The posterior compartment was defined as zone II, which includes the posterior cul-de-sac and uterosacrals, as proposed in a laparoscopic classification recently published by one of the authors (MB).[11,12] This zone II corresponds to zone III in the classification of the World Endometriosis Research Foundation for the Endometriosis Phenome and Biobanking Harmonization Project.[22]

We divided the zone II posterior compartment into 3 parts: (a) central zone II (central cul-de-sac); (b) right zone II (right uterosacral with the adjacent right cul-de-sac; and (c) left zone II (left uterosacral with the adjacent left cul-de-sac). To test our hypothesis, we refined the sample by selecting participants who fit one of the 3 stringently phenotyped groups: a study group and 2 control groups (Table 1). As described in Table 1, stringent phenotyping was based on history (deep dyspareunia), examination (zone II tenderness on endovaginal ultrasound-assisted pelvic examination), and laparoscopy (zone II findings at laparoscopic surgery with excision and histological confirmation).

The study group (tender endometriosis) consisted of women meeting the following criteria: (a) presence of deep dyspareunia; (b) tenderness of the right, central, or left zone II on endovaginal ultrasound-assisted pelvic examination; and (c) histologically confirmed endometriosis of the corresponding right/central/left zone II excised at laparoscopy. The surgically

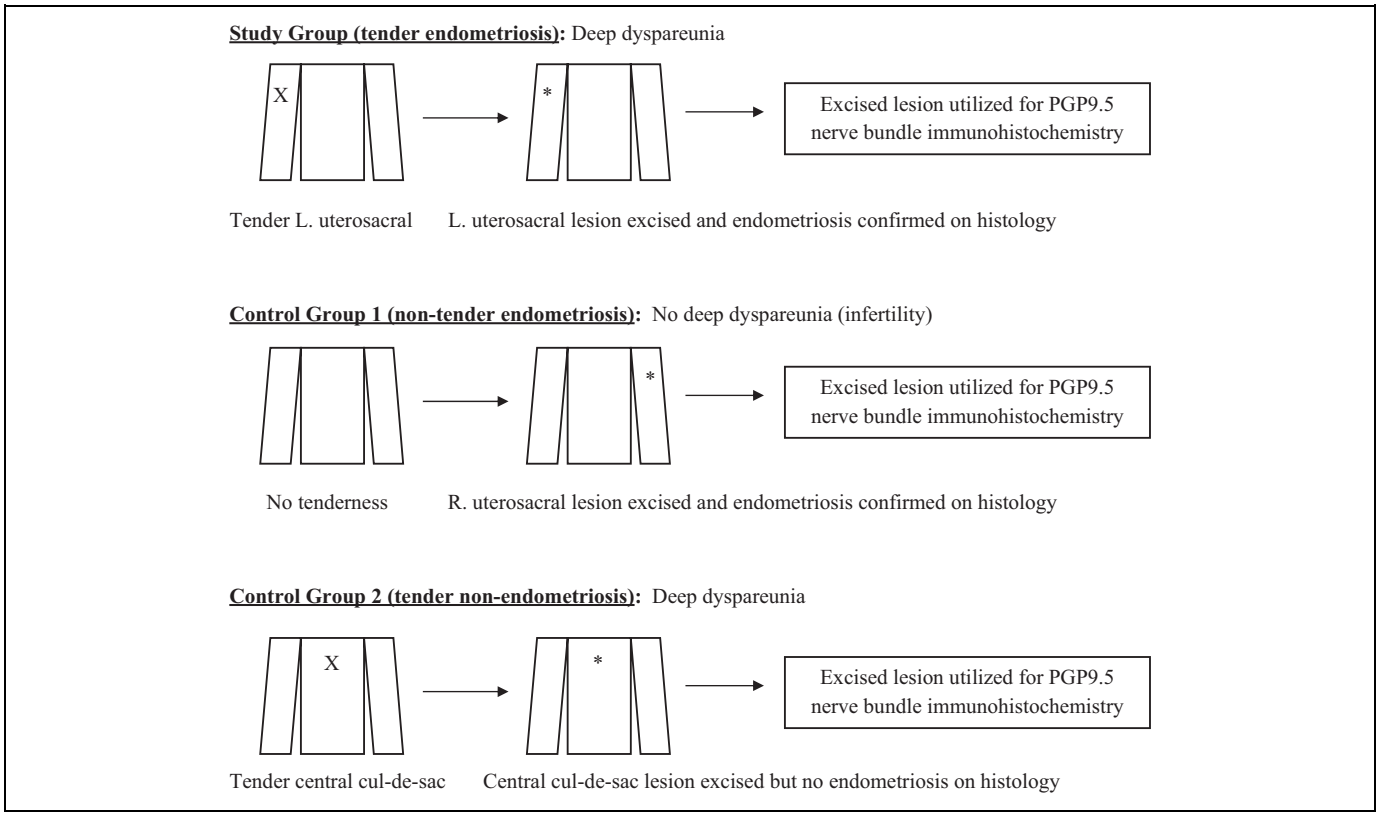

Figure 1. Examples of lesions utilized for protein gene product 9.5 (PGP9.5) nerve bundle immunohistochemistry.

excised lesion meeting these criteria was then used for PGP9.5 nerve immunohistochemistry. For example, a patient in the study group may have deep dyspareunia, a tender left uterosacral, and histologically confirmed endometriosis of the left uterosacral, with the left uterosacral lesion being utilized for PGP9.5 immunohistochemistry (Figure 1).

Control group 1 (nontender endometriosis) consisted of women with infertility trying for pregnancy, who met the following criteria: (a) absence of deep dyspareunia; (b) absence of zone II tenderness on endovaginal ultrasound-assisted pelvic examination; and (c) histologically confirmed endometriosis of zone II excised at laparoscopy. The surgically excised lesion meeting these criteria was then used for PGP9.5 nerve immunohistochemistry. For example, a patient in control group 1 may have no deep dyspareunia and no tenderness on examination, but histologically confirmed endometriosis of the right uterosacral, with the right uterosacral lesion being utilized for PGP9.5 immunohistochemistry (Figure 1). The purpose of control group 1 was to identify zone II endometriosis that was nontender on examination and that did not manifest as deep dyspareunia, in order to determine whether local nerve density was specific to exam tenderness/deep dyspareunia.

Control group 2 (tender nonendometriosis) consisted of women without endometriosis, who met the following criteria: (a) presence of deep dyspareunia; (b) tenderness of the right, central, or left Zone II on endovaginal ultrasound-assisted pelvic examination; and (c) visual suspicion of endometriosis lesions of the corresponding right/central/left zone II at laparoscopy, but the excised lesion was found to be histologically negative for endometriosis (ie, histologically normal or chronic inflammation). The surgically excised lesion meeting these criteria was then used for PGP9.5 nerve immunohistochemistry. For example, a patient in control group 2 may have deep dyspareunia, a tender central cul-de-sac, and a central cul-de-sac lesion suspicious for endometriosis seen at laparoscopy but found to be histologically negative for endometriosis, with the central cul-de-sac lesion being utilized for PGP9.5 immunohistochemistry (Figure 1). The purpose of control group 2 was to identify zone II nonendometriosis lesions that were tender on examination and manifested as deep dyspareunia in order to determine whether local nerve density was specific to endometriosis.

Exclusion Criteria

We excluded patients with an obliterated cul-de-sac. The reason is that severe deep endometriosis nodules producing cul-de-sac obliteration are known to be associated with high numbers of local nerves.[17] We only included patients with superficial endometriosis and/or isolated deep endometriosis without an obliterated cul-de-sac.

PGP9.5 Immunohistochemistry and Blinded Nerve Bundle Density Scoring

For each participant in the study group and control groups, we chose the surgically excised lesion meeting the above

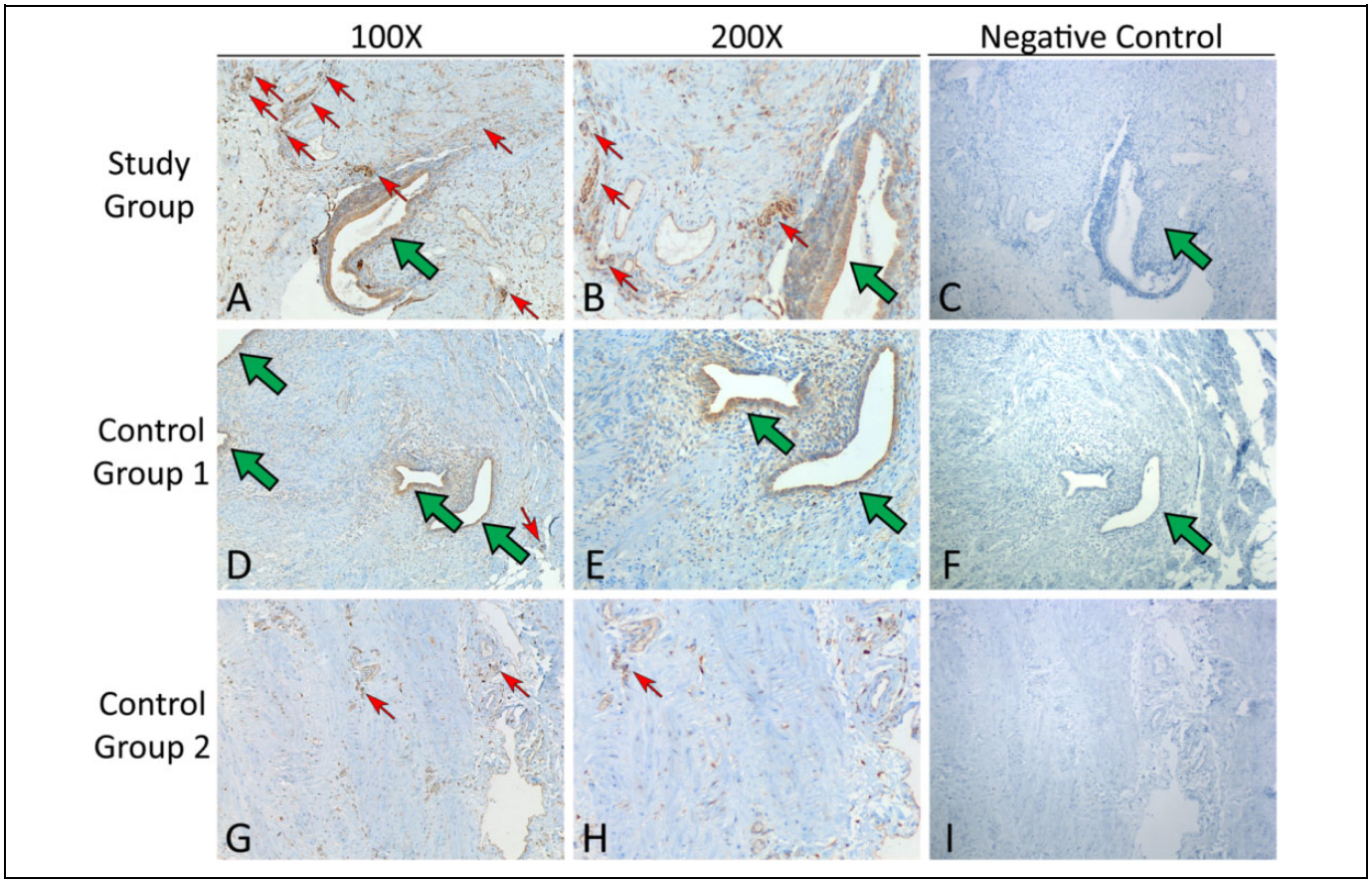

Figure 2. PGP9.5 nerve bundle immunohistochemistry.
PGP9.5 positive nerve bundles (small arrows) in the Study Group, Control Group 1, and Control Group 2, at 100X, 200X, and negative control (no primary antibody). Endometriosis glands/stroma also shown in the Study Group and Control Group 1 (large arrows).

inclusion/exclusion criteria for PGP9.5 nerve immunohistochemistry (Table 1). For the excised lesion in each participant, two additional tissue sections were cut from the formalin-fixed paraffin-embedded block and mounted onto charged glass slides. For the first slide, immunohistochemistry for the pan-neuronal marker PGP9.5 was carried out using a rabbit polyclonal anti-human PGP9.5 antibody (Ventana, Roche Diagnostics, 760-4434; predilute). Heat-induced epitope retrieval was utilized. Routine diaminobenzidine secondary antibody staining was done on the automated Ventana stainer. For the second slide, the same staining protocol was carried out except the primary antibody was omitted (negative control).

PGP9.5 positive nerve density in the surgically excised lesion in each participant was scored by one of the 2 pathologists with academic interest in gynecologic pathology (AL and LH). The pathologists were blinded to the group (study or control) and other patient characteristics; however, they could not be completely blinded to presence/absence of endometriosis, as histological endometriosis would be seen when scoring nerve density. Each pathologist scored PGP9.5 positive nerves using the following standardized method. High powered fields (HPFs) were scanned from top to bottom and then from left to right, for the entire portion of surgically excised tissue on the slide. This was done using a 20× objective and a 10× ocular, such that each HPF was 200× (area = 3.53mm^2). Both the number of PGP9.5 positive nerves and the number of HPFs were scored.

We chose to focus on PGP9.5 positive nerve bundles, defined as PGP9.5 positive nerve fibers surrounded by a perineurium, rather than individual PGP9.5 positive nerve fibers. The reason is that the nerve bundles were easily identifiable due to their large size and highly visible perineurium (Figure 2). To measure interobserver reliability, slides in 5 participants were examined by both pathologists blinded to each other's findings. The 2 pathologists had an intraclass correlation of .94 for the scoring of PGP9.5 positive nerve bundles, which indicates excellent interobserver reliability.

Primary Analysis

The primary outcome was PGP9.5 positive nerve bundle density, defined as the total number of PGP9.5 positive nerve bundles divided by the total number of HPFs examined in the slide for each participant (bundles/HPF).

For the primary analysis, PGP9.5 nerve bundle density was compared between the study group (tender endometriosis),

control group 1 (nontender endometriosis), and control group 2 (tender nonendometriosis; analysis of variance (ANOVA), followed by post hoc Tukey testing). In addition, PGP9.5 nerve bundle density was tested for a correlation with the severity of deep dyspareunia (0-10 numeric rating scale; Spearman rank correlation test).

The characteristics of each participant and excised lesion were also described in the study group and control groups to check for potential confounding. For patient characteristics, we looked at age, American Fertility Society (AFS) stage, AFS endometriosis score, AFS total score, and hormonal suppression. For example, previous studies have shown a reduction in nerve density with hormonal suppression, although not with phase of the menstrual cycle in natural cycles.[23-26] For characteristics of the excised lesion used for PGP9.5 immunohistochemistry, we looked at the amount of the excised tissue, and whether the excised tissue was taken from a superficial endometriosis lesion or deep endometriosis lesion (without cul-de-sac obliteration).

Secondary Analyses

Two secondary analyses were carried out. First, we repeated the comparison of PGP9.5 nerve bundle density in the study group and control group 1, correcting for the amount of histological endometriosis glands/stroma in the excised lesion. In this correction, we divided the PGP9.5 nerve bundle density by the number of HPFs involved by histological endometriosis. This adjusted PGP9.5 nerve bundle density was expressed as bundles/HPF/HPF. Second, we determined whether the relationship with PGP9.5 nerve bundle density was specific to deep dyspareunia, by testing whether nerve bundle density was also correlated with severity of chronic pelvic pain (rated from 0-10).

Statistics and Power Analysis

All statistics were performed using SPSS 22.0. Significance was $P = .05$ (two-tailed). Data were provided as n (%) or mean $\pm$ 1 standard deviation (range). Any missing data points were excluded, without imputation. Sensitivity analysis was not performed.

For the primary analysis, the primary outcome (PGP9.5 nerve bundle density) was compared between the study group, control group 1, and control group 2, using ANOVA followed by post hoc testing with the Tukey test. The assumption of normality was confirmed by using graphical inspection, the Kolmogorov-Smirnov test, and the Shapiro-Wilk test. The assumption of homogeneity of variances was confirmed using the Levene test. For the correlation between PGP9.5 nerve bundle density and severity of deep dyspareunia (0-10), the Spearman rank correlation test was used due to nonnormality in severity of deep dyspareunia.

For the other analyses, depending on the variables involved, we used ANOVA (with Welch correction for lack of homogeneity of variances, followed by the Games-Howell test for post hoc testing) or the Kruskal-Wallis test (for nonnormality, followed by Dunn post hoc testing with Bonferroni correction); the 2 sample t test (with Welch correction for lack of homogeneity of variances) or Mann-Whitney test (for nonnormality); and the chi-square test (for a 2×3 table) or the Fisher exact test (for a 2×2 table).

In the initial power analysis, a total sample size of 38 had 90% power to detect a correlation coefficient ≥ 0.5 for the primary outcome (PGP9.5 nerve bundle density) and severity of deep dyspareunia.[27]

Results

Sample Description

A total of 58 participants met the stringent inclusion/exclusion criteria and were included and analyzed in the study: 29 participants in the study group, 17 participants in control group 1, and 12 participants in control group 2 (Table 1). For the total sample, the mean age was 31.1 $\pm$ 6.2 years (range 17-45). For the primary outcome (PGP9.5 positive nerve bundle density), the mean density in the total sample was 0.92 $\pm$ 0.56 bundles/HPF (range 0-3.02).

Pain symptoms are shown in Table 2. Almost all patients had a history of dysmenorrhea. In contrast, severity of deep dyspareunia (0-10) was 0 in control group 1 (as expected based on the inclusion criteria in Table 1), which was significantly lower than the study group and control group 2. Similarly, severity of chronic pelvic pain (0-10) was significantly lower in control group 1 compared to the study group and control group 2.

Primary Analysis

Analysis of variance showed a significant difference in PGP9.5 nerve bundle density between the 3 groups ($F_{2,55} = 6.39$, $P = .003$; Figure 3). In the study group, the mean PGP9.5 positive nerve bundle density was 1.16 $\pm$ 0.56 bundles/HPF (range 0.02-3.02), which was significantly higher than in control group 1 (0.65 $\pm$ 0.36 bundles/HPF, range 0.15-1.40; post hoc Tukey test, $P = .005$) and in control group 2 (0.72 $\pm$ 0.56 bundles/HPF, range 0-1.74; post hoc Tukey test, $P = .044$). Representative PGP9.5 immunohistochemistry images are shown in Figure 2. PGP9.5 nerve bundle density was also significantly correlated with the severity of deep dyspareunia rated 0 to 10 (Spearman $r = .43$, n = 54, $P = .001$; Figure 4).

To check for confounding, clinical characteristics stratified by the 3 groups are shown in Table 3. The groups were similar in patient characteristics (AFS stage, AFS endometriosis score, AFS total score, and hormonal suppression) and in characteristics of the lesion used for PGP9.5 immunohistochemistry (size of lesion and whether the excised lesion was taken from superficial endometriosis or deep endometriosis without cul-de-sac obliteration). Only age was significantly different between the groups, with the study group being younger than control group 1 on post hoc testing ($P = .006$; Table 3). Nevertheless, there was no evidence of confounding between age and PGP9.5 nerve bundle density (Spearman $r = -.14$, n = 58, $P = .28$).

Table 2. Symptoms in the Phenotypic Groups.

	Total sample (n = 58)							
	Study Group	Control Group 1	Control Group 2			Post hoc Testing[a]		
Characteristic	Tender Endometriosis n = 29	Nontender Endometriosis (Infertility) n = 17	Tender Nonendometriosis n = 12	Statistical Test	*P* Value	Study vs Control 1	Study vs Control 2	Control 1 vs Control 2
Dichotomous (present/absent)	n (%)	n (%)	n (%)					
History of dysmenorrhea	27 (93%)	15 (88%)	10 (83%)	$\chi^2(df = 2)^b = 0.93$	.63	-	-	-
Severity scores (0-10)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)					
Deep dyspareunia severity	7.8 ± 2.3 (2-10)[c]	± 0.0 (0-0)	7.3 ± 2.9 (1-10)	$\chi^2(df = 2)^d = 36.0$	.001	*P* < .001	*P* = 1.00	*P* < .001
Chronic pelvic pain severity	7.7 ± 1.7 (4-10)[e]	0.5 ± 1.4 (0-5)[e]	8.0 ± 2.6 (2-10)	$\chi^2(df = 2)^d = 32.4$	.001	*P* < .001	*P* = 1.00	*P* < .001

Abbreviation: SD, standard deviation.
[a]Dunn post hoc testing with Bonferroni correction.
[b]Chi-square test for comparison of the 3 groups.
[c]Severity score missing for n = 4 (present/absent only).
[d]Kruskal-Wallis test (due to lack of normality) for comparison of the 3 groups.
[e]Severity score missing for n = 2 (present/absent only).

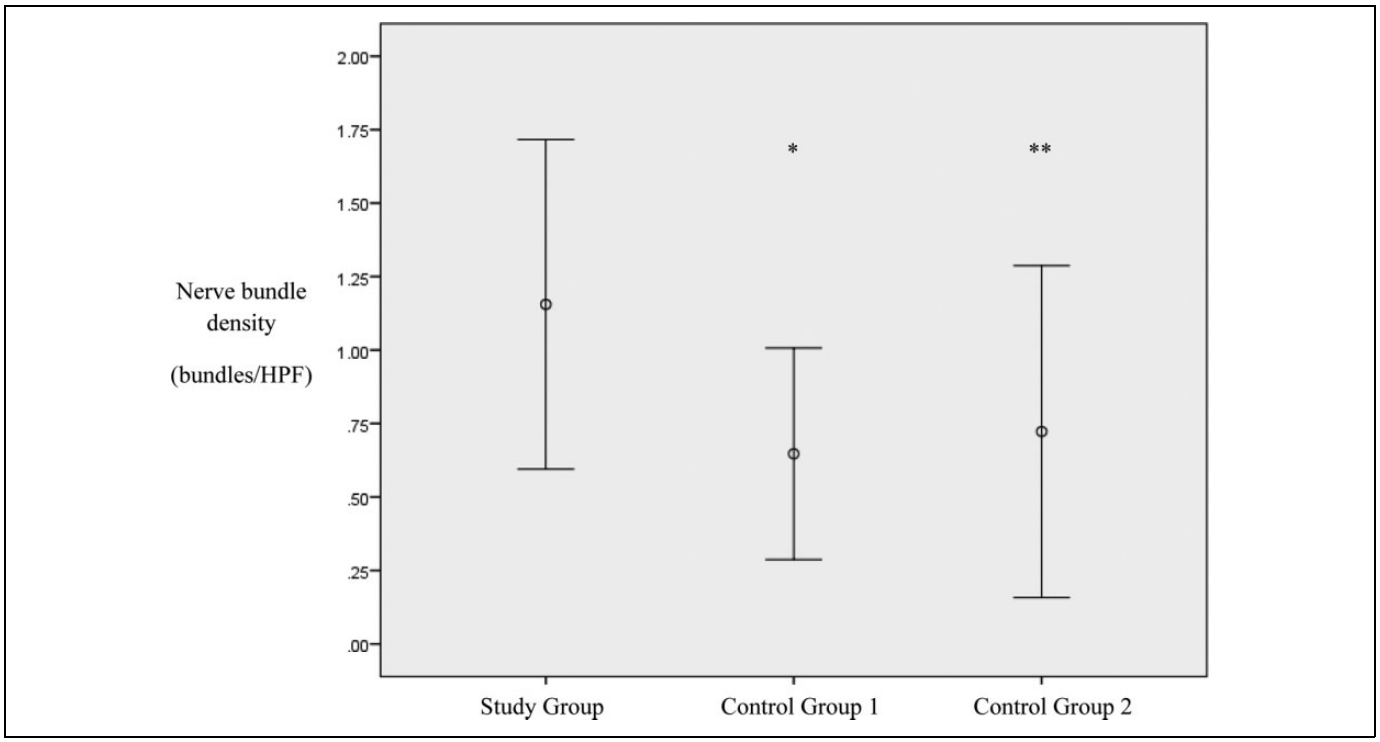

Figure 3. PGP9.5 nerve bundle density in the Study and Control Groups.
Mean +/- 1 standard deviation. ANOVA: F[2,55] = 6.39, p = 0.003. Post-hoc Tukey testing: *p = 0.005 **p = 0.044 (compared to Study Group).

Secondary Analyses

We repeated the analyses of PGP9.5 nerve bundle density in the study group and control group 1, correcting for the area of the excised lesion involved by histological endometriosis glands/stroma (see Material and Methods section). This adjusted PGP9.5 nerve bundle density was still significantly higher in the study group (1.42 ± 0.98 bundles/HPF/HPF, range

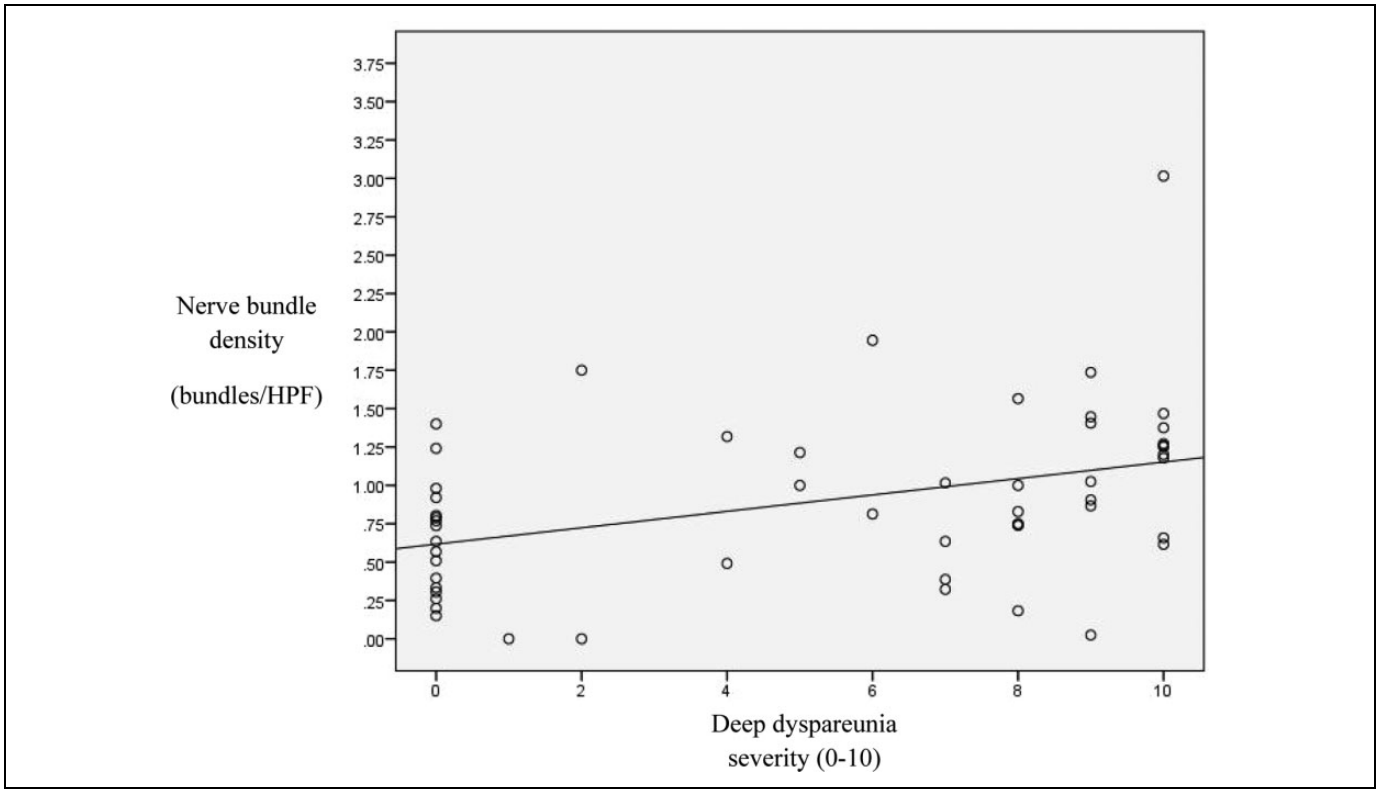

Figure 4. PGP9.5 nerve bundle density and severity of deep dyspareunia.
Scatterplot with linear fit line showing correlation between PGP9.5 nerve bundle density and deep dyspareunia severity (Spearman r = 0.43, n = 54, p = 0.001).

0.05-3.89) compared to control group 1 (0.47 $\pm$ 0.50, range 0.02-1.84; Mann-Whitney test, $U = 398$, $P = .001$). Similarly, the adjusted PGP9.5 nerve bundle density was still significantly correlated with severity of deep dyspareunia (0-10; Spearman $r = .49$, n = 42, $P = .001$).

In contrast to severity of deep dyspareunia, PGP9.5 nerve bundle density had a weaker correlation with severity of chronic pelvic pain (0-10; Spearman $r = .23$, n = 54, $P = .09$).

Discussion

This study provides evidence in favor of our hypothesis that an increase in nerve bundle density is associated with tenderness of zone II posterior compartment endometriosis and thus the symptom of deep dyspareunia. We found that PGP9.5 nerve bundle density, assessed in blinded standardized manner by pathologists, was higher in the study group (tender zone II endometriosis in women with deep dyspareunia) compared to the 2 control groups. Moreover, PGP9.5 nerve bundle density was not only associated with the study group but was also correlated with the severity of deep dyspareunia (0-10). Together, these findings suggest that local neurogenesis in the zone II posterior compartment may be associated with deep dyspareunia in endometriosis.

The strengths of this study include its use of stringently phenotyped groups (combining history, physical examination, and surgical and histological findings) in order to test our hypothesis. In addition, we employed blinding so that scoring of nerve bundle density would not be biased by knowledge of the groups. Also, the scoring of nerve bundles showed high interobserver reliability (intraclass correlation = .94) due to the bundles being large structures surrounded by perineurium that were easily identifiable and confirmed by PGP9.5 pan-neuronal immunohistochemistry. This study should also be generalizable to any patient with deep dyspareunia who has tenderness of the cul-de-sac/uterosacrals and who is found to have histological endometriosis of the corresponding cul-de-sac/uterosacral at laparoscopy. Furthermore, we reviewed 3 years of surgeries to select participants for the phenotypic groups, and the total sample size (n = 58) is higher than previous studies looking at nerve density and symptoms in endometriosis (see below).

Limitations of the study include its retrospective nature. Also, the groups were not matched for age, although there was no evidence of confounding between age and nerve bundle density. In addition, we utilized a pan-neuronal marker and thus do not have data on whether nerve subtypes may also differ between the groups, although endometriosis-associated nerve fibers are known to include the sensory A-delta fibers and C fibers involved in nociception.[14] It is also important to emphasize that nerve bundle density is just one factor in the etiology of deep dyspareunia, with other anatomic variables (eg, bladder tenderness) and psychological variables (eg, depression) also being important.[28,29]

Table 3. Characteristics of the Phenotypic Groups.

	Total Sample (n = 58)							
	Study Group	Control Group 1	Control Group 2			Post hoc Testing[a]		
Characteristic	Tender Endometriosis n = 29	Nontender Endometriosis (Infertility) n = 17	Tender Nonendometriosis n = 12	Statistical Test	*P* Value	Study vs Control 1	Study vs Control 2	Control 1 vs Control 2
Characteristics of the patients	n (%) or mean ± SD (range)	n (%) or mean ± SD (range)	n (%) or mean ± SD (range)					
Age, years	29.3 ± 6.8 (17-45)	34.2 ± 3.3 (26-40)	31.0 ± 6.8 (21-41)	$F_{2,26.5}$[b] = 5.63	.009	*P* = .006	*P* = .76	*P* = .31
AFS stage I/II	26 (90%)	15 (88%)	N/A[c]	Fisher[d]	1.00	-	-	-
AFS stage III/IV	3 (10%)	2 (12%)	N/A[c]	Fisher[d]	1.00	-	-	-
AFS endometriosis score	5.9 ± 3.5 (2-15)	6.6 ± 5.3 (2-25)	N/A[c]	*U* = 237[e]	.82	-	-	-
AFS total score	8.7 ± 10.3 (2-54)	8.1 ± 7.7 (2-31)	N/A[c]	*U* = 240[e]	.88	-	-	-
Hormonal suppression	16 (55%)	N/A[f]	8 (67%)	Fisher[d]	.73	-	-	-
Characteristics of the excised lesion used for PGP9.5 immunohisto-chemistry	n (%) or mean ± SD (range)	n (%) or mean ± SD (range)	n (%) or mean ± SD (range)					
Size of excised tissue for PGP9.5 (# HPF)	78.0 ± 39.6 (15-151)	68.5 ± 36.9 (19-120)	75.9 ± 65.8 (7-186)	$F_{2,24.5}$[b] = 0.33	.72	-	-	-
Excised tissue for PGP9.5 taken from superficial endometriosis	22 (76%)	10 (59%)	N/A[c]	Fisher[d]	.32	-	-	-
Excised tissue for PGP9.5 taken from deep endometriosis (without cul-de-sac obliteration)[g]	7 (24%)	7 (41%)	N/A[c]	Fisher[d]	.32	-	-	-

Abbreviations: SD, standard deviation; AFS, American Fertility Society; PGP9.5, protein gene product 9.5; HPF, high powered field; ANOVA, analysis of variance; N/A, not applicable.

[a]Games-Howell post hoc testing, due to lack of homogeneity of variances.

[b]ANOVA (with Welch correction for lack of homogeneity of variances) for comparison of the 3 groups.

[c]N/A because control group 2 did not have endometriosis.

[d]Fisher exact test for 2 × 2 table.

[e]Mann-Whitney test due to nonnormality.

[f]N/A because by definition, all control group 1 patients had infertility and were trying for pregnancy. Hormonal suppression is known to reduce nerve density, while absence of hormonal suppression is associated with higher nerve density.[23-26] Since our hypothesis was that the study group would have more nerve bundles than control group 1, the absence of hormonal suppression in control group 1 is a conservative control (ie, it would be expected to increase the nerve bundles in control group 1).

[g]We only used isolated deep endometriosis lesions of zone II without cul-de-sac obliteration (ie, we excluded severe deep nodules with an obliterated cul-de-sac).

Previous studies have shown initial evidence for an association between nerve density and pain symptoms (including dyspareunia) in women with endometriosis. In a study of 28 cases of deep rectovaginal endometriosis, the authors found that nerve fibers within endometriosis lesions were more frequent in cases with dysmenorrhea, pelvic pain, or deep dyspareunia categorized as binary variables (>7/10 vs ≤7/10).[17] In a study of 51 cases, it was found that endometriosis-associated nerve fibers were more likely to be present versus absent in cases with dysmenorrhea or pelvic pain categorized as a binary variable (>2/10 vs ≤2/10), but there was no association with deep dyspareunia also categorized as a binary variable (present vs absent).[18] In a Chinese language study, it was reported that posterior compartment nerve fibers in endometriosis were associated with dyspareunia and dyschezia.[20] Therefore, 2 of these 3 previous studies also showed an association between local nerves and dyspareunia.

We propose a model where a quantitative increase of nerve bundles in the zone II posterior compartment leads to amplification of afferent nociceptive signaling,[13-15] which results in

hyperalgesia in the posterior compartment. Thus, deep-hitting contact of the posterior compartment becomes painful, which predisposes to tenderness on examination and to deep dyspareunia with intercourse. It should be noted that there was variability in nerve bundle density in the study group and control groups (Figures 3 and 4), which indicates that nerve bundle density is not the sole etiological factor in deep dyspareunia. Other multifactorial contributors likely interact with local nerve bundle density to produce tenderness of the posterior compartment and deep dyspareunia, which may include the degree of local inflammation from endometriosis, peripheral sensitization, central sensitization, and psychological factors.[13-15]

This study has several clinical implications. For example, surgical ablation of endometriosis may not have the same effect on nerve bundles surrounding endometriosis compared to wide excision of the entire lesion. In fact, long-term results of a randomized controlled trial showed that excision was superior to ablation specifically for the outcome of dyspareunia.[30] In addition, local neurogenesis may provide another therapeutic target for endometriosis.[31] For future research, we are investigating the factors that mediate the increase in nerve bundles in some women with endometriosis (eg, nerve growth factor and its receptors).[13-15]

In conclusion, nerve bundle density in the cul-de-sac/uterosacrals was associated with tenderness and deep dyspareunia. Thus, local neurogenesis may be an etiological factor for deep dyspareunia in endometriosis.

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Authors' Note

This study was performed at University of British Columbia (UBC). Data and tissue blocks can be accessed by contacting the PI (P. Yong).

Declaration of Conflicting Interests

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