

Distribution of Nerve Fibers in Abdominal Wall Endometriosis and Their Clinical Significance

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Objective: This study aimed to explore the distribution of nerve fibers in abdominal wall endometriosis (AWE) and discern their association with pain.

Methods: A retrospective case-control study was conducted. The cases comprised 30 patients diagnosed with AWE, while the control group consisted of 17 patients who had undergone laparotomy without any history of endometriosis. We analyzed clinical characteristics and examined the innervation patterns in samples using stains for S-100, neuron-specific enolase (NSE), protein gene product 9.5 (PGP9.5), neurofilament (NF), and substance P (SP) antibodies.

Results: There was a notable increase in the density of S-100, NSE and PGP9.5 immunoreactive nerve fibers and a higher proportion of SP positivity in AWE lesions compared to standard abdominal wall scars ($p < 0.05$). However, there were no significant differences in the density or proportion of NF-immunoreactive nerve fibers between the cases and the controls. Moreover, no statistically significant correlation was observed between the density of S-100, NSE, PGP9.5, NF, or SP-positive nerve fibers and pain scores.

Conclusion: This study demonstrated an increased immunoreactive nerve fiber density located in AWE lesions compared to normal abdominal wall scars. Further high-quality studies are needed to investigate the mechanisms responsible for pain in women with endometriosis.

Keywords: abdominal wall endometriosis, pain, nerve fibers, immunohistochemistry

Introduction

Endometriosis (EMs) is characterized by the ectopic presence of endometrial tissue outside the uterine cavity, which can respond to ovarian hormonal stimuli. Predominantly observed in women of reproductive age, it leads to various pain manifestations such as dysmenorrhea, rectalgia, dyschezia, dyspareunia, and chronic pelvic pain.¹ EMs can manifest outside the genital tract and has been reported in nearly all organs, including the skin, brain, and lungs.² The abdominal wall endometriosis (AWE) remains the most prevalent form of extragenital EMs.³

Patients diagnosed with AWE often report palpable pain around scars from prior gynecological procedures.⁴ This pain, which tends to intensify in severity during menstruation, significantly impacts the patient's quality of life. Nevertheless, the precise relationship between pain and AWE is yet to be elucidated. Notably, deep infiltrating pelvic endometriosis frequently results in severe pain and discomfort.⁵ Prior clinical studies have indicated a potential correlation between pain severity and nerve fiber distribution in endometriotic lesions,^{6,7} prompting our research team to investigate the pain mechanisms in AWE. To date, few researchers have delved into the innervation of these nodules.

Painful scars could result from nerve injury or entrapment following surgical intervention or traumatic injury, leading to disruption of the structural integrity of the dermis and underlying soft tissues.⁸ Our objective is to perform a comparative analysis of the collagen-rich and fibrous characteristics exhibited by both scar subcutaneous tissue and AWE lesion while also investigating the underlying factors contributing to pain associated with AWE. In this investigation, we will utilize the painless subcutaneous

tissue of scars as a control group. The presence of various nerve fibers was confirmed through immunohistochemistry using specific markers, including S-100, neuron-specific enolase (NSE), protein gene product 9.5 (PGP9.5), neurofilament (NF), and substance P (SP), to differentiate between myelinated, unmyelinated, and other types of nerve fibers. Additionally, we collected clinical data with a focus on pain symptoms to explore potential associations with AWE lesion innervation.

Methods

Study Protocol

A retrospective case–control study was conducted. The specimens were gathered during routine surgical procedures at the Department of Gynecology and Obstetrics, Peking Union Medical College Hospital, from March to December 2009. The participants consisted of women within the reproductive age range, specifically between 18 and 45 years old. All participants were required to abstain from any endometriosis-related treatment for a period of three months prior to surgery. The AWE group comprised individuals with severely agonizing nodules that exhibited resistance to conservative treatment. The control group consisted of participants who had undergone a previous laparotomy for benign gynecological conditions (eg, uterine leiomyoma) but did not have EMs. They subsequently underwent a secondary laparotomy and did not report any pain at the surgical scar. All the patients underwent standardized laparotomy by the same senior surgical team. Based on histopathological findings, participants were categorized into an AWE (case) group and non-AWE (control) group. The presence of various nerve fibers, including S-100, NSE, PGP9.5, NF, and SP, was confirmed by immunohistochemistry analysis of the paraffin section. Clinical data on patients' ages, initial symptoms, examines, surgery details, and follow-up were analyzed. The study received approval from the Human Ethics Committees of Peking Union Medical College Hospital, and informed consent was obtained from all participants. Our research adheres to the principles outlined in the Declaration of Helsinki.

Clinical Outcomes

The dataset included preoperative data, including demographic details, such as age and parity, and clinical characteristics, such as asymptomatic duration (from previous surgery to symptom occur), symptomatic duration, number of abdominal masses, diameter of mass, blood flow surrounded the lesion, location of the lesion, pain of the lesion, dysmenorrhea, type of incision, and depth of infiltration. The assessment of pain using the visual analogue scale (VAS) involves the utilization of a ruler equipped with precise markings, enabling patients to accurately indicate their pain intensity by marking the corresponding position on the ruler. Subsequently, physicians assign a score based on the marked position, facilitating a relatively objective evaluation of pain levels before and after treatment while accounting for potential individual variations.⁹ The postoperative follow-up data were extracted from the medical records of outpatients. The documentation of pain relief was also collected.

Immunohistochemistry

Surgical tissue samples, from either the AWE lesions or control subcutaneous tissue of scars, were immediately preserved in 10% neutral buffered formalin and forwarded to the Pathomorphology Department. Two experienced pathomorphologists independently assessed the hematoxylin and eosin (H.E.) stained slides obtained from paraffin-embedded tissues, meticulously selecting appropriate samples for immunohistochemistry. Subsequently, sections measuring 4 μm in thickness were carefully prepared for further analysis.

Sections from each sample were immunostained overnight at 4°C using specific antibodies, including polyclonal rabbit anti-S-100 protein (dilution 1: 100; Abcam Inc. USA), monoclonal mouse anti-human NSE (dilution 1: 100; Abcam Inc. USA), monoclonal mouse anti-human PGP9.5 protein (dilution 1: 100; Abcam Inc. USA), monoclonal mouse anti-human NF protein (dilution 1: 100; Zeta Corporation USA), and polyclonal rabbit anti-human SP protein (dilution 1: 100; Abcam Inc. USA). After washing with Phosphate Buffered Saline (PBS), sections were incubated with PV9001/9002 agent (GBI, USA) for 30 minutes at 37°C. The antigen–antibody reactions were visualized using 3,3'-diaminobenzidine (DAB). Sections were counterstained with hematoxylin, dehydrated, and mounted. PBS was employed as a negative control to the antibodies.

Specimens were visualized using a Nikon OPTIPHOT-2 optical microscope and images captured with the ACT-2U Digital Image Acquisition System. Nerve fibers in EMs lesions or control group subcutaneous tissues were enumerated per low-power field ($\times 100$). The total number of nerve fibers was divided by actual squares of low-power field (each

square of 0.425 mm²) to obtain an average of nerve fibers per mm². Results were presented as mean (\pm SD) nerve fibers per mm² for all EMs and control sections. Two histopathologists, blinded to sample origins, independently assessed the immunohistochemical reactions, focusing on areas with high nerve density.

Statistical Analysis

Continuous variables were presented as the mean \pm standard deviation and compared using Student's *t*-test. Categorical variables were presented as counts (percentages) and were compared using the chi-squared test. The Spearman correlation analysis was employed to ascertain the association between nerve fibers count in EMs lesions and pain severity. Statistical analyses were finished with Statistical Package for the Social Sciences (SPSS, Version 22.0. Armonk, NY: IBM Corp). A *p*-value <0.05 were deemed statistically significant.

Results

Clinical Characteristics of Participants

A total of 47 eligible patients were included in the study. Among the participants, thirty individuals (average age: 32.7 years; range: 27–45 years) in the AWE group presented with a painful nodule that enlarged during menstruation, located either on a previous cesarean section scar (27 cases) or another laparotomy scar (3 cases) following excision of an endometriotic lesion. In the control group, seventeen patients (average age: 34.2 years; range: 30–42 years) without any pain on their surgical scars were recruited. Their demographic and clinical data are detailed in Table 1.

Table 1 Clinical Features of the Patients in AWE and Control Groups

	AWE (n=30)	Control (n=17)	P
Age (y)	32.7 \pm 7.5	34.2 \pm 6.2	0.488*
Parity (n)	0.9 \pm 0.3	0.8 \pm 0.4	0.336*
Incisions			
Pfannenstiel (n, %)	24(80%)	14(82.4%)	0.850**
Vertical midline (n, %)	6(20%)	3(17.6%)	
Dysmenorrhea (n, %)	21(70.0%)	4(23.5%)	0.002**
VAS of dysmenorrhea	4.8 \pm 2.4	1.5 \pm 1.2	<0.001 *
Asymptomatic duration (month)	25.0 \pm 16.8	–	
Symptomatic duration (month)	26.8 \pm 17.6	–	
No. abdominal mass			
Solitary (n, %)	21(70.0%)	–	
Multiple (n, %)	9 (30.0%)	–	
Diameter of mass (cm)	1.8 \pm 1.1	–	
Blood flow surrounded the lesion			
High impedance flow (n, %)	25(83.3%)	–	
Low impedance flow (n, %)	5(16.7%)	–	
Location of lesion			
In scar (n, %)	33(82.5%)	–	
Away from scar (n, %)	7(17.5%)	–	
Pain of lesion (n, %)			
Cyclic pain	26(86.7%)	–	
Noncyclic pain	4(13.3%)	–	
VAS of pain	4.1 \pm 2.9	–	
Depth of infiltration			
Subcutaneous only (n, %)	19(63.3%)	–	
Affect the fascia (n, %)	11(16.7%)	–	

Notes: *Student's *t*-test. **Chi-squared test.

Abbreviation: VAS, visual analogue scale.

Table 2 Nerve Fiber Density and Composition in AWE Lesions and Control Scars

Groups	Positive n (%)			Density (/mm ²)			Pearson Correlation p value ^a
	AWE (n=30)	Control (n=17)	p	AWE (n=30)	Control (n=17)	p	
S-100	30(100%)	17(100%)	–	7.00±4.68	3.05±1.92	0.002*	0.61
NSE	26(86.7%)	12(70.6%)	0.337**	7.99±3.97	2.89±2.06	<0.001*	0.42
PGP9.5	29(96.7%)	15(88.2%)	0.606**	7.60±5.15	4.02±2.83	0.011*	0.30
SP	17(56.7%)	3(17.6%)	0.009**	0.57±0.26	0.42±0.30	0.079*	0.11
NF	19(63.3%)	10(58.8%)	0.760**	2.60±1.23	1.98±1.05	0.087*	0.32

Notes: *Student's *t*-test. **Chi-squared test. ^aThe p value of Pearson correlation between nerve fiber density and visual analogue scale (VAS) score of pain.

Abbreviations: AWE, abdominal wall endometriosis; NSE, neuron-specific enolase; PGP9.5, protein gene product 9.5; NF, neurofilament; SP, substance P.

Seventy percent (21/30) of AWE patients experienced dysmenorrhea, with a visual analogue scale (VAS) score of 4.8±2.4. In contrast, only 23.5% (4/17) of control group patients reported dysmenorrhea with a VAS score of 1.5±1.2. Among the AWE patients, the average time from previous surgery to symptom onset was 25.0±16.8 months, with an average symptom duration of 26.8±17.6 months. Thirty percent (9/30) of patients exhibited multiple endometriomas, 17.5% (7/30) had masses distanced from the scar, 86.7% (26/30) experienced cyclic pain on the lesions, and the remainder reported noncyclic pain. The average mass diameter was 1.8±1.1 cm. Eleven patients had masses that infiltrated the fascia, while four required synthetic mesh for abdominal wall reconstruction.

Immunohistochemistry Findings

S-100 antibody displayed positive immunorexpression in all 47 patients. A significant difference in nerve fiber density stained with S-100 existed between the AWE group (7.00±4.68/mm²) and the control group (3.05±1.92/mm², *p* = 0.002; Table 2; Figure 1a). Immunohistochemical analysis using NSE antibody was positive in 38 (80.9%) patients. Among

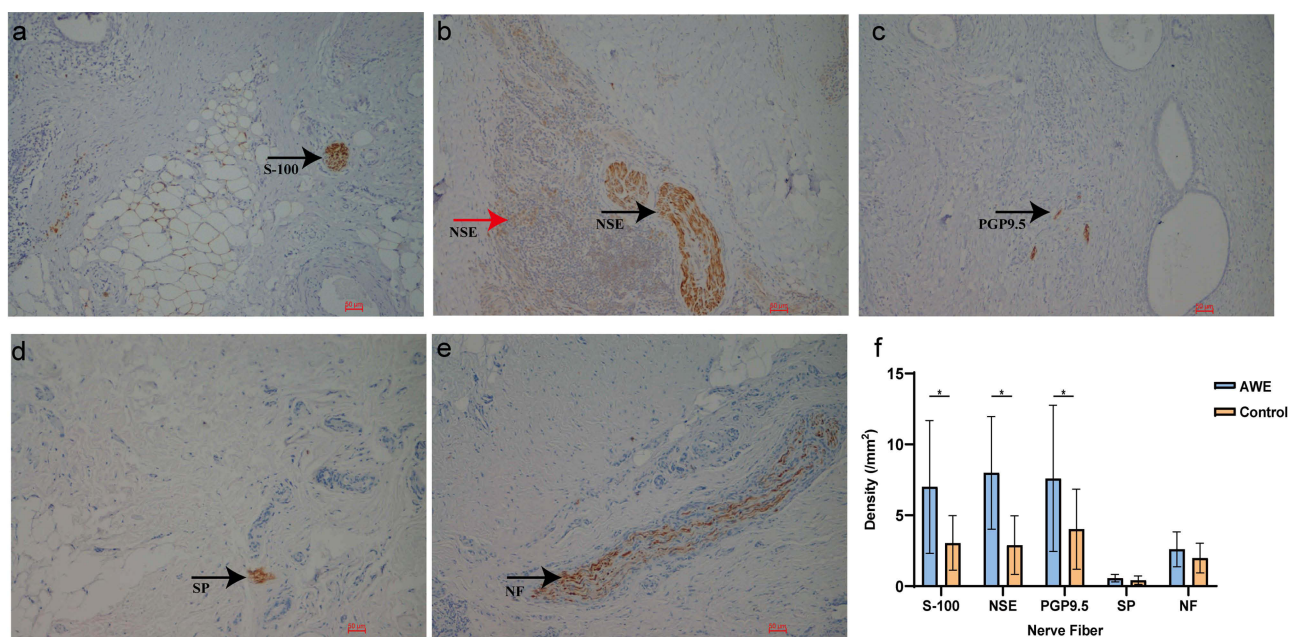


Figure 1 Nerve fibers in abdominal wall endometriotic lesions stained with S-100 (a), NSE (b), PGP9.5 (c), SP (d) and NF (e). Black arrows denote tiny positive multiple nerve fibers. The red arrow denotes neuroendocrine cells stained for NSE in ectopic endometrium. Magnification ×100 for all photomicrographs. (f) Nerve fiber density in AWE lesions and control scars.

Notes: *Indicates a significance level of *p* < 0.05.

Abbreviations: NSE, neuron-specific enolase; PGP9.5, protein gene product 9.5; NF, neurofilament; SP, substance P; AWE, abdominal wall endometriosis.

these, 86.7% (26/30) were from the AWE group and 70.6% (12/17) from the control. The nerve fiber density in AWE lesions ($7.99 \pm 3.97/\text{mm}^2$) significantly surpassed that in control ($2.89 \pm 2.06/\text{mm}^2$, $p < 0.001$). NE cells intensely stained with NSE were evident in the glands of ectopic endometrium of all 30 AWE patients (Table 2; Figure 1b). The density of PGP9.5 in the AWE group was $7.60 \pm 5.15/\text{mm}^2$, whereas it was $4.02 \pm 2.83/\text{mm}^2$ in the control group, with a significant p value of 0.011 observed between the two groups. However, there was no significant difference in the positive ratios between the two groups ($p = 0.606$; Table 2; Figure 1c).

SP positivity was observed in 56.7% (17/30) of AWE samples, substantially higher than the 17.6% (3/17) in the control group ($p = 0.009$). However, there was no significant difference in SP-immunoreactive nerve fiber density between the groups ($0.57 \pm 0.26/\text{mm}^2$ in AWE vs $0.42 \pm 0.30/\text{mm}^2$ in control; $p = 0.079$; Table 2; Figure 1d). Similarly, we found no statistical difference in NF-immunoreactive nerve fiber densities between the two groups ($p > 0.05$; Table 2; Figure 1e). The discrepancy in nerve fiber density between AWE and control groups was illustrated in Figure 1f. While no significant correlation was identified between nerve fiber densities and pain VAS scores ($p > 0.05$; Table 2).

Follow-Up Details

The average follow-up post-procedure was 19.2 months. Over 96% (29/30) of AWE patients remained asymptomatic at their last examination. One patient still reported mild pain around the scar, without a palpable mass. Combined oral contraceptives alleviated the discomfort.

Discussion

Our study identified increased densities of S-100, NSE and PGP9.5 immunoreactive nerve fibers and a greater proportion of SP immunoreactive nerve fibers in AWE lesions compared to normal abdominal wall scars. However, there were no significant differences in NF-immunoreactive nerve fiber densities between groups. Furthermore, no significant correlation emerged between nerve fiber densities and pain severity as quantified by the visual analogue score.

Previous reports have identified nerve fiber proliferation in various endometriotic lesions.^{7,10–13} Multiple factors can contribute to nerve fiber growth in ectopic endometrium, including hormonal stimulations,^{14–16} inflammatory mediators,^{17–19} and potential interactions between nerve fibers, blood vessels, and ectopic endometrium.^{20,21} In this study, several markers of nerve fibers, such as S-100, NSE, PGP9.5, NF and SP, were chosen, and the clinical meanings of these markers were tried to explain the potential mechanism of the pain of AWE lesions.

S-100 is a dimer intracellular calcium-binding protein, expressed in melanocytes, glial cells and Schwann's cells.²² The S-100 family has been reported involved in the regulation of a cellular processes,²³ such as the participation in mediating fibroproliferative remodeling through the RAGE-NA-kB axis,²⁴ the vascular remodeling in endometriotic angiogenesis,²⁵ and the activating NF- κ B signaling pathway in endometrial stromal cells which promotes the development of EMs.²⁶ NSE is a dimer intracellular enzyme of glucose metabolism²⁷ and exists in neurons and peripheral nervous system tissue. NSE is a sensitive marker of neuronal damage. These two biochemical markers above have been widely used in several central nervous system diseases, including hypoxic brain injury, acute ischemic stroke, head injury and epilepsy, as potent predictors of neurological outcome.²⁸ In our study, the elevated expression of S-100 and NSE may be related to the presence of possible neuroendocrine pathways and the injury and regeneration of nerve fibers in AWE lesions.

NSE is also one of the most commonly used immunohistochemical markers of neuroendocrine (NE) cells. The NE system is defined as cells present either in endocrine organs or dispersed throughout the human body. NE cells can produce neurotransmitters, neuromodulators, neuropeptide hormones, paracrine regulators and other bioactive substances.²⁹ Vittoria et al³⁰ presented that a few NE cells might be contained in the normal human endometrium and increased in proliferative conditions, such as endometrial carcinomas. In this study, large number of NSE-stained cells in ectopic endometrium were observed, which was consistent with Wang's observation,³¹ and he also reported no NSE-positive NE cells were detected in proliferative phase endometrium in women without EMs and very low density in secretory and menstrual period. We postulated that the intense expression of NSE in NE cells in AWE lesions may stimulate nerve fibers and nociceptors to induce pain signals.

PGP9.5, a cytoplasmic thiol-esterase restricted to nervous and neuroendocrine cells, has been proved to be increased in the proliferative phase of endometriotic women with pain.³² NF is a specific marker for myelinated nerve fibers,³³

whereas SP³⁴ is sensory nerve fiber marker that represents both A δ and C fibers. Published articles proved a higher nerve fiber density with the positive presentation of the above markers in EMs patients compared with controls.^{35–37} Our data revealed a significant difference in PGP9.5 expression between AWE and control groups, along with a relatively high proportion of SP expression but no difference in NF expression. These findings suggest that the involvement of type A δ and C fibers in pain generation associated with abdominal wall endometriosis.

The pain caused by EMs lesions can be variable. Some lesions are not painful, while others can cause neuroinflammation at a distance up to 28 mm.³⁸ In this study, no significant connection was proved between nerve fiber density and the severity of patients' pain. This result might also be restricted by the small number of patients, and the correlation might change with increased patients.

This is the first time to observe the distribution of nerve fiber in abdominal wall endometriotic lesions. The increased innervation of AWE gives us another explanation of the typical experience a palpable mass with cyclic or noncyclic pain. Besides the mechanical and thermal irritation caused by cyclic congestion, they may also combine with hyperalgesia, which is an exacerbation of pain or initiation of painful sensation to a non-painful stimulus. In fact, EMs have been proposed to be a consequence of neurological dysfunction, and possibly involved in a process of denervation and reinnervation.³⁹ The infiltrating of endometrium in abdominal wall subcutaneous tissue might impair the local nerve fiber and lead to a series of denervation and reinnervation process.

Our study has limitations. The sample size was modest, pain severity assessments were subjective, and we only considered certain immunoreactive nerve fibers. It is essential to understand that pain in EMs may result from a combination of factors, including hormonal interactions, angiogenesis, neurotransmitters, inflammation, and others. Further comprehensive studies could provide a more nuanced understanding of these mechanisms.

Conclusion

In summary, this study demonstrated an increased immunoreactive nerve fiber density in AWE lesions compared to normal abdominal wall scars. However, no correlation was observed with clinical pain. The presence of augmented nerve fibers in AWE lesions may play a substantial role in the pathogenesis of pain and tenderness. Further comprehensive, high-quality studies are essential to elucidate the mechanisms underlying pain in women with EMs.

Abbreviations

EMs, endometriosis; AWE, abdominal wall endometriosis; NSE, neuron-specific enolase; PGP9.5, protein gene product 9.5; NF, neurofilament; SP, substance P; VAS, visual analogue scale.

Data Sharing Statement

The article does not involve sequencing data. Demographic information and scientific results are already shown in the main text. All the data used in the study are available from the corresponding author upon reasonable request.

Details of Ethics Approval

Approved from The Institutional Review Board (IRB) of Peking Union Medical College Hospital.

Informed Consent Addressed

All participants gave their informed consent for participation.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declared no potential conflicts of interest.

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