SCIE DOI: 10.14744/scie.2018.96168
South. Clin. Ist. Euras. 2017;28(4):261-265

Original Article

Diagnostic Value of Endometrial Nerves in the Endometrial Tissue of Patients with Endometriosis

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Submitted: 22.01.2018
Accepted: 29.01.2018

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Keywords: Endometriosis; immunohistochemistry; nerve fibers.

ABSTRACT

Objective: The aim of the present study was to determine the density of nerve fibers in the functional layer of the endometrium in patients with endometriosis in order to determine if it could be used as a minimally invasive diagnostic method for endometriosis. A secondary goal was to assess the relationship between the severity of pelvic pain due to endometriosis and the density of nerve fibers in endometrial sampling materials.

Methods: Endometrial sampling was performed in 67 patients who presented at the hospital between August 2011 and November 2012. Endometriosis was diagnosed by surgical histopathological examination. A total of 34 patients diagnosed with endometriosis were selected as the study group. Thirty-three patients who were operated on for benign conditions other than endometriosis were selected as a control group. Immunohistochemical detection of the nerve fiber marker protein gene product 9.5 (PGP 9.5) was used to analyze the endometrial samples for nerve fibers. Visual Analogue Scale scores were used to evaluate the correlation between nerve fiber density and the severity of pelvic pain. Statistical analyses were calculated with SPSS Statistics for Windows, Version 17.0 (SPSS, Inc., Chicago, IL, USA).

Results: The mean density of nerve fibers was 1.85±1.74 fibers/mm^2 in the endometriosis group. In the control group, the mean density was 1.15±1.48 fibers/mm^2. There was no statistically significant difference between the groups (p=0.08). The pelvic pain score did not correlate with PGP 9.5 intensity.

Conclusion: The detection and measurement of nerve fibers in the eutopic endometrium using the PGP 9.5 marker was not found to be a decisive noninvasive method to diagnose endometriosis.

INTRODUCTION

Endometriosis is an important problem of women's health that affects quality of life.[1] Pelvic pain and dysmenorrhea are among the most common complaints.[2]

Diagnosis can be made based on clinical symptoms, imaging methods, and surgical pathological examinations. The clinical complaints of the patient are sometimes consistent with endometriosis but may not be definitively diagnosed with any diagnostic method. The most valuable method for diagnosis is a laparoscopic examination and pathological sampling.

However, as laparoscopy cannot be performed on every patient, minimally invasive methods are of particular importance in this group of patients.[3,4]

A non-invasive, inexpensive, reliable, and easily applicable method is needed to diagnose these patients.

There are some differences in the eutopic endometrium of women with endometriosis compared with the normal endometrium. Various abnormalities may manifest, such as structural abnormalities, enhanced proliferation ability, immune components, adhesion molecules, proteolytic enzymes, steroids, and cytokine production.[5–7]

It was determined in some studies that there was a significant increase in the number of nerve fibers in endometrial tissue in endometriosis patients. This increase in nerve fibers was investigated in relation to pelvic pain and dysmenorrhea.

The relationship of nerve fibers to pain is controversial and there is still much related to endometriosis that is not clear.[8–16] Progesterone treatment revealed a decrease in endometrial nerve fibers, but no clinical significance was identified.[17] As a result, further studies were recommended in recent publications for these patient groups.[18,19]

The objective of this study was to investigate the relationship between these nerve fibers and the diagnosis and symptoms of endometriosis patients.

MATERIAL AND METHODS

This study was performed with patients who underwent a laparotomy or laparoscopy for benign indications (infertility, chronic pelvic pain, dysmenorrhea, or ovarian cysts) between August 2011 and November 2012 in the Obstetrics and Gynecology Department of the hospital. Women of reproductive age between 19 and 45 years were included. Patients receiving hormonal treatment in the 3 months prior to surgery, with a history of malignancy or systemic disease, or who were pregnant, were excluded from the study. The Dr. Lutfi Kirdar Kartal Education and Research Hospital ethics committee granted approval of the study and all of the women participating provided written, informed consent.

The patients were divided into 2 groups: the study group and the control group. Women who were diagnosed with endometriosis surgically and histologically, staged according to the revised staging system of American Society for Reproductive Medicine (ASRM, 1996) comprised the study group. Patients who were operated on for various benign gynecological indications (persistent cystic mass, paratubal cyst, tubal ligation, etc.) and in whom the presence of endometriosis had been ruled out were selected as the control group.

The severity of endometriosis-associated pain symptoms was documented before surgery using a standardized questionnaire and the Visual Analogue Scale (VAS). The pain scale was subdivided into 10 parts. "No pain" was indicated on the left side of the scale and "the maximum pain you can imagine" was on the right side of the scale.

In both groups, endometrial samples were taken during the operation under sterile conditions from all the endometrial surfaces using a pipella cannula. Tissue was stored in polypropylene tubes at -80°C. Endometrial biopsies were analyzed by the Pathology Department of the hospital. The protein gene product 9.5 (PGP 9.5) antibody was used to immunohistochemically assess all myelinated and unmyelinated nerve fibers.

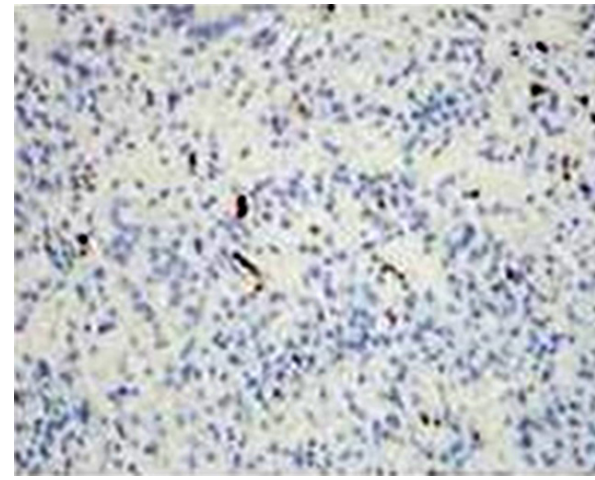

Figure 1. Nerve fibers staining with PGP9.5 in the functional layer of endometrium with endometriosis (magnification ×400).

All samples underwent endometrial evaluation by the same experienced pathologist and hematoxylin and eosin staining analysis was used for conventional histological assessment. Three micron thick-tissue sections were cut from paraffin blocks and were deparaffinized at 60°C in the oven. The next stages were performed using tissue microarray (Leica Microsystems GmbH, Wetzlar, Germany).

The sections were stored in 5% hydrogen peroxide for 15 minutes allow for an endogenous peroxidase block. After that, the sections were incubated for 30 minutes with PGP 9.5, a highly specific, pan-neuronal marker that recognizes all types of nerve fibers. Respectively, post-primary antibody, polymer antibody and DAB mixtures (LOT 11776; Leica Microsystems GmbH, Wetzlar, Germany;) were applied for 10 minutes. Contrast staining was used with Mayer's hematoxylin and sections were closed with a closing substance.

A normal skin biopsy was used to validate PGP9.5 by stain-

Table 1. Demographic characteristics of the patients

	Endometriosis (n=34)	Controls (n=33)
Age, years (Mean±SD)	33.7±6.8	34.5±6.4
Gravidity (Mean±SD)	1.7±1.5	2.6±1.8
Parity (Mean±SD)	1.5±1.2	2.1±1.5
History of infertility, n (%)	7 (10.4)	8 (11.9)
Chronic pelvic pain, n (%)	23 (34.3)	7 (10.4)
Dysmenorrhea, n (%)	25 (37.3)	9 (13.4)
History of operation, n (%)	15 (22.4)	10 (14.9)

SD: Standard deviation.

Table 2. Endometrial nerve fiber density staining with protein gene product 9.5

Marker	Endometriosis (n=34)	Controls (n=33)	p
	Mean±SD	Mean±SD	
PGP 9.5 (density/mm²)	1.85±1.74	1.15±1.48	0.08

PGP 9.5: Protein gene product 9.5; SD: Standard deviation.

Table 3. Distributions according to menstrual cycle phase

	Endometriosis (n=34)		Controls (n=33)		p
	n	%	n	%	
Menstrual	9	13.4	8	11.9	0.92
Proliferative	19	28.4	18	26.9	
Secretory	6	9	7	10.4	

ing nerve fibers, as it reliably contains myelinated and unmyelinated nerve fibers expressing PGP 9.5. Histopathological and immune staining of endometrial samples was evaluated blindly by the pathologist. Specimens without nerve fibers in the sections were defined as a negative result. Images of the sections were evaluated using an Olympus BX53 microscope (Olympus Corp., Tokyo, Japan) with magnification of 200 [×200] and objective of 20 [×20]. (Fig. 1) Nerve fibers in a 1-mm square were counted. The results were expressed as the number of nerve fibers per 1 mm² in each section.

Statistical analyses were calculated using SPSS Statistics for Windows, Version 17.0. (SPSS Inc., Chicago, IL, USA). The results were expressed as the mean±SD number of nerve fibers per mm² in each section. Statistical significance was established at p-values of <0.05, The Student's t-test and a chi-square test were used for the comparison of the 2 groups. Spearman analysis was used to analyze the correlation between the density of PGP 9.5 in endometrial biopsies and the severity of pain symptoms.

RESULTS

The study group consisted of a total of 67 patients, and all underwent surgical assessment. The control group comprised 33 patients and the study group was made up of 34 patients. The study group had stage 3 or 4 endometriosis, according to the revised staging system of the American Society of Reproductive Medicine. The mean age of the study group was 33.79±6.86 years, and in the control

Table 4. The relationship between protein gene product 9.5 positivity and the presence of endometriosis

Group	Protein gene product 9.5 positive	Protein gene product 9.5 negative	Total	p
Endometriosis, n (%)	25 (73.5)	9 (26.5)	34	
Control, n (%)	20 (60.6)	13 (39.4)	33	
Total	45	22	67	0.26

Table 5. The relationship between protein gene product 9.5 positivity and the presence of pelvic pain

Pelvic pain	Protein gene product 9.5 positive	Protein gene product 9.5 negative	Total	p
Present, n (%)	22(73.3)	8(26.7)	30	
Absent, n (%)	23(62.2)	14(37.8)	37	
Total	45	22	67	0.33

Table 6. The relationship between protein gene product 9.5 positivity and the presence of dysmenorrhea

Dysmenorrhea	Protein gene product 9.5 positive	Protein gene product 9.5 negative	Total	p
Present, n (%)	26 (76.5)	8 (23.5)	34	
Absent, n (%)	19 (57.6)	14 (42.4)	33	
Total	45	22	67	0.44

group, the mean age was 34.57±6.48 years. The demographic characteristics of both groups are shown in Table 1. Other than pelvic pain and dysmenorrhea, both groups were statistically similar.

The patients with endometriosis and the control group had a mean PGP 9.5 density staining of 1.85±1.74 mm^2 and 1.15±1.48 mm^2, respectively. Though the PGP 9.5 staining was higher in the study group, there was no statistically significant difference found (p=0.08) (Table 2).

All endometrial samples exhibited histological features consistent with normal menstrual cycle phases (menstrual: n=13 cases; proliferative: n=17 cases; secretory: n=37 cases). As illustrated in Table 3, there was no difference in the endometrial sampling time between groups (p=0.92).

The relationship between PGP 9.5 positivity and the presence of endometriosis, pelvic pain, and dysmenorrhea is demonstrated in Table 4. No statistically significant relationship between PGP 9.5 positivity and the presence of endometriosis was observed in this study. Further, we did not find a correlation between the presence of PGP 9.5 and pelvic pain or dysmenorrhea in our patients. The pelvic pain scores of the patients were not correlated to PGP 9.5 staining density (p=0.07; r=0.219) (Table 5 and 6).

DISCUSSION

In the present study, endometrial samplings obtained through a minimally invasive procedure, were analyzed for the diagnosis of endometriosis. We had 34 patients with endometriosis and 33 patients in a control group. The mean nerve density of both groups was not significantly different (Table 2). When we examined the presence of nerve fibers in the endometrial tissue, we did not find a significant relationship between endometriosis and the number of nerve fibers. We investigated the relationship between the density of nerve fibers and the severity of pelvic pain. Our results indicated no relationship between the severity of pelvic pain and nerve fiber-density. Our dysmenorrhea findings were similar.

In some studies, the density of nerve fibers in endometrial tissue was found to be correlated with endometriosis8-10. Bokor et al. 9 found a 14-times higher nerve density in the endometrial tissue of patients with endometriosis. Al-Jefout et al. 10 analyzed endometrial biopsies from 64 endometriosis and 35 non-endometriosis patients. They found that women with endometriosis and pain symptoms had a significantly higher nerve fiber density in comparison with women with infertility with no pain. They also found that nerve fiber density did not differ between different menstrual cycle phases.

Cetin et al.[11] examined the results of endometrial curettage in 31 endometriosis patients and 29 controls. They found that the detection of nerve fibers in the eutopic endometrium with PGP 9.5 and neurofilament was not helpful in the diagnosis of endometriosis. Zevallos et al.[12] studied the eutopic endometrial curettage of 29 patients: 16 women with endometriosis and 13 women without endometriosis. They did not find a significant difference in PGP 9.5 demonstrated fibers.

Leslie et al.[13] analyzed 68 endometrial samples before surgery. They found no correlation between the presence of functional layer nerve fibers and the presenting symptoms, endometrial histology, or current hormonal therapy. It was determined to be neither specific nor sensitive for the diagnosis of endometriosis.

De Andrade et al.[14] reported that an increase in interleukin 6 (IL-6) expression was associated with the severity of endometriosis. Elgafor El Sharkwy et al.[15] examined endometrial samples from 74 endometriosis patients for PGP 9.5 and blood samples from 40 control group participants for IL-6. They found that a combination of both serum IL-6 and the presence of nerve fibers in the endometrium was a more reliable method to diagnose minimal-mild endometriosis than a single test.

Recent publications in the literature have indicated that further research is needed in these patient groups.[18,19] As a result of these findings and our research, we found that the mean density of nerve fibers in endometrial tissue is higher in patients with endometriosis than in patients with other benign conditions, but the difference was not significant statistically. Our study results did not support determining nerve fiber density in endometrial tissue as a valuable method to diagnose endometriosis.

Conclusion

In conclusion, we did not find nerve fiber density to be a valuable diagnostic indicator in endometrial sampling in patients with endometriosis. Neither did we determine any relationship between severity of disease and density of nerve fibers in endometrial tissue.

In the light of the literature and our study findings, we conclude that more studies are needed to determine reliable and sensitive, noninvasive methods to diagnose endometriosis.

Acknowledgement

There is acknowledgement.

Ethics Committee Approval

Ethics committee approved.

Informed Consent

Informed consent was obtained.

Peer-review

Internally peer-reviewed.

Authorship Contributions

Concept: Y.T.B., Z.M.P., E.E.B.; Design: Y.T.B., Z.M.P., E.E.B.; Data collection &/or processing: Y.T.B., S.O., K.B., A.O.Y.; Analysis and/or interpretation: Y.T.B., O.S.; Literature search: O.S.; E.E.S.; Writing: Y.T.B., O.S., E.E.S.; Critical review: O.S., E.E.S.

Conflict of Interest

None declared.

REFERENCES

1. Marinho MCP, Magalhaes TF, Fernandes LFC, Augusto KL, Brilhante AVM, et al. Quality of Life in Women with Endometriosis: An Integrative Review. J Womens Health (Larchmt). 2017 Oct 24 [Epub ahead of print], doi: 10.1089/jwh.2017.6397. [CrossRef]
2. Bernardi M, Lazzeri L, Perelli F, Reis FM, Petraglia F. Dysmenorrhea and related disorders. F1000Res 2017;6:1645. [CrossRef]
3. Ugwumadu L, Chakrabarti R, Williams-Brown E, Rendle J, Swift I, John B, et al. The role of the multidisciplinary team in the management of deep infiltrating endometriosis. Gynecol Surg 2017;14:15.
4. Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, et al. ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod 2005;20:2698–704. [CrossRef]
5. Evans S, Moalem-Taylor G, Tracey DJ. Pain and endometriosis. Pain 2007;132 Suppl 1:S22–5. [CrossRef]
6. Berbic M, Schulke L, Markham R, Tokushige N, Russell P, Fraser IS. Macrophage expression in endometrium of women with and without endometriosis. Hum Reprod 2009;24:325–32. [CrossRef]
7. Tran LV, Tokushige N, Berbic M, Markham R, Fraser IS. Macrophages and nerve fibres in peritoneal endometriosis. Hum Reprod 2009;24:835–41. [CrossRef]
8. Medina MG, Lebovic DI. Endometriosis-associated nerve fibers and pain. Acta Obstet Gynecol Scand 2009;88:968–75. [CrossRef]
9. Bokor A, Kyama CM, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A, et al. Density of small diameter sensory nerve fibres in endometrium: a semi-invasive diagnostic test for minimal to mild endometriosis. Hum Reprod 2009;24:3025–32. [CrossRef]
10. Al-Jefout M, Dezarnaulds G, Cooper M, Tokushige N, Luscombe GM, Markham R, et al. Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study. Hum Reprod 2009;24:3019–24. [CrossRef]
11. Cetin C, Serdaroglu H, Tuzlali S. The importance of endometrial nerve fibers and macrophage cell count in the diagnosis of endometriosis. Iran J Reprod Med 2013;11:405–14.
12. Zevallos HB, McKinnon B, Tokushige N, Mueller MD, Fraser IS, Bersinger NA. Detection of the pan neuronal marker PGP9.5 by immuno-histochemistry and quantitative PCR in eutopic endometrium from women with and without endometriosis. Arch Gynecol Obstet 2015;291:85–91. [CrossRef]
13. Leslie C, Ma T, McElhinney B, Leake R, Stewart CJ. Is the detection of endometrial nerve fibers useful in the diagnosis of endometriosis? Int J Gynecol Pathol 2013;32:149–55. [CrossRef]
14. De Andrade VT, Nácul AP, Dos Santos BR, Lecke SB, Spritzer PM, Morsch DM. Circulating and peritoneal fluid interleukin-6 levels and gene expression in pelvic endometriosis. Exp Ther Med 2017;14:2317–22. [CrossRef]
15. Elgafor El Sharkwy IA. Combination of non-invasive and semi-invasive tests for diagnosis of minimal to mild endometriosis. Arch Gynecol Obstet 2013;288:793–7. [CrossRef]
16. McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation and nerve fiber interaction in endometriotic pain. Trends Endocrinol Metab 2015;26:1–10. [CrossRef]
17. Kim YA, Kim JY, Chang SH, Chang DY, Chun KC, Koh JW. Progesterone reduces neurofilament (NF)-positive nerve fibers in eutopic endometrium of patients with endometriosis and myomata. J Reprod Med 2014;59:481–7.
18. Ellett L, Readman E, Newman M, McIlwaine K, Villegas R, Jagasia N, et al. Are endometrial nerve fibres unique to endometriosis? A prospective case-control study of endometrial biopsy as a diagnostic test for endometriosis in women with pelvic pain. Hum Reprod 2015;30:2808–15. [CrossRef]
19. Yan D, Liu X, Guo SW. Nerve fibers and endometriotic lesions: partners in crime in inflicting pains in women with endometriosis. Eur J Obstet Gynecol Reprod Biol 2017;209:14–24. [CrossRef]

Endometriozisli Hastaların Endometrial Dokudaki Sinir Liflerinin Tanısal Değeri

Amaç: Çalışmanın amacı, endometriozisli hastalarda endometriyum fonksiyonel tabakasında sinir liflerinin yoğunluğunu belirlemektir. Bu sayede endometrioziste minimal invaziv bir tanı yöntemi olarak kullanılabileceğini anlamaya çalışmaktır. Ayrıca endometriozisten dolayı pelvik ağrı şiddeti ile sinir lifi yoğunluğu arasında bir ilişki olup olmadığını endometrial örnekleme materyalinde bulmayı hedeflemektir.

Gereç ve Yöntem: Ağustos 2011 ve Kasım 2012 tarihleri arasında hastanemize başvuran 67 hastada endometriyal örnekleme yapıldı. Endometriozis tanısı cerrahi histopatolojik inceleme ile yapıldı. Endometriozis saptanan 34 hasta çalışma grubu olarak seçildi. Endometriozis dışındaki benign sebepler nedeniyle ameliyat edilen 33 hasta da kontrol grubu olarak seçildi. Endometrial örnekler sinir lifleri için sinir lifi markerı immünohistokimyasal olarak saptanması ile araştırıldı; PGP9.5 (Protein Geni Ürünü 9.5). Sinir lif yoğunluğu ve pelvik ağrı şiddeti arasındaki korelasyonu değerlendirmek için Vizuel Analog Skala (VAS) skorları kullanıldı. İstatistiksel analizler SPSS 17.0 programı ile hesaplandı.

Bulgular: Endometriozis grubunda sinir liflerinin ortalama yoğunluğu 1.85±1.74 bulundu. Kontrol grubunda ortalama olarak 1.15±1.48 bulundu. Gruplar arasında istatistiksel olarak anlamlı fark yoktu (p=0.08). Pelvik ağrı skoru PGP 9.5 şiddetiyle korelasyon göstermemekteydi.

Sonuç: Sonuç olarak, ötopik endometriyumda sinir liflerinin PGP 9.5 işaretleyicisi ile saptanması, endometriozisi teşhis etmek için belirleyici noninvaziv bir yöntem olarak bulunmamıştır.

Anahtar Sözcükler: Endometriozis; immünohistokimya; sinir lifleri.