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Density of nerve fibres in eutopic endometrium in women with endometriosis

Review Article

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Abstract: Endometriosis is the disease which usually takes a wide range of time to be diagnosed. Recently, the investigators found that endometriosis could be diagnosed using the density of nerve fibers in eutopic endometrium after taking endometrium bioptate. The aim of the article is to summarize the existing literature on density of nerve fibers in eutopic endometrium in women with and without endometriosis. In this review were involved only those studies which used the same exclusion criteria and the same technology to detect nerve fibers in eutopic endometrium. Our review confirmed the position of all studies' results that detection of specific nerve fibers within eutopic endometrium using minimally invasive endometrial biopsy technique could be widely used in clinical practice to diagnose endometriosis with high specificity and sensitivity.

Keywords: *Endometriosis • Density of nerve fibers • Endometrial biopsy*

1. Introduction

Endometriosis is a chronic, benign gynaecological disease of reproductive aged women, characterized by endometrial-like tissue found outside the uterine cavity. The prevalence of endometriosis in women of reproductive age is between 10% and 15%. Women with endometriosis usually suffer from dysmenorrhoea, dysuria, dyschezia, dyspareunia and infertility. However, the relationship between different pains and endometriosis is not clear and there is poor correlation between the severity of pain symptoms and anatomical staging of the disease [1].

Even today the pathogenesis of endometriosis is not well understood, but there are a lot of theories trying to explain it: implantation of endometrial fragments transported via the Fallopian tubes by retrograde menstruation, mechanical transplantation, lymphatic and vascular metastasis, direct extension or invasion, coelomic metaplasia, embryonic rests, and a composite theory [2]. Moreover, today many clinical studies have discovered biochemical factors that are involved in immune responses linked to endometriosis, for example, expression of gene products, cytokines and other immunological components [3-5]. Recently accumulated scientific findings claim that nerve fibre density differs between women with and without endometriosis.

Unfortunately, there is still a substantial delay in the diagnosis of endometriosis. It takes a lot of time (approximately 6-10 years) from the onset of symptoms to the definite diagnosis for endometriosis. It depends on multiple factors of individual patient and medical levels. At the moment, laparoscopic visualization followed by histological confirmation is a gold standard for the diagnosis of endometriosis, but this surgical approach has complications and side effects, in addition to physician variability in detecting lesions or ectopic endometrial tissue [6-7]. Therefore, current medical researchers are trying to find a simple but reliable and non-invasive way to diagnose endometriosis. Only a few studies have already been carried out to compare the differences between the density of nerve fibres in eutopic endometrium in women with and without endometriosis. However, no comparative analysis has been performed so far.

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2. Material and methods

In order to make expansive review of density of nerve fibres in endometrium in women with and without endometriosis electronic database search (PubMed, Medline) was used. In our acta review we included only those studies which analysed nerve fibre density within endometrium. Consequently, five studies, i.e. three case-controlled trials, one pilot and one double-blind, were selected as they followed the same inclusion criteria. All the studies were approved by national Human Ethics Committees and written informed consent was obtained from all participants. Any reproductive age women undergoing laparoscopy for suspected endometriosis/ pelvic pain/ or infertility and not currently receiving hormonal treatment for at least 3 months prior to laparoscopy [2,8-11] were involved in those studies. Different menstrual phases were not regarded to be exclusion criteria because no significant differences were found for density of nerve fibres related to different menstrual phases [10]. The reference standard in all studies for diagnosing endometriosis was laparoscopy and visualization of endometriotic lesions with surgical staging of the disease by 3 gynaecologists. Selection of participants in control and case groups was performed according to laparoscopic findings and histopathologic confirmation.

All selected biopsies had been fixed in 10% neutral-buffered formalin immediately after collection for 18-24 h and they were embedded in paraffin blocks. For the studies paraffin blocks were sectioned at 4μm and then stained with haematoxylin and eosin for morphological evaluation. Immunohistochemistry was performed using polyclonal rabbit anti-PGP 9.5 (protein gene product) as a special pan neural marker. PGP9.5 – specific marker for unmyelinated and myelinated nerve fibres. In all studies normal skin was used as a positive control as it reliably contains myelinated and unmyelinated nerve fibres expressing PGP9.5. The obtained data were analyzed using Excel or SPSS17. The results were expressed as the mean (±SD) number of nerve fibres per mm² in each specimen from all endometrial samples, stained with anti-PGP9.5. Statistical significance was established at p values of <0.05.

However, some differences between the methodologies of the studies were observed. Methods for collecting endometrial tissues for examination varied. Endometrial biopsies after hysteroscopy or before laparoscopy were obtained using different curettes (exp.: Endosampler, Pipelle or Novak curette). In one study, full-thickness uterine blocks were selected from women who had undergone hysterectomy [2].

The studies focused on the technique of endometrial biopsy in order to ensure that a narrow and deep endometrial strip was obtained. During the studies it was ensured for the curette to be inserted in line with the contour of the uterus and then rotated 180°, so that the curette was still firmly pressed against the endometrium. Then the curette should be slowly withdrawn in a straight line with full suction [9,11]. Consequently, a solid column of endometrium could be obtained and assessed immunohistochemically.

Only in one study endometrium samples were collected during secretory phase of the cycle, since it was thought that the density of multiple small nerve fibres was higher during that phase than during other phases of the cycle [2]. In other studies endometrium samples were collected from women of varying severities of the disease according to the Revised American Fertility Society Scoring System and different phases of menstrual cycle because no significant differences for density of nerve fibres related to stage of the disease or different menstrual phases were detected.

In this acta review, a total of 355 endometrium samples were analysed: 169 of them from women with proven endometriosis and 186 from women without endometriosis. It was not possible to calculate the average age of women because some studies failed to indicate the average age. However, the age of women ranged from 26 to 46 years.

Furthermore, the pilot study which analysed 38 endometrium samples from women with endometriosis and 34 control cases was excluded from further analyses because of the observed unexpectedly high values of density of nerve fibres (the range of nerve fibres of Endosampler was 1.6 -125 and for the curetting was 0.8-250). As the authors mentioned such wide range in mean nerve fibre densities for both techniques can be explained by the non-homogeneous distribution of these endometrial nerve fibres [9].

In order to compare the differences in density of the nerve fibres in women with and without endometriosis we calculated the mean values of these already observed studies.

3. Results

As a result, totally 283 endometrium samples were analysed (Table 1): 131 of them from women with proven endometriosis and 152 – with no endometriosis. In women with laparoscopically confirmed endometriosis, mean (±SD) nerve fibres density of the endometrial biopsy was 5.67±12.08 per mm² compared with 0.78±3.39 per mm² for women without endometriosis ($p < 0.05$) (Table 2).

Table 1. Nerve fibre density per mm² in the functional layer of endometrial biopsy

Biopsy method	Endometriosis positive women			Endometriosis negative women			Sensitivity	Specificity
	N	Mean	SD	N	Mean	SD		
Curettage [2]	25	10	7	47	0	0	100	100
Hysterectomy [2]	10	11	5	35	0	0	100	100
Pipelle [8]	20	2.6	2.2	20	0.2	0.3	90	60
Endosampler [10]	64	2.7	3.4	35	3.1	1.7	98	83
Curettage [11]	12	13.1	3.3	15	2.2	4.7	100	80

Table 2. Nerve fibre density per mm² in the functional layer of endometrial biopsy

	Endometriosis positive women	Endometriosis negative women	p
Number	131	152	<0.005
Mean	5.67	0.78	
SD	12.08	3.39	

In all the studies small unmyelinated nerve fibres stained for PGP9.5 were present in the functional layer of the endometrium in all patients with confirmed endometriosis except only three cases with no nerve fibres in the functional layer but with the clear evidence of endometriosis at laparoscopy. One of these cases (age 43 years) had severe pain symptoms and no previous history of endometriosis. At laparoscopy she had stage IV endometriosis involving bowel and both ovaries and adhesions [10]. The other two cases were not explained.

No nerve fibres were found in women without endometriosis in the majority of cases. But in 17 women's endometrial biopsies, nerve fibres were detected in spite of negative endometriosis diagnosis. In 6 cases there was no definite evidence of endometriosis at laparoscopy: 3 of these cases had severe dysmenorrhoea and dyspareunia with history of infertility, 1 case had a single spot of adhesions on the Pouch of Douglas which was not considered convincing for endometriosis and another 2 cases underwent laparoscopy for secondary infertility. So it is possible that some of those 6 women had endometriosis which was not visible at laparoscopy. The remaining 11 cases were not analyzed in greater detail.

Furthermore, the double-blind study including the biggest number of participants confirmed that there were no significant differences for density of nerve fibres related to stage of disease, quality of specimens and different menstrual phases as it had been thought earlier. Furthermore, during that study it was observed that a history of previous endometriosis had a close relationship with a higher density of nerve fibres in the functional layer of the endometrial biopsy [10].

Similar results were observed in a small scale study in Lithuania. The results of the study were published in a Lithuanian medical journal. In spite of a small number of participants (10 women with and 10 women without endometriosis) the results of the study confirmed the fact that the density of nerve fibres in eutopic endometrium is higher in women with endometriosis (1.27 ± 0.05 mm²) compared with women having no endometriosis (0.04 ±0.03 mm²) with a sensitivity of 100% and specificity of 50% [12].

4. Discussion

The results of all studies conclude that it is possible to diagnose endometriosis by using micro-anatomical endometrial markers and suggest that immunohistochemical analysis of endometrial biopsy may be a reliable means to diagnose endometriosis in women who are not currently on any hormonal treatment. However, endometrial nerve fibres may no longer be detectable in women getting hormonal treatment [8,13].

Moreover, through analysis of those trials we confirm that the assessment of nerve fibre density in eutopic endometrium can be used as a reliable diagnostic test for minimal to mild endometriosis with high sensitivity and specificity.

However, meticulous attention must be paid to the technique of endometrial biopsy in order to ensure that a deep and narrow endometrial strip was obtained. It provides good quality tissue for evaluation as a solid column of endometrium is easier to assess immunohistochemically compared with a superficial and fragmented biopsy. However, as it was observed earlier, sometimes it is possible to get a variable density of nerve fibres in the functional layer. It was explained that a narrow endometrial biopsy may sometimes pass between nerve fibres, and therefore more than 1 section through the biopsy may sometimes need to be inspected [9].

Consequently, the question arises if laparoscopy and histopathologic confirmation is still the gold standard in diagnosis of endometriosis because laparoscopy has some limitations in terms of false negative findings, e.g. missing a peritoneal or deep lesion in difficult locations. It means that the sensitivity is not always reliable. Other issues include the accuracy of histopathological

confirmation of diagnosis, as sometimes the lesion is not included in biopsies that are taken or the pathologist has limitations of his experience in identifying the characteristic features of endometriosis especially in cases of mild disease where glandular elements may not be obvious [10]. Moreover, laparoscopy is a gynaecological procedure which needs local or general anaesthesia and may have various complications during or after the procedure.

It is established that carefully planned laparoscopic excision of endometriosis can improve fertility of infertile women [14]. But fertility can't be changed by laparoscopy for women who have no endometriosis. And those women suffer the cost, discomfort and complications of surgical procedure.

Summarising, endometrial biopsy is a less invasive procedure than laparoscopy and this test could be used for the diagnosis of endometriosis which would allow gynaecologists to triage infertile patients (with no other indications for surgery) and plan for a potentially valuable laparoscopy or no laparoscopy [10]. Because of PGP9.5 diagnostic test's high specificity and sensitivity, it would allow in an infertility workup to reduce the number of laparoscopies performed without reducing the number of women whose endometriosis is diagnosed and surgically treated. A further benefit for patients would be that most endometrial biopsies could be performed without general anaesthesia and good biopsies could easily be obtained using local anaesthesia.

5. Conclusion

The results of this analysis demonstrate that studying the density of nerve fibres in endometrial biopsy samples stained with PGP9,5 and identifying them immunohistochemically can be used as a less invasive and yet accurate method for diagnosing endometriosis compared to laparoscopy. This test could help to reduce the current lengthy delay in diagnosis of the condition and could allow more effective planning for formal surgical or long-term medical management. Therefore, further research is required to confirm the efficiency and usefulness of performing endometrial biopsy and detection of nerve fibres in eutopic endometrium for diagnosing endometriosis.

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