The Clinical Usefulness of Endometrial Biopsy for the Diagnosis of Endometriosis in the Work up of Women with Infertility

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Abstract

Background & Aims: The eutopic endometrium in women with endometriosis harbor small nerve fibres. This study aimed to explore the clinical usefulness of office endometrial biopsy to detect endometrial nerve fibres as diagnostic test for endometriosis in the work up of women with infertility and no endometrioma.

Material & Methods: A cohort of 28 Jordanian women with infertility and without endometriomas was prospectively followed up for 1 year. All women underwent clinical evaluation and an office endometrial biopsy. Those with positive nerve fibres using immunohistochemistry with PGP9.5 were considered endometriosis patients and underwent planned laparoscopy while those with negative results were considered endometriosis free and underwent assisted reproductive techniques. All patients were followed up at 3, 6 and 12 months.

Results: Eighteen women had positive nerve fibres and underwent planned laparoscopy. At laparoscopy 14 patients had endometriosis, two cases had no visible endometriosis (sensitivity 88.9 (16/18). In women with endometriosis and pain symptoms (n- 12) the density of nerve fibres was higher than in those with endometriosis and no pain symptoms (n-6) 2.1 and 0.6 per mm2 respectively (P = 0.005). Women with negative results at biopsy (n-10) underwent either ovulation induction or IVF. In both groups at one year follow up the pregnancy rate was similar (60%).

Conclusions: The proposed method may be useful in the work up of patients with infertility and without endometrioma and would allow gynecologists to triage infertility patients and plan for a potentially valuable laparoscopy and will also shorten the delay of endometriosis diagnosis.

Keywords: Eutopic endometrium; Endometriosis diagnosis; IVF; Endometrial biopsy; Infertility; Nerve fibres.

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Introduction

Endometriosis is a benign gynecological disease defined as the presence of endometrial-like glands and stroma outside the uterine cavity. Although the exact prevalence of endometriosis in the general population is not clear, the prevalence in women of reproductive

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age is estimated to range between 10 and 15%[1]. Endometriosis may contribute to excruciating pain and/or infertility[2,3]. However, the relationship between different types of pain and endometriosis is not well understood and there is poor correlation between the severity of pain symptoms and anatomical staging of the disease[4].

A significant diagnostic delay is often reported with an estimated average delay of 7 years in the USA, 8 years in the UK[5,6]. Furthermore, the delay before surgical diagnosis of deep infiltrating endometriosis (DIE) is significantly longer for patients with advanced stage IV disease than for those with stage I, II or III disease[7]. High index of suspicion is important in order to diagnose endometriosis. The gold standard for the diagnosis of endometriosis is currently diagnostic laparoscopy and peritoneal biopsy for histological confirmation or exclusion. The advantage of such procedure is that it allows direct visualisation of interior of abdominal and pelvic cavities and can be used for both diagnostic and therapeutic purposes. The patient's baseline condition and subsequent progress can be staged according to location, diameter and depth of endometriotic lesion, as well as density of adhesion[8]. Moreover, transvaginal ultrasound (TVU) has been used in the diagnosis of ovarian endometrioma, however, it is not useful in diagnosis of other types of endometriosis. Moreover, recently a clinical report suggested a clinical score for the diagnosis of DIE which based on clinical findings and symptoms in women with endometrioma[9].

Neurogenesis mechanisms involvement in endometriosis pathophysiology and clinical presentation had attracted a lot of attention recently. Increased amounts of nerve growth factor (NGF)[10,11], TGF-α[12] and prostaglandin I2[13] are found in the eutopic endometrium of women with endometriosis in comparison to women without endometriosis. This stimulates growth of fine unmyelinated sensory nerve fibres in the endometrium of women with endometriosis, which are not present in women without the disease[10, 14-18].

Infertility in endometriosis remains a complex and controversial topic in the reproductive field. A couple are deemed to be infertile after failure to conceive with 12 months of unprotected intercourse[19]. Studies have shown that 25% to 40% of infertile women have endometriosis in comparison to 0.5%-5% of fertile women but with endometriosis[20]. While it is clear that endometriosis is associated with reduced fertility[21], the true cause of the relationship between endometriosis and infertility is unknown. Mechanisms of endometriosis-associated infertility include impaired folliculogenesis[22], poor oocyte quality[23], low fertilisation rates, disturbed sperm function[24], abnormal endometrial environment and failure of implantation pelvic anatomical distortion[20], impaired implantation[25], immune dysfunction[26] and increased inflammatory process[27,28]. The big dilemma facing gynaecologists in cases of infertility is when to do laparoscopy? Especially if there is no imaging evidence of endometriosis in the presence of pain symptoms suggestive of mild to moderate endometriosis. Medical treatment of endometriosis associated infertility is almost exclusively limit reproductive options due to their contraceptive effects. Therefore, it is agreed that surgery is the most beneficial approach in patients suspected with endometriosis. However, most of the available

data is for patients with mild to moderate disease. There are no randomized controlled trials to determine the efficacy of surgical management of DIE. The Canadian Collaborative Group on Endometriosis studied 341 infertile women with minimal or mild endometriosis who were randomized to diagnostic laparoscopy alone or laparoscopic treatment of endometriosis by ablation or resection, and found a significantly higher 36-week cumulative probability of pregnancy continuing beyond 20 weeks in the treatment group (30.7% versus 17.7%, p=0.006) suggesting improved fecundity with surgical treatment of endometriosis[29]. A recent Cochrane review concluded that there is a benefit of surgical treatment compared to diagnostic laparoscopy alone for clinical pregnancy, and ongoing pregnancy after 20 weeks (OR 1.66, 95% CI 1.09–2.51 and OR 1.64 95% CI 1.05–2.57, respectively)[30].

Assisted reproductive technology (ART) has been widely adopted to improve fertility in women with endometriosis[20,31,32]. ART incorporates clinical and laboratory technology in order to assist couples who have trouble conceiving. *In vitro* fertilisation (IVF) is a reproductive technology that involves a series of hormone injections to stimulate the growth of oocytes, removal and fertilisation of the oocytes and finally, embryo transferred into the uterus to establish pregnancy[33].

Theoretically, the use of this technology in women with endometriosis gives the women a higher chance of achieving a pregnancy[34]. This can be accomplished by controlling folliculogenesis and allowing selection of good quality oocytes, increasing chances of fertilisation of the oocyte and promoting a uterine environment receptive to embryo implantation. However, the success rate of IVF in women with endometriosis is lower in comparison to women with other infertility causes (33.3% vs 41.5%)[35-37]. This is due to a number of reasons, such as decreased numbers of oocytes retrieved (5.3 vs. 9.5), low fertilisation rates (9% vs 16.3%) and poor endometrial receptivity[35,38-43]. As not all women with endometriosis-associated infertility are able to conceive with IVF, a strong need to improve our understanding of this condition and explore other management options is indicated.

Most gynecologists are not sure whether endometriosis is present when a woman has, for example, infertility since more than 1 year, a regular cycle, no or limited pain during menses or intercourse, and male infertility. The main problems with surgery in infertile patients with endometriosis in cases they have endometrioma is the fear of affecting ovarian reserve, incomplete excision, repeat surgeries and possible rare but serious complications.

The first surgery for endometriosis is the most important surgery and should be planned and performed by expert surgeons. The main benefits of planned surgery that the surgeon will be prepared for possible advanced surgery, identification of women with pelvic inflammatory disease (PID), who would benefit from surgical laparoscopic adhesiolysis and removal of hydrosalpinx to improve fertility and to reduce pain.

The aim of this study was to determine the clinical usefulness of endometrial biopsy for the detection of nerve fibres in the functional layer of the endometrium in the work up of women with infertility and no endometrioma and identification of women with possible

endometriosis or no endometriosis and plan their treatment plan as for surgery or ovulation induction or IVF.

METHODOLOGY

Patients

Between the years; 2012-2014, a total of 28 Jordanian women with infertility were offered to participate in this prospective observational study and all study participants gave their written informed consent. Ethics approvals obtained from Mutah University Ethics Committee. Inclusion criteria were: reproductive age women with infertility, no presence of endometrioma on ultrasound scan and willingness to participate in the study. Exclusion criteria were: age >45 years, known endometriosis diagnosis, history of intrauterine intervention (endometrial biopsy, hysteroscopy) in the 3 months before consultation, presence of endometrioma or hydrosalpinx identified by pelvic sonography, an abnormal uterine cavity, (endometrial polyp, fibroid), and male factor infertility.

Procedures

All women underwent vaginal U/S scan, speculum and bimanual examinations, an office endometrial biopsy (Endosampler) explained in our other report[15] regardless of menstrual phase, conventional H&E and IHC staining with ready to use PGP 9.5 (Ventana Medical Systems, Inc. Roche) standard protocol as explained in our other report[15]. All patients were followed up at 3, 6 and 12 months. Those with positive PGP 9.5 results identified at IHC underwent planned laparoscopy while those with negative PGP 9.5 underwent ovulation induction or IVF.

Results

The average age of all participants (Mean years± SD) was 28.3 ± 4.3 years. Other patients' clinical data are shown in table 1. The results of physical and speculum examination are shown in table 2. Signs suggestive of endometriosis during speculum examination (bluish lesions or nodules) n= 4 (14%). Signs suggestive of endometriosis during bimanual examination (retroverted fixed uterus) n= 3 (10%). Presence of dysmenorrhea as leading symptom n= 16 (57%). Presence of deep dyspareunia as leading symptom= 12 (42%). Presence of dysuria as leading symptom n= none. Presence of dyschesia n= 3 (10%). No pain symptoms n=12 (42%).

Table 1. Patient clinical data

Characteristic	Mean ± SD
Age (years)	28.3 ± 4.3
BMI (kg/m2)	23.5 ± 3.9
Past pregnancies	1.3 ± 1.2
Infertility duration (years)	3.5 ± 1.9

Results of the endometrial biopsy

In women with positive PGP 9.5 group (n-18) the H7E results showed: normal endometrium in 17 cases, one cases with chronic endometritis which was diagnosed with Fitz-Hugh–Curtis syndrome at laparoscopy and was positive for PGP 9.5.In women with negative PGP 9.5 group (n-10) (considered endometriosis negative): H&E showed normal endometrium in5 cases , one case with endometrial hyperplasia which had PCOS and was negative for PGP 9.5and four cases showed hormonal imbalance.

In the group with positive PGP9.5 (Figure1) the density of nerve fibres (mean per mm2 ± SD) was not affected by menstrual phase: in the proliferative and secretory phases

the density was 2.0 (± 4.3) and 1.5 (± 1.3) respectively (K–W $\chi 2 = 0.95$, df = 3, P = 0.78). However, when we explored the nerve fibres density between those with pain or no pain symptoms the results showed that women with pain symptoms (n-12) had density of nerve fibres (mean per mm2 ± SD) 2.1(±3.2) while in women without any pain symptoms (n-6) 0.6(±1.0) with a significant higher density in the pain group (M–W U z = − 2.82, P = 0.005).

Table 2. Nerve fibre density among women according to the presence or absence of pain symptoms

	Main nerve fibres density per mm2 (± SD)	**Statistics**
Women with pain symptoms (n-12)	2.1(±3.2)	M–W *U* z = − 2.82, *P* = 0.005
Women without any pain symptoms (n-6)	0.6 (±1.0)	

Table 3. Follow results of pregnancy out come in both groups

Groups	**3 months**	**6 months**	**12 months**	**Total**
Positive PGP 9.5	none	3/18	8/15	11/18 (61%)
Negative PGP 9.5	none	4/10	2/6	6/10 (60 %)

Those with positive PGP 9.5 were considered as possible endometriosis patients and underwent well planned laparoscopy- number -18 (64%). Those with negative PGP 9.5 were considered as highly negative for endometriosis and had either ovulation induction (n-5) or IVF (n-5)- number -10 (36%). At laparoscopy women with mild to moderate endometriosis- 14 underwent excision. DIE- 2 cases: One case had open laparatomy due to difficulty of removal while second case had wide excision of the lateral pelvic wall deep lesions and shaving of bowel endometriosis with the help of a surgeon. There was one case with Fitz-Hugh–Curtis syndrome with perihepatic adhesions which were removed. One case had no visible endometriosis and no procedure was done.

At the follow up visits at 3, 6 and 12 months (Table 3); in the group who underwent laparoscopy; the pregnancy outcome was: at 3 months visit- none, at 6 months visit- n=3/18, and at 12 months-n=8/15, with total number of those who got pregnant is 11/18 (61%). In the group who were negative for PGP 9.5 in the endometrial biopsy and underwent ovulation induction (n= 5) or IVF (n=5): at three months visit none got pregnant, at 6 months visit only four cases got pregnant (4/10), and at 12 months visit two cases got pregnant (2/6), with total number of women who got pregnant n= 6/10 (60 %).

Discussion

The results of this study clearly showed that the use of endometrial biopsy for the diagnosis of endometriosis would allow gynecologists to triage fertility patients (with no other indication for surgery) and plan for a potentially valuable laparoscopy or no laparoscopy. Using this approach will indeed avoid patients having surgical procedure that is invasive and expensive, and carries a risk of morbidity[44]. Moreover, endometrial biopsy in the work up of patients with infertility, would allow to discover cases with other endometrial pathology- Endometrial hyperplasia, chronic endometritis etc.

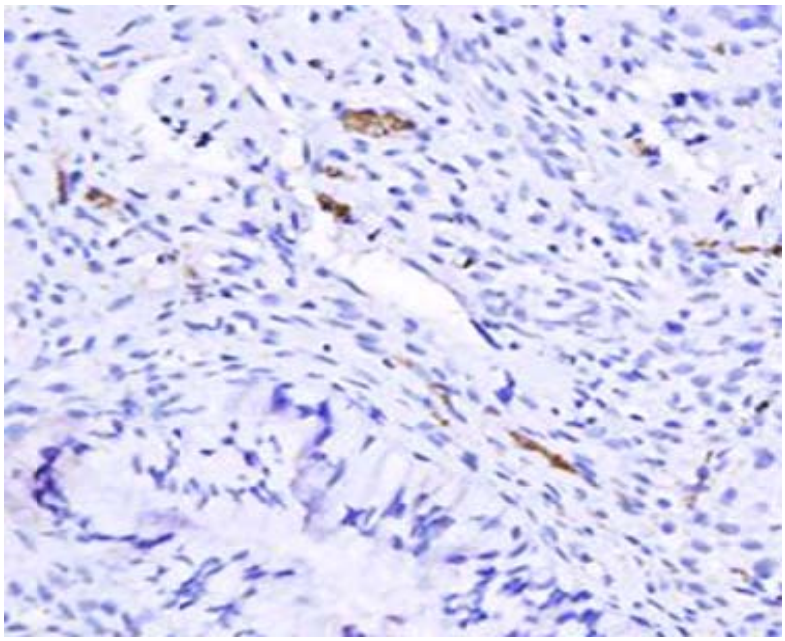

Figure 1: Nerve fibres stained with PGP 9.5 in the functional layer of the eutopic endometrium

Our previous results indicated that a negative endometrial biopsy result would miss endometriosis in only 4% of women[18]. In fact, such a test would then be "false positive" for endometriosis but still "true positive" for pelvic adhesions that can be managed by surgery. This would include nearly all cases of minimal-to-mild endometriosis, some cases of moderate-to-severe endometriosis without clearly visible ovarian endometrioma, and women with pelvic adhesions and/or other pelvic pathology, who might benefit from surgery to improve their pelvic pain and/or infertility.

Performing a planned laparoscopy only on a woman with a positive endometrial biopsy would result in endometriosis being confirmed in 90% of these women. Thus, using the PGP9.5 diagnostic test in an infertility workup would significantly reduce the number of laparoscopies performed without reducing the number of women whose endometriosis is diagnosed and surgically treated.

A further benefit for patients is the reduction in the cost of their infertility problem and avoidance of unnecessary procedures. Using this approach in the infertility work up

would also be cost effective. The average cost of surgical laparoscopy in Jordan is around 3000$ while the proposed endometrial biopsy cost is around 60$. In addition, this would shorten the need for lengthy wait for planned laparoscopies and will certainly shorten time before ART.

This study showed that the density of nerve fibres was not different during different menstrual phases which confirms our previous results[18]. This finding allows clinicians to take biopsy independent at menstrual phase. However, other reports found that nerve fibres density is higher in the secretory phase[19]. In addition results showed that women with pain symptoms have more nerve fibres density which may explain the pain generation mechanisms in endometriosis.

A further benefit for patients is that most endometrial biopsies can be performed without local or general anaesthesia, and good biopsies can easily be obtained without the use of anaesthetic. However, it is of high importance that clinicians using this technique should be familiar of taking good quality biopsies as a meticulous biopsy is an important issue, in addition to careful IHC technique.

A major weakness of this study is the small sample size. However, this is the first study that has utilized this approach clinically and will encourage others to explore it's usefulness in a larger sample size clinical studies.

In conclusion; a noninvasive test such endometrial biopsy for endometriosis would be useful for women with or without pelvic pain and infertility with normal ultrasound results. Endometrial biopsy is clearly less invasive than laparoscopy, and this test could help to reduce the current lengthy delay in diagnosis of the condition, as well as allow more effective planning for formal surgical or long-term medical management and will allow women to avoid unnecessary surgical procedures. It saves time and money.

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الفائدة السريرية من استخدام خزعة بطانة الرحم لتشخيص بطانة الرحم المهاجرة عند النساء المصابات بالعقم

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الملخص

الخلفية والأهداف: إن بطانة الرحم عند النساء اللواتي يعانين من بطانة الرحم المهاجرة تأوي بعض الألياف العصبية الصغيرة. هدفت هذه الدراسة إلى استكشاف الفائدة السريرية من استخدام خزعة بطانة الرحم المكتبية للكشف عن الألياف العصبية في بطانة الرحم لتشخيصي مرض بطانة الرحم المهاجرة عند النساء المصابات بالعقم مع عدم وجود الأكياس الدموية المبيضية.

مواد وطريقة البحث: تم متابعة فوج من 28 امرأة أردنية من المصابات بالعقم وبدون الأكياس الدموية المبيضية لمدة عام واحد. خضعت جميع النساء للتقييم السريري وخزعة بطانة الرحم المكتبية. عدت النسوة اللواتي وجدت عندهن الألياف العصبية في الخزعة الرحمية باستخدام الصبغة PGP9.5 مرضى بطانة الرحم المهاجرة وخضعن للتنظير البطني في حين عدت النسوة ذوات النتائج السلبية خاليات من بطانة الرحم المهاجرة وخضعن للتقنيات الإنجابية المساعدة. تم متابعة جميع المرضى على فترات 3 و6 و12 شهراً.

النتائج: وجدنا ثمانية عشر امرأة إيجابية لوجود الألياف العصبية الرحمية وخضعن للتنظير البطني المخطط له. عند التنظير وجدت 14 حالة من بطانة الرحم المهاجرة، بينما لم نجد بطانة الرحم المهاجرة في حالتين (الحساسية 88.9 (18/16). وبالنسبة للنساء اللواتي عانين من بطانة الرحم المهاجرة وأعراض الألم عند الدورة الشهرية (عدد 12) كانت كثافة الألياف العصبية أعلى من أولئك النسوة اللواتي يعانين من بطانة الرحم المهاجرة و بدون أي ألام عند الدورة الشهرية (عدد 6) 2.1 و0.6 mm2 على التوالي (P = 0.005). النسوة ذوات النتائج السلبية في الخزعة (عدد 10) خضعن إما لتحريض الإباضة أو عملية أطفال الأنابيب. في كلتا المجموعتين خلال سنة واحدة من المتابعة كان معدل الحمل مماثل (60٪)

الاستنتاجات: الطريقة المقترحة قد تكون مفيدة في التعامل مع المرضى الذين يعانون من العقم وبدون الأكياس الدموية المبيضية وسيسمح لأطباء الأمراض النسائية فرز مرضى العقم والتخطيط للتنظير البطني بشكل مناسب وكما أنها قد تقصر من التأخير في تشخيص مرض بطانة الرحم المهاجرة.

الكلمات الدالة: الفائدة السريرية، خزعة بطانة الرحم، الرحم المهاجرة، العقم، النساء.