The clinical usefulness of endometrial biopsy in women with infertility related to endometriosis

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Paris-2015
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Infertility and endometriosis

It is rather an association than causality
Usefulness of laparoscopy in women with infertility with or without pain

- In cases with infertility and no endometrioma; is there endo?
- Do we need to do lap?
- If yes for what?
- The value of a diagnostic laparoscopy in:
  - Women with pelvic pains
  - Women without pelvic pains
The ideal situation

- The best operation is the first one
- There is a need for elimination of diagnostic laps for endometriosis
- Laps should be well planned and performed by experienced surgeons
The problem of surgery for endometriosis

- In cases with endometrioma - Ovarian reserve
- Incomplete excision
- Repeat surgeries
- Possible complications
Noninvasive or semi-invasive diagnostic test

- A noninvasive or semi-invasive diagnostic test would be useful to discriminate between women without endometriosis (for whom the surgery would be unnecessary) and those with endometriosis, most likely minimal-to-mild disease, who may benefit from surgical therapy for both infertility and pain and from controlled ovarian stimulation in combination with intrauterine insemination for subfertility

(S. Kennedy, 2005; T.M. D'Hooghe, 2006; T.M. D'Hooghe, 2005)
Other benefits of a well planned laparoscopy

- Surgeon will be prepared for possible advance surgery
- Women with Pelvic inflammatory disease (PID), would also benefit from surgical laparoscopic adhesiolysis to improve fertility and to reduce pain.
- 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
Benefits of planned lap

- 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
Endometrial Nerve fibers

- An increasing body of evidence suggests that endometriosis may be diagnosed on the basis of an increased density of nerve fibers in the endometrium of women with endometriosis as compared with controls who lack the disease (Al-Jefout, 2007, 2009; Bokor, 2009; F AMeibody, 2011; HBV Zevallos, 2014)

- Various neural transmitters such as substance P (SP), vasoactive intestinal polypeptide (VIP), and neural proteins such as protein gene product 9.5 (PGP9.5), neurofilament (NF), neuropeptide Y (NPY), and calcitonin gene-related protein (CGRP) (N. Tokushige, 2006; Bokor, 2009)
Endometrial nerve fibres

- In a proteomic study, researchers found that NT-4/5 and brain-derived neurotrophic factor proteins were significantly higher in the eutopic endometrium from endometriosis patients (n = 18) than in controls (n = 15), whereas nerve growth factor levels were similar (A.S. Browne, 2012).

- A higher density of small unmyelinated C nerve fibers has been observed in the functional layer of endometrium from women with confirmed endometriosis when compared with women without endometriosis (IS Fraser Group 2006, 2007).
PGP 9.5 in the eutopic endometrium-  
(Al-Jefout, 2009)
Nerve fibres in peritoneal and deep infiltrating lesions of endometriosis (PGP9.5; fast red)

Large nerve trunk (multiple different types of nerve fibres in peritoneal lesion)

Tokushige et al, 2006; Wang et al, 2009
Endometrial biopsy for the diagnosis of endometriosis

- On this basis, the detection of endometrial nerve fibers has been proposed by the Fraser group as a diagnostic tool for endometriosis in a pilot study and was confirmed in a blinded study (Al-Jefout, 2007, 2009).

- Another group found that endometrial nerve density was 14 times higher in women with minimal-to-mild endometriosis (n = 20) than in controls with a laparoscopically confirmed absence of endometriosis and normal pelvis (n = 20); the combined analysis of neural markers PGP9.5, VIP, and SP could predict the presence of minimal-to-mild endometriosis with 95% sensitivity, 100% specificity, and 97.5% accuracy (Bokor, 2009).
A pilot study to evaluate the relative efficacy of endometrial biopsy and full curettage in making a diagnosis of endometriosis by the detection of endometrial nerve fibers

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Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study

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+ Author Affiliations
Other investigators have disputed these findings and reported that the presence of nerve fibers in the functional layer of endometrium does not depend only on the presence of endometriosis but rather is associated with a diagnosis of pelvic pain.

(M.L. Barcena de Arellano, 2012; X. Zhang, 2010)
Patients

- Between 2012-2013, a total of 28 Jordanian women with infertility were offered to participate in this prospective study and all study participants gave their written informed consent.
- Ethics approvals obtained from Mutah University Ethics Committee
- 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/Scan
  - speculum and bimanual examinations.
  - an endometrial biopsy (Endosampler) regardless of menstrual phase

- Conventional H&E and IHC staining with PGP 9.5 (Ready to use Roche) standard protocol
  (Al-jefout et al, 2009)

- Follow up of patients at 3, 6 and 12 months
Positive staining of nerve fibres - PGP 9.5-DAB chromogen
Baseline Participants’ Characteristics
Total number 28 patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>28.3 ± 4.3</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.5 ± 3.9</td>
</tr>
<tr>
<td>Past pregnancies</td>
<td>1.3 ± 1.2</td>
</tr>
<tr>
<td>Parity</td>
<td>0.57 ± 0.56</td>
</tr>
<tr>
<td>Infertility duration (years)</td>
<td>3.5 ± 1.9</td>
</tr>
</tbody>
</table>
## Main findings and symptoms

<table>
<thead>
<tr>
<th>Values</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Signs suggestive of endometriosis during speculum examination</td>
<td>4 (14%)</td>
</tr>
<tr>
<td>Signs suggestive of endometriosis during bimanual examination</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Presence of dysmenorrhea as leading symptom</td>
<td>16 (57%)</td>
</tr>
<tr>
<td>Presence of deep dyspareunia as leading symptom</td>
<td>12 (42%)</td>
</tr>
<tr>
<td>Presence of dysuria as leading symptom</td>
<td>none</td>
</tr>
<tr>
<td>Presence of dyschesia</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>No pain symptoms</td>
<td>12 (42%)</td>
</tr>
</tbody>
</table>
Results of H&E staining

• In the 28 biopsies:
  • In the positive PGP 9.5 group (n=18):
    • Normal endometrium- 17- in the positive PGP 9.5 group
    • Chronic endometritis- 1 case- *Fitz-Hugh–Curtis syndrome* at lap and was positive for PGP 9.5
  • In the negative PGP 9.5 group (n=10):
    • Normal endometrium- 5 cases (assumed endo negative)
    • Endometrial hyperplasia- 1 case- PCOS- negative for PGP 9.5
    • Hormonal imbalance- 4 cases-
Main nerve fibres density of positive results in IHC with PGP 9.5 by menstrual phase (N= 18)

<table>
<thead>
<tr>
<th></th>
<th>Proliferative phase</th>
<th>Secretory phase</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive IHC with PGP9.5 (Mean nerve fibre density per mm² ± SD)</td>
<td>2.0 (± 4.3)</td>
<td>1.5 (± 1.3)</td>
<td>K–W $\chi^2 = 0.95$, df = 3, $P = 0.78$</td>
</tr>
</tbody>
</table>
Comparison of nerve fibre density between women with or without pain symptoms

<table>
<thead>
<tr>
<th></th>
<th>Main nerve fibres density per mm²(± SD)</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women with pain symptoms</td>
<td>2.1(±3.2)</td>
<td>M–W U z = −2.82, P = 0.005</td>
</tr>
<tr>
<td>(n-12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women without any pain symptoms</td>
<td>0.6(±1.0)</td>
<td></td>
</tr>
<tr>
<td>(n-6)</td>
<td></td>
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</tbody>
</table>
Further actions

- Those with positive PGP 9.5 were considered as endo patients and underwent well planned laparoscopy - number -18 (64%)
- Those with negative PGP 9.5 were considered as negative for endo and had either ovulation induction (n-5) or IVF (n-5) - number -10 (36%)
Laparoscopy findings in those with positive PGP 9.5-18 patients

<table>
<thead>
<tr>
<th>Finding at Laparoscopy</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild to moderate endometriosis</td>
<td>14 (78%)</td>
</tr>
<tr>
<td>DIE</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>No endometriosis and One case had Fitz-Hugh–Curtis syndrome</td>
<td>2 (11%)</td>
</tr>
</tbody>
</table>
Surgical management

- Mild to moderate endometriosis - 14
  - excision
- DIE - 2 cases
  - One case had open laparotomy due to difficulty of removal
  - Second case had wide excision lateral pelvic wall deep lesions and shaving of bowel endometriosis with the help of a surgeon
- One case had Fitz-Hugh–Curtis syndrome with perihepatic adhesions which were removed
- The other case had no procedures done
Follow up of all cases regarding pregnancy outcome

<table>
<thead>
<tr>
<th>Groups</th>
<th>3 months</th>
<th>6 months</th>
<th>12 months</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>none</td>
<td>3/18</td>
<td>8/15</td>
<td>11/18 (61%)</td>
</tr>
<tr>
<td>PGP 9.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>none</td>
<td>4/10</td>
<td>2/6</td>
<td>6/10 (60%)</td>
</tr>
<tr>
<td>PGP 9.5</td>
<td></td>
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</table>
Discussion

- The use of endometrial biopsy for the diagnosis of endometriosis would allow gynaecologists to triage fertility patients (with no other indication for surgery) and plan for a potentially valuable laparoscopy or no laparoscopy.

- Biopsy would allow to discover cases with other endometrial pathology - Endometrial hyperplasia, chronic endometritis etc.
Discussion

- Our previous results indicated that a negative endometrial biopsy result would miss endometriosis in only 4% of women.
- 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.
Cost effective and saving time

- The average cost of surgical lap in Jordan is around 3000$
- The proposed EB cost is around 60$
- No need for lengthy wait for planned laps and will certainly shorten time before ART.
Endometrial biopsy for the detection of NF using PGP 9.5 as part of infertility work up
A woman with pelvic pains and infertility with no evidence of endometrioma

A woman with long standing infertility, but no evidence of endometrioma
1- Office Endometrial biopsy

2- Positive PGP 9.5 for nerve fibres- high probability of endo

3- Well planned laparoscopy

4- Negative PGP 9.5 for nerve fibres- low probability of endo- IO or IVF
Conclusion

- A noninvasive test such as endometrial biopsy for endometriosis would be useful for women with or without pelvic pain and/or 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.
- It saves time and money.
Thank you