Stereometric evaluation of peritoneal endometriosis and endometriotic nodules of the rectovaginal septum

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Introduction

The incidence of endometriosis has increased progressively over the years with the recognition and awareness of subtle lesions. During the last decade, attention has been focused on subtle lesions such as white and red lesions (Chatman, 1981; Jansen and Russel, 1986; Redwine, 1981; Stripling et al., 1988; Martin et al., 1989; Nisolle et al., 1990; Brosens, 1994). Typical and subtle endometriotic lesions are histologically characterized by both epithelium and stroma of the endometrial type. Recently, the three-dimensional evaluation of peritoneal endometriosis demonstrated two different types of peritoneal endometriosis. Mitotic activity, stromal vascularization and the epithelium/stroma ratio were found to be significantly different in peritoneal and rectovaginal endometriosis. The evaluation revealed a major role of glandular epithelium in rectovaginal nodules where the stroma sometimes appeared absent around glandular epithelium. The study demonstrated opposite effects of gonadotrophin-releasing hormone agonists (GnRHa) and lynestrenol on the two lesions. Indeed, in peritoneal endometriosis, after GnRHa therapy, our study demonstrated a lower rate of mitosis and poor stromal vascularization. The same drug was unable to induce the same effects in the nodule although, in contrast, lynestrenol has a strong effect on nodule vascularization. In conclusion, it is suggested that the rectovaginal adenomyotic nodule is a specific disease, different from peritoneal endometriosis. It is not the consequence of 'deep infiltrating' endometriosis but can probably develop from Müllerian rests present in the rectovaginal septum.

Key words: adenomyosis/endometriosis/peritoneal/rectovaginal nodule/stereometry

Materials and methods

In a first series of 225 women who were undergoing laparoscopy for infertility, peritoneal biopsies of 3–5 mm in size were taken from vesicular papular red peritoneal lesions (n = 43) and from typical black endometriotic implants (n = 182) with a biopsy punch forceps (26–175 DH; Storz, Tutlingen, Germany). Among the 182 black lesions, 133 were biopsied during the luteal phase, 36 after a 3 month gonadotrophin-releasing hormone agonist (GnRHa) therapy and 13 after a 3 month lynestrenol (5 mg daily) therapy. All red lesions were biopsied during the luteal phase.

In a second series of 65 women complaining of pelvic pain and/or infertility, an endometriotic nodule of the rectovaginal septum was diagnosed by palpation. In this second series, 35 women underwent surgery during the luteal phase, 14 women were treated with GnRHa therapy and 16 with lynestrenol for >3 months before laparoscopy. A laparoscopy was carried out and the nodule removed using the surgical technique previously described (Donnez et al., 1994, 1995). The rectovaginal endometriotic nodule was defined, in our series, as a large and deep nodule (>2 cm in size) whose largest area was under the peritoneal surface. The lesions visible on the peritoneal surface through the laparoscope are often minimal.

Peritoneal endometriotic biopsies and the rectovaginal septum nodule were fixed in formaldehyde and embedded in paraffin. Serial sections (6 μm) were stained with Gomori's trichrome and examined on a blind basis with a Leitz Orthoplan microscope (Leitz, Wetzlar, Germany). An endometriotic lesion was considered 'active' when typical glandular epithelium that appeared either proliferative or completely unresponsive to progestogens was found with typical stroma. The epithelium was not flattened, was >10 μm thick and showed no signs of pyknosis (Nisolle et al., 1988, 1990). In all cases, the mitotic index was calculated, as previously described (Donnez et al., 1985), by counting mitotic figures (prometaphase, metaphase, anaphase and telaphase) for 2000 epithelial cells per biopsy.
A morphometrical investigation of stromal vascularization was carried out by image analysis programs set on a Vidas 21 computer (Kontron Bildanalyse GmBH, Eching, Germany). All samples were analysed using an Axioskop light microscope (Zeiss, Oberkochen, Germany) through a CCD 72 E camera (Dage-MTI, Michigan City, IL, USA). The image features were displayed on a Red/Green/Blue (RGB) monitor and stored for processing by the image analysis program. Data management and evaluation were checked according to specific search criteria on the Videoplan (Kontron Bildanalyse GmBH) and displayed on a Vido Graphic Array (VGA) monitor and printed. All cases were analysed field by field using the objective ×40 of the Axioskop light microscope. Histological structures of interest such as the stroma, the glandular epithelium and lumen and the capillaries, were drawn moving a cursor, discriminated, and the grey level images were transferred to binary images. The interactive measurements of the selected parameters (number of structures, area of the structures per field) were appended and stored at the end of an existing data base as previously described by Nisolle et al. (1993). The χ² test, the median test and Student's t-test were used for statistical analysis.

Results

Peritoneal biopsy

Biopsies taken from typical puckered black or bluish peritoneal lesions showed the presence of endometrial elements (glands and stroma) in all cases (100%). 'Active' endometriosis was found in 75% of cases in the non-treated group, in 78% after GnRHa therapy and in 85% after lynestrenol. The incidence of active endometriosis was significantly (P < 0.01) lower when compared to red lesions (100%). Areas of oviduct-like epithelium with ciliated cells were demonstrated in respectively 63, 71, 44 and 69% of cases. No significant differences were observed among the subgroups. The mitotic activity was calculated in glandular epithelium and its value was 1.8% in red lesions, 0.6% in the non-treated black lesions, 0.3% after 12 weeks of GnRHa therapy, and 0.4% after lynestrenol. The value observed after GnRHa therapy was significantly (P < 0.01) lower than that observed in the red lesions. The epithelial height was respectively 23.9 ± 6.5, 15.1 ± 3.6, 14.3 ± 5.3 and 11.6 ± 1.8 μm. The value was significantly (P < 0.001) higher in red lesions when compared to other subgroups.

The results concerning the vascularization are shown in Table I. The number of capillaries per mm² of stroma, their mean surface area, and the surface area ratio (capillaries/stroma) were calculated. In red lesions, the number of capillaries per mm² of stroma was 174. Their mean surface area was 209 ± 181 μm and the ratio of capillaries/stroma surface area was 3.1%. The number of capillaries was respectively 230 in non-treated black lesions, 225 after GnRHa therapy and 281 after lynestrenol therapy. All values were significantly different (P < 0.01) from those observed in red lesions. The capillary mean surface area was significantly (P < 0.001) reduced in these three subgroups when compared to red lesions. The capillary mean surface areas after GnRHa therapy and after lynestrenol therapy were significantly (P < 0.001) lower when compared to black lesions. The capillaries/stroma relative surface area was significantly (P < 0.01) lower after GnRHa therapy when compared to all other subgroups. After GnRHa therapy, the number of capillaries per mm² of stroma was similar to that observed without therapy, but the ratio of capillaries/stroma surface area was found to be significantly (P < 0.01) reduced after the administration of GnRHa. After lynestrenol, the number of capillaries, the capillary surface area and the ratio of capillaries/stroma surface area were similar to the values observed without therapy.

Nodules

Biopsies taken from endometriotic nodules showed the presence of endometrial elements in all cases (100%). Histologically, scanty endometrial-type stroma and glandular epithelium are disseminated in muscular tissue (Figure 1). Cellular activity was found in 97% of cases and cellular differentiation in phase with the eutopic endometrium was never observed. Infiltration of surrounding fibromuscular tissue by endometrial glands with some signs of hyperplasia can be found (Figure 1). Very often, endometriotic glands and stroma were found by serial section up to the vaginal mucosa (Figure 2) which was sometimes replaced by endometrial epithelium.

It is obvious that the invasion process of the smooth muscle
Figure 1. Rectovaginal adenomyosis (Gomori’s trichome). Endometrial glands with signs of hyperplasia in the smooth muscle. Original magnification ×240.

Figure 2. Rectovaginal adenomyosis (Gomori’s trichome). Serial sections reveal the presence of endometrial glands and stroma up to the vaginal mucosa. Original magnification ×85.

Figure 3. Rectovaginal adenomyosis (Gomori’s trichome). Invasion of the smooth muscle by only the glandular epithelium (without stroma). Original magnification ×240.

by glandular epithelium did not require the presence of stroma (Figure 3). Indeed, the glandular epithelium was often seen without any surrounding stroma very deep in the nodular

fibromuscular tissue; it consisted of whorled anastomosing fascicles of uniform, fusiform, smooth muscle cells.

‘Active’ endometriosis was demonstrated in 97, 86 and 81% of cases in the different subgroups (without therapy, after GnRHa therapy, after lynestrenol therapy) respectively. No difference was observed between the different subgroups. When compared to the mitotic index observed in peritoneal endometriosis (0.6%), it was found to be significantly lower ($P < 0.001$) in glandular epithelium of nodules (0.09%). No significant differences were noted after GnRHa and lynestrenol therapy. The results concerning the vascularization are shown in Table II. In the untreated group, the number of capillaries per mm$^2$ of stroma was 161, their mean surface area was $140 \pm 80 \, \mu m$ and the ratio of capillaries/stroma surface area was 1.8%.

After GnRHa therapy, the number of capillaries per mm$^2$ of stroma (208), their mean surface area ($120 \pm 60 \, \mu m$) and the ratio of capillaries/stroma surface area (2.2%) were found to be similar to the values observed in the non-treated group. After lynestrenol, the number of capillaries (139), the capillary/surface area ($76 \pm 70 \, \mu m$) and the capillaries/stroma surface ratio (0.9%) were found to be significantly ($P < 0.01$) different from the non-treated group and from the GnRHa group.

### Table II. Rectovaginal endometriosis. Figures in parentheses are percentages

<table>
<thead>
<tr>
<th></th>
<th>Control group (n = 35)</th>
<th>After GnRHa (n = 14)</th>
<th>After lynestrenol (n = 16)</th>
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<tbody>
<tr>
<td>Typical glandular epithelium and stroma</td>
<td>35 (100)</td>
<td>14 (100)</td>
<td>16 (100)</td>
</tr>
<tr>
<td>Active endometriosis</td>
<td>34 (97)</td>
<td>12 (86)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>Oviduct-like epithelium</td>
<td>27 (77)</td>
<td>3 (21)</td>
<td>7 (44)</td>
</tr>
<tr>
<td>Mitotic index (%)</td>
<td>0.09 ± 0.25</td>
<td>0.03 ± 0.60</td>
<td>0.03 ± 0.10</td>
</tr>
<tr>
<td>Epithelial height (µm)</td>
<td>16.5 ± 4.1</td>
<td>12.9 ± 3.3</td>
<td>11.4 ± 2.9</td>
</tr>
</tbody>
</table>

Vascularization

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>After GnRHa</th>
<th>After lynestrenol</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. capillaries per mm$^2$ stroma</td>
<td>161</td>
<td>208*</td>
<td>139</td>
</tr>
<tr>
<td>Capillary mean surface area (µm$^2$)</td>
<td>140 ± 80</td>
<td>120 ± 60</td>
<td>76 ± 70$^b$</td>
</tr>
<tr>
<td>Capillaries/stroma relative surface area</td>
<td>1.8</td>
<td>2.2$^a$</td>
<td>0.9$^b$</td>
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GnRH$\alpha$s = gonadotrophin-releasing hormone agonists.

$^a$Significantly different from lynestrenol group.

$^b$Significantly different from the control group.

Discussion

It is generally believed that endometriosis is caused by the implantation of retrograde menstrual endometrial cells, or by metaplasia. In the pelvis, three different forms of endometriosis must be considered: (i) peritoneal; (ii) ovarian; and (iii) rectovaginal septum (Donnez et al., 1995). The early manifestations of the disease are believed to be subtle or non-coloured lesions such as white lesions (white opacification) (Jansen and Russel, 1986; Nisolle et al., 1990; Donnez et al., 1992; Brosens, 1994). The presence of a lower mitotic activity and poor stromal vascularization in white lesions suggests that this type of lesion is a quiescent form of the disease. Red lesions (red vesicles, polypoid lesions, flame-like lesions) (Jansen and
called typical or black 'lesions which must be considered as
portion and uniting the cervix or the lower portion of the
disease is 'deep-infiltrating endometriosis of the rectovaginal
more active forms of the disease. In women, our hypothesis
1994) are
et al, et al, et al,
septum'. Sampson (1922) defined cul-de-sac obliteration as
in which epithelial glands are systematically surrounded by
smooth muscle by very active glandular epithelium widiout
glandular epithelium and scanty stroma. The invasion of the
smooth muscle is like an adenomyoma, a circumscribed nodular aggregate of
myometrial-type stroma. The very similar histological descrip-
tions to uterine adenomyosis lead us to suggest, like Brosens
(1995) that the so-called endometriotic nodule of the recto-
vaginal septum is, in fact, just like an adenomyoma or an
adenomyotic nodule. In our study, whatever the hormonal
treatment used, a high incidence of active endometriosis
without signs of degeneration was found, but differences could
be observed between peritoneal endometriosis and rectovaginal
'adenomyosis'. After GnRHa therapy, the incidence of active
endometriosis and the mitotic activity were significantly lower
in peritoneal endometriosis but not in rectovaginal endo-
metriosis. The ectopic foci are more or less autonomous, not
governed by the normal control mechanisms governing the
uterine endometrial glands and stroma. The exact reason why
a number of implants or cells do not respond to hormonal
therapy is not known, but at least four hypotheses have been
proposed: (i) the drug does not gain access to the endometriotic
foci because fibrosis surrounding the foci prevents access
locally; (ii) endometriotic cells may have their own genetic
programming, while endocrine influence appears to be only
secondary and dependent on the degree of differentiation of
the individual cell; (iii) the lower oestrogen receptors in
peritoneal ectopic endometrium and in rectovaginal nodule
endometrium than in eutopic endometrium (Bergqvist et al.,
1993; Bergqvist and Ferno, 1993; Nakamura et al., 1993;
Howell et al., 1994; Nisolle et al., 1994; Bergqvist, 1995);
and (iv) the different regulatory mechanisms of endometriotic
steroid receptors may result in deficient endocrine dependency
(Nisolle et al., 1994) because the receptors, although they are
present, are biologically inactive (Metzger, 1992).
In peritoneal endometriosis, the stromal vascularization was
found to be significantly lower after GnRHa therapy. This
change was due to a decrease in the volume occupied by the
vessels, as proved by both the mean capillary surface area
and the ratio of capillaries/stroma surface area. This last parameter
which is the stromal vascularization index (Nisolle et al.,
1993) was not reduced after lynestrenol therapy. In rectovaginal
endometriosis, lynestrenol, on the contrary, appeared more
active in the reduction of vascularization than GnRHa.
In peritoneal endometriosis, after GnRHa administration,
our study demonstrated a low rate of mitosis and poor stromal
vascularization. Our hypothesis is that under GnRHa treatment,
lesions are in latent stages. They are probably non-active
lesions that could be quiescent as long as the oestrogen
secretion is suppressed. But the fact that residual areas of
active endometriosis are present in 75% of cases explains the
quick recurrence observed after cessation of therapy.
When data from rectovaginal endometriosis are evaluated,
we can see that GnRHa is unable to suppress the mitotic
activity and to decrease the stromal vascularization. All these
parameters were strongly in evidence in peritoneal endo-
metriotic lesions. The absence of any lynestrenol effect on the
peritoneum was in contrast to the strong effect of the same
drug on rectovaginal endometriotic nodule vascularization.
The completely different responses of peritoneal lesions and
nodular rectovaginal lesions to GnRHa therapy or lynestrenol
therapy suggest that peritoneal endometriosis and rectovaginal
endometriosis are two different diseases and have a different
physiopathology. Peritoneal endometriosis is probably caused by the implantation of regurgitated menstrual cells. Rectovaginal endometriosis is a lesion of adenomyosis and can possibly develop from Mullerian rests. The very low mitotic activity observed in this pathology can explain the relatively slow evolution of the adenomyoma.

In conclusion, it is suggested that rectovaginal adenomyosis is a specific disease, different from peritoneal endometriosis.

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References


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