Colour Doppler analysis of ovarian and uterine arteries in women with hypoestrogenic amenorrhoea

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BACKGROUND: This is a clinically-controlled study designed to investigate uterine and ovarian blood flow in patients with hypoestrogenic amenorrhoea. METHODS: Twelve women with hypoestrogenic amenorrhoea and 13 eumenorrhoeic subjects (controls) were enrolled. Colour and pulsed Doppler was used to visualize the uterine and ovarian arteries and the blood vessels within the ovarian stroma in both groups. Four blood flow indices were calculated: the pulsatility index, the resistance index, the peak systolic velocity and the end-diastolic velocity. RESULTS: Peak systolic velocity underwent the most significant change in amenorrhoeic patients, being significantly lower in comparison with that of controls, both in the uterine (P = 0.0009) and ovarian (P = 0.001) arteries. Compared with controls, the end-diastolic velocity of the ovarian artery was significantly lower (P = 0.039) in amenorrhoeic patients, and was also lower in the uterine artery (though not statistically significantly so). A reduction in blood flow was also evident in the ovarian stroma in amenorrhoeic patients. CONCLUSIONS: The significant reduction in blood flow observed in hypoestrogenic amenorrhoea suggests that estrogens play an important role in regulating both uterine and ovarian blood flow.

Key words: amenorrhoea/ovarian and uterine blood flow/pulsed and colour Doppler/transvaginal colour Doppler

Introduction

It has been shown repeatedly in animal studies that important blood flow modifications occur in the female reproductive organs either in normal or pathological conditions. Initial studies in primates showed that a normal blood flow profile of the ovary was correlated with the maturation and selection of the dominant follicle (Zeleznick et al., 1981).

The introduction of non-invasive technology such as transvagal colour and pulsed Doppler techniques has made it possible to study uterine and ovarian perfusion directly in humans. This is important because even if vascularization of the reproductive organs is similar in all mammals, there are important differences in complexity between humans and other mammals (Reynolds, 1973). Pulsed and colour Doppler technology have been used to show that blood flow directed to the uterus and ovary undergoes important modifications in relation to cyclic hormonal changes, and can be easily detected using colour Doppler. For example, increased blood flow characterizes the pre-ovulatory follicle in spontaneous cycles (Campbell et al., 1993), with a peak in blood flow velocity occurring during the day of ovulation (Kupesic and Kurjak, 1993).

Moreover, a clear relationship between follicular vascularity and establishment of clinical pregnancy after IVF has been shown (Coulam et al., 1999). Vascularization of the reproductive organs also undergoes remarkable modifications in many pathological conditions. Blood flow study of vascular resistance of the stromal vessels seems to predict the risk of severe ovarian hyperstimulation syndrome in half of all IVF-stimulated patients (Moohan et al., 1997). Other endocrinological diseases such as anovulation or missed implantation showed important vascular modifications in the uterus and ovary compared with normal fertile women (Goswamy et al., 1988; Merce et al., 1990).

The aim of the present study was to determine whether, in hypoestrogenic amenorrhoea, there were different blood flow profiles in the uterine and ovarian arteries compared with those observed in eumenorrhoeic subjects.

Materials and methods

Study population

A total of 12 patients affected by hypoestrogenic amenorrhoea was selected among those attending the Endocrinological Reproductive Center of the University of Padua. All patients had had amenorrhoea for at least 6 months and had not received any hormonal treatments within the past 6 months. Among the amenorrhoeic cases, two resulted from premature ovarian failure, six were hypothalamic amenorrhoea (including three cases of anorexia) and four were pituitary amenorrhoea (one case of beta-thalassaemia and three cases of hyperprolactinaemia). Patients with amenorrhoea caused by polycystic ovary syndrome were excluded from the study.
Thirteen volunteers who had a regular menstrual cycle (length between 26 and 30 days), normal uteri and ovaries at transvaginal ultrasound examination and without any signs of hyperandrogenism were asked to participate in the study as a control group. The study was approved by the Institutional Review Board of the University, and the participants provided their informed consent to be enrolled.

An accurate personal medical history was taken for each subject and the following parameters were evaluated: age, weight, height and body mass index (BMI, calculated as body weight (kg)/height$^2$ (m$^2$)).

**Biochemical analysis**

Radioimmunoassays for estradiol, LH, FSH, prolactin and thyroid-stimulating hormone (TSH) were performed in all amenorrhoeic patients in a reliable laboratory. The mean serum estradiol level was $25.51 \pm 16.1$ pg/ml.

**Ultrasound and Doppler examination technique**

Each patient underwent single transvaginal ultrasonography in a quiet and comfortable location; patients were asked to empty their bladder, to normalize blood pressure and pulse rate. All subjects underwent ultrasonography between 11:00 and 12:00 h am.

Patients in the control group were scanned between the 3rd and 8th days of the menstrual cycle in order to avoid interference with blood flow modifications due to ovulation and corpus luteum. A Siemens Sonoline Elegra fitted with a 6.5 MHz probe for B-mode and colour imaging, as well as pulsed Doppler spectral analysis, was used. All examinations were performed by the same physician. The lowest colour pulsed repetition frequency of 400 Hz was used. The high-pass filter was set from 50 to 100 Hz, and the spatial peak temporal average was 80 mW/cm$^2$ (based on limits recommended by the American Food and Drug Administration).

A two-dimensional mode scan was first performed to evaluate the shape, dimension and morphology of the uterus and ovaries.

Endometrial thickness was measured in all patients and included both layers at the thickest point in the longitudinal section of the uterus. In the control group, endometrial thickness was evaluated during the early follicular phase, between the 3rd and 8th days of the cycle.

Ovarian volume and morphology were evaluated in both ovaries in all patients. An estimation of ovarian volume was performed by applying the formula for a prolate ellipse ($\text{length} \times \text{depth} \times \text{width} \times 0.533$) (Sample et al., 1977). No statistical difference in volume was noted between the right and left ovary in any patient; therefore the mean volume was calculated and considered for further analysis.

Ovarian morphology was assessed by counting the number of follicles and considered polycystic if more than 10 follicular cysts of 2–8 mm diameter were retrieved for each ovary.

Transvaginal colour and pulsed Doppler was used to visualize the uterine and ovarian arteries and the blood vessels within the ovarian stroma in all patients. The uterine artery was localized laterally to the uterus at the level of the corpuscervical junction, and the ovarian artery was found lateral to the upper pole of the ovary, near the infundibulopelvic ligament. The stromal vessels of the ovary were identified as colour signals within the ovarian stroma of both ovaries.

A pulsed Doppler range gate was placed across each vessel, aiming for an angle of insonation close to 0$^\circ$ between the Doppler beam and the vessel. After detection of blood flow and visualization of the waveform of the uterine and ovarian arteries, four blood flow indices were automatically calculated: the pulsatility index (PI); the resistance index (RI); the peak systolic velocity (Vp, units of cm/s); and the end-diastolic velocity (Vd, units of cm/s). At least three consecutive correctly imaged blood flow velocity waveforms were analysed, and

### Table I. Differences in vascular indices in the uterine artery in the two subject groups

<table>
<thead>
<tr>
<th>Vascular index</th>
<th>Amenorrhoeic group $n=12$</th>
<th>Control group $n=13$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsatility index</td>
<td>$3.92 \pm 1.569$</td>
<td>$3.78 \pm 1.31$</td>
</tr>
<tr>
<td>Resistance index</td>
<td>$0.89 \pm 0.007$</td>
<td>$0.91 \pm 0.004$</td>
</tr>
<tr>
<td>Peak systolic velocity (cm/s)</td>
<td>$29.27 \pm 9.56^a$</td>
<td>$41.99 \pm 12.24^a$</td>
</tr>
<tr>
<td>End-diastolic velocity (cm/s)</td>
<td>$3.10 \pm 2.26$</td>
<td>$3.82 \pm 2.21$</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

$^aP = 0.009$.

### Table II. Differences in vascular indices in the ovarian artery in the two subject groups

<table>
<thead>
<tr>
<th>Vascular index</th>
<th>Amenorrhoeic group $n=12$</th>
<th>Control group $n=13$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulsatility index</td>
<td>$4.00 \pm 2.27$</td>
<td>$4.25 \pm 0.91$</td>
</tr>
<tr>
<td>Resistance index</td>
<td>$0.78 \pm 0.38$</td>
<td>$0.93 \pm 0.005$</td>
</tr>
<tr>
<td>Peak systolic velocity (cm/s)</td>
<td>$22.89 \pm 16.06^a$</td>
<td>$48.33 \pm 14.87^a$</td>
</tr>
<tr>
<td>End-diastolic velocity (cm/s)</td>
<td>$1.29 \pm 1.27^b$</td>
<td>$2.64 \pm 1.75^b$</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

$^aP = 0.001$; $^bP = 0.039$.

mean values of PI, RI, Vp and Vd calculated. No significant difference was observed between the right and left uterine arteries, or between the right and left ovarian arteries in the value of each vascular index. Thus, the uterine and ovarian artery of one side were selected for further analysis.

Since it is difficult to obtain an angle between the ultrasound beam and ovarian stromal vessels, it is possible that blood flow velocities might be inaccurate; therefore, stromal blood flow was evaluated in terms of the presence or absence of colour Doppler signals within the ovary.

**Statistical analysis**

Values of the four vascular indices were expressed as mean ($\pm$SD). The degree of significance was determined by analysis of variance (ANOVA), with a $P$-value $< 0.05$ being considered statistically significant. Statistical analysis was performed using SPSS software (Chicago, IL, USA).

**Results**

The mean age of patients with amenorrhoea was $27.92 \pm 6.82$ years, while that for controls was $24.15 \pm 1.57$ years. The mean BMI in these groups was $20.25 \pm 0.53$ and $21.08 \pm 0.57$ respectively. No significant difference was found between the two groups in either age or BMI, and mean ovarian volume was also similar in both groups ($4.42 \pm 3.17$ cm$^3$ in amenorrhoeic patients and $5.15 \pm 1.99$ cm$^3$ in controls). Endometrial thickness in amenorrhoeic patients ($2.76 \pm 0.94$ mm) was significantly less ($P = 0.001$) than in the control group ($5.43 \pm 2.2$ mm).

Mean values of vascular indices for the uterine and ovarian arteries are listed in Tables I and II respectively. There were no significant inter-group differences in PI and RI for either the uterine or ovarian arteries.
Figure 1. Example of a waveform of the ovarian artery in an amenorrhoeic patient.

Figure 2. Example of a waveform of the ovarian artery in a control subject.

Both the peak systolic and end-diastolic velocities of the ovarian artery were significantly lower in amenorrhoeic patients than in controls ($P = 0.001$ and $P = 0.039$ respectively).

The blood flow velocity waveforms of an ovarian artery in an amenorrhoeic patient and in a normal menstruating patient are shown in Figures 1 and 2 respectively. In the uterine artery, the peak systolic velocity was significantly reduced in amenorrhoeic patients compared with controls ($P = 0.009$), but there was no significant inter-group difference in end-diastolic velocity. Typical flow velocity waveforms of a uterine artery in an amenorrhoeic patient and in a normal menstruating patient are shown in Figures 3 and 4 respectively.

The intra-ovarian blood flow was bilaterally absent in nine of the amenorrhoeic patients; in two cases, blood flow was registered in only one ovary, and in one patient the flow was bilateral. Among controls, intra-ovarian blood flow was bilateral in 11 cases, monolateral in one case and absent in one case.

Among amenorrhoeic patients, ovarian morphology was normal in four cases, polycystic in six, and without follicle in two.

Discussion

Mean ovarian volume in patients affected by amenorrhoea was not significantly lower compared with controls, whereas mean serum estrogen levels in the pathological group were rather low throughout the study. This might be explained by the fact that in some patients with hypoestrogenic amenorrhoea the ovaries had a polycystic appearance with many follicles, which caused a slight increase in the size of the organ. In healthy fertile women, the ovarian volume is generally about 4–6 cm$^3$ (Rossato and Pecorari, 1996), though significant changes may occur at the beginning and end of a woman’s reproductive life. Likewise, changes may also occur in certain extreme pathological conditions such as anorexia and premature ovarian failure in which a remarkable and prolonged hypoestrogenic status is present. A positive correlation between estradiol serum level and ovarian volume was clearly demonstrated only in puberty (Orbak et al., 1998) and many years after onset of the menopause (Higgins et al., 1989; Tepper et al., 1995). A remarkable reduction in ovarian volume with an immature morphological appearance and either without or with few small follicles has been noted in anorectic patients. In
these patients the recovery of body weight normalized both the volume and morphology of the ovary (Sobanski et al., 1997).

In the present study, the mean endometrial thickness was less in patients affected by amenorrhoea compared with controls. The endometrium is in fact the target organ for estrogens and progestins; the former have a proliferative effect such that, when they are reduced as in post-menopausal and amenorrhoeic women, the atrophy of the tissue occurs (Zichella et al., 1996).

Blood flow analysis of the uterine and ovarian arteries revealed interesting modifications of their haemodynamic profiles in patients with amenorrhoea. In this group, pronounced variations of blood flow velocities were noted while the values of RI and PI remained unchanged compared with controls.

Peak systolic velocity was the vascular parameter which underwent greater modification in amenorrhoeic patients, being significantly lower when compared with that of controls in both the uterine and ovarian arteries. The end-diastolic velocity of the ovarian artery was significantly lower in amenorrhoeic patients than in controls, and also appeared lower in the uterine artery, though this difference was not statistically significant.

The resistance indices of the uterine and ovarian arteries were not statistically different between the two groups. This may relate to the fact that all control patients were scanned during the early proliferative phase, when the vascular resistance of the uterine and ovarian vessels is physiologically high. Vascular resistance is high during the initial part of the menstrual cycle and declines progressively in line with increasing estrogen levels until the time of ovulation and during the luteal phase both in the uterus and ovary carrying the dominant follicle and subsequent corpus luteum. In particular, it was noted that the PI from the ovarian artery began to decline at day 13 of the cycle in the ovary carrying the ovulatory follicle, and this reduction was determined by a rise in end-diastolic velocity. These vascular modifications are not evident in the contralateral ovary and in the anovulatory cycles (Scholtes et al., 1989; Merce et al., 1990; Zaidi et al., 1995). Moreover, others (Tan et al., 1996) demonstrated that although the PI was not significantly different between the dominant and non-dominant uterine arteries during the follicular phase, it became significantly lower in the dominant uterine artery when compared with the contralateral vessel during the mid-luteal phase.

The results of the present study showed that, under hypoestrogenic conditions, the blood supply directed to the reproductive organs underwent a marked reduction. The effects of estrogens on the uterine vascular bed have been demonstrated both in human and animal studies (Greiss and Anderson, 1970; Resnik et al., 1974; Hillard et al., 1992; Pirhonen et al., 1993).

In particular, a negative linear correlation between resistance to uterine blood flow and increased estrogen levels has been noted throughout the cycle (Weiner, 1993). Estrogens induce a remarkable decrease in vascular resistance of the uterus, increasing blood perfusion of the organ (Bourne et al., 1990) and causing vasodilatation, most likely by the action of catecholamines (Ford, 1982) and perhaps directly acting on receptors localized in the tunica media of the vessel wall (Perrot-Apllanat et al., 1988; De Ziegler et al., 1991). The effect of estrogens on uterine perfusion is dose-dependent (Goswamy and Steptoe, 1988).

Ovarian circulation is under the control of different mechanisms. The ovarian vascular bed is constituted by two vascular systems: (i) the extrinsic system, which is represented by the ovarian artery and the uterine-ovarian artery; and (ii) the intrinsic system, which is formed by the vascular network inside the stroma. Both systems are under the control of neuronal fibres, but they are also influenced by the events of the menstrual cycle, ovulation and luteinization. The growth and maturation of the follicle and corpus luteum induce haemodynamic changes mainly represented by a decrease in vascular resistance, especially at the level of the stromal ovarian network and at the perifollicular vessels by physiological angiogenesis (Aguardo and Ojeda, 1984; Folkman, 1992; Kurjak, 1995).

The chronic depletion of estrogens in the present patients determined a marked reduction in blood supply, represented by a pronounced decrease in blood flow velocities in the uterine and ovarian arteries. The reduction in blood flow was also evident in the ovarian stroma, where in amenorrhoeic patients very few colour Doppler signals were registered. The significant reduction in blood perfusion of the ovary in amenorrhoeic patients suggests that estrogens also have an important role in regulating ovarian blood flow supply (Taylor et al., 1985; Scholtes et al., 1989; Fatma and Mladen, 1996).

The results of the present study have demonstrated that although uterine and ovarian morphology were slightly modified in hypoestrogenic amenorrhoea, the vascular system of the two organs underwent much more marked changes, the most significant being that of blood flow velocity. Hence, it is possible that these criteria could be used as markers to monitor the efficiency of hormonal treatment and to optimize the ratio between the therapeutic efficacy and adverse effects of hormone replacement therapy in the treatment of certain endocrinological disorders. These preliminary results confirmed the role of transvaginal colour and pulsed Doppler analysis in evaluating the functional state of the reproductive organs.

References


