Doppler Study of Uterine Arteries Blood Flow in Patients with Unexplained Recurrent Pregnancy Loss and the Effects of Nitric Oxide Donors

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Abstract

Objectives: To study the uterine artery resistance and pulsation indices during the mid luteal phase in patients with history of unexplained recurrent pregnancy loss and the effects of the nitric oxide donor isosorbid mononitrite on these indices.

Study Design: A cross-section, controlled observational study.

Setting: Benha University Hospital, Egypt. July 2009 to July 2010.

Subjects and Methods: Two groups of women each 30 cases were studied. The control group included women with no history of abortion who delivered at least one living child. The study group included nulliparous women with history of unexplained recurrent pregnancy loss. Vaginal color Doppler ultrasound was done in the mid luteal phase of the menstrual cycle to measure uterine arteries resistance and pulsation indices, then 20 mg isosorbid mononitrate tablet was applied vaginally in the study group and the Doppler indices were measured again after two hours.

Outcome Measures: Comparison between uterine artery resistance and pulsation indices in both groups and the effects of isosorbid mononitrate on these indices in the study group.

Results: Women with recurrent pregnancy loss had significantly higher uterine artery resistance and pulsation indices. Isosorbid mononitrate applied vaginally causes significant decrease in these indices.

Conclusions: Patients with history of unexplained recurrent pregnancy loss had decreased uterine arteries perfusion during the mid luteal phase. Nitric oxide donors increases uterine arteries blood flow and may have a role in the treatment of these patients.

Key Words: Recurrent pregnancy loss – Nitric oxide donors.

Introduction

RECURRENT pregnancy loss (RPL) is defined by the American Society for Reproductive Medicine [1] as two or more failed pregnancies documented by ultrasound or histopathology. The condition affects 3-5% of couples and represents a major concern for reproductive medicine, and despite extensive endocrine, chromosomal, serologic and anatomic evaluation 30-40% of RPL cases remain unexplained [2].

Uterine perfusion regulates uterine receptivity and influences the success of implantation and maintenance of early pregnancy [3]. Studies [4] showed that during the normal menstrual cycle the impedance to uterine arteries blood flow diminishes progressively during the luteal phase, reaching the lowest values in the period that coincide with the implantation window. Other investigators [5] found that high blood flow resistance is associated with a reduced conception rate and that women with lower pulsation index values have the highest possibility of becoming pregnant.

In 1998 Furchgott et al. [6], awarded the Nobel Prize for their contribution to science in the field of nitric oxide research. Recent studies [7] provide evidences that nitric oxide (NO) generated in vivo from L-arginine by the vascular endothelium plays a major role in vascular smooth muscle relaxation, decrease in utero-placental vascular resistance and increase in uterine artery blood flow that is observed in early pregnancy. Based on these observations, it can be speculated that an impaired uterine perfusion due to deficient production of endogenous NO could play a central role in the pathogenesis, and is considered a causative factor in unexplained recurrent abortion [8]. Nonetheless, few studies
have been published on whether uterine artery impedance during the luteal phase of the menstrual cycle in patients with history of RPL differs from that in normal fertile women and whether nitric oxide donors, which are drugs capable of releasing NO in vivo \[9\], may improve this impaired uterine blood flow to be of therapeutic value in these patients.

**Subject and Methods**

The study was approved by the ethical committee and consent was taken from every patient. Two groups of women each 30 cases selected from the outpatient clinic of Banha university hospital during the period from July 2009 to July 2010 were studied. The control group was women without previous abortion who delivered at least one living child. The study group was nullipara with history of two or more successive unexplained RPL according to the following investigations \[10\]: Normal uterine cavity by hysterography, normal glucose tolerance test, normal thyroid function tests (TSH, T3 and T4), normal blood picture, normal levels of lupus anticoagulant measured by the activated partial thromboplastin time and normal levels of anticardiolipin IgG and IgM antibodies measured by ELISA.

Women in both groups were asked to attend during the 21-23 day of the cycle and were subjected to history taking, full clinical examination, mean arterial pressure calculated and vaginal US to exclude any pelvic pathology. Pulsed color Doppler vaginal US (Voluson, PRO 720, GE Medical System) was done to measure the resistance index (RI) and pulsation index (PI) of the both uterine arteries. A mid-sagittal section of the uterus was obtained and the cervical canal was identified. The probe was then moved laterally until the paracervical vascular plexus was observed, then the color Doppler was used to identify the uterine artery as it turned cranially along the uterine body. The gate was adjusted to 2 mm with an angle of isonation less than 60 degree, then pulsed wave Doppler was used to obtain three similar consecutive waveforms and the PI and the RI of both arteries were recorded and the mean was calculated.

The effect of nitric oxide donor was evaluated in the study group by placing 20 mg tablet isosorbid mononitrate (IMN), (Effox, Mina Pharma Co, Egypt; under license of Schwartz Pharma, Germany), high in the vagina, and the uterine artery RI and PI were measured again after two hours as the period of maximum effect of the drug recommended by the manufacturer.

**Statistical analysis:**

Data were tabulated and analyzed using Statistical Package of Social Science (SPSS) version 16. Result was represented by the mean and the standard deviation. The Student \(t\)-test was used to compare between two independent groups and the Paired \(t\)-test was used to compare the same group before and after intervention. \(p\) 0.05 was considered significant.

**Results**

Table (1) shows non significant difference between the bio data of the control and the study groups.

Table (2) shows that the RI and PI of the uterine arteries were significantly higher in the patients with unexplained recurrent pregnancy loss (\(p<0.01\) and \(<0.001\) respectively).

Table (3) shows highly significant decrease in the RI and PI (\(p<0.001\)) after vaginal application of 20 mg IMN in patients with unexplained recurrent pregnancy loss.

Figs. (1,2,3) show uterine artery waveform in normal fertile women, in patients with RPL before and 2 hours after vaginal application of IMN respectively.

### Table (1): Comparison between the bio data of the study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Study group N=30</th>
<th>Control group N=30</th>
<th>(t)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP</td>
<td>85.87±5.9</td>
<td>84.90±6.7</td>
<td>0.6</td>
<td>0.55*</td>
</tr>
<tr>
<td>Age</td>
<td>31.6±1.4</td>
<td>30.6±2.5</td>
<td>1.8</td>
<td>0.07*</td>
</tr>
<tr>
<td>BMI</td>
<td>24.3±2.3</td>
<td>23.8±1.9</td>
<td>0.9</td>
<td>0.36*</td>
</tr>
</tbody>
</table>

*Non significant.

MAP: Mean arterial pressure.

BMI: Body mass index.

### Table (2): Comparison between the Doppler parameters of the uterine arteries in the study and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Study group N=30</th>
<th>Control group N=30</th>
<th>(t)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI</td>
<td>0.80±0.02</td>
<td>0.78±0.03</td>
<td>2.9</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>PI</td>
<td>2.64±0.07</td>
<td>2.08±0.09</td>
<td>26.9</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

**Significant; RI: Resistance index; PI: Pulsation index.

### Table (3): Comparison between the Doppler parameters of the uterine arteries in the study group before and after vaginal administration of isosorbid mononitrate.

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
<th>(t)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI</td>
<td>0.80±0.02</td>
<td>0.77±0.03</td>
<td>5.4</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>PI</td>
<td>2.64±0.07</td>
<td>2.19±0.10</td>
<td>20.4</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

**Significant; RI: Resistance index; PI: Pulsation index.
Discussion

In the present study the control and the study groups were comparable as regard the mean arterial pressure, the maternal age and the body mass index, but the RI and PI of the uterine arteries at 21-23 day of the cycle were significantly higher in patients with RPL compared to normal fertile women ($p < 0.01-0.001$ respectively). These results are in agreement with Habara et al. [11] that investigated patients with RPL and found that in the mid luteal phase the pulsation index in women with antinuclear antibodies was significantly higher than that in women without, and that among women without antinuclear antibodies the PI was also significantly higher than that in the control group. The same observation was reported by Nakatsuka et al. [12] who found higher PI in recurrent abortion patients compared to a control group and recommended that pulsed Doppler ultrasound provided a means for non invasive evaluation of uterine impedance and may identify patients with recurrent pregnancy loss associated with impaired uterine perfusion. In another study [13] the mid luteal PI of the uterine artery was significantly higher in RPL patients compared to a control group and there were no significant correlation between the PI and the endometrial thickness, serum progesterone, serum estradiol or maternal age. Ferreira et al. [14] found that in the second half of the cycle the PI was significantly higher and there was a higher incidence of flow velocity waves of the A and B types in women with RPL than those in the control group. In another study [15] the PI was significantly higher in the mid luteal phase in women with RPL compared to normal fertile women and when patients were grouped according to the different RPL causes, the highest PI values were found among patients with uterine anomalies, antiphospholipid syndrome and unexplained RPL. The authors found also that there were no differences observed between fertile women and those with RPL due to thyroid abnormalities, inherited thrombophilia, genetic anomalies or when patients were grouped according to primary or secondary RPL.

Nitric oxide generated in vivo from L-arginine is produced in excess during pregnancy mainly in the uterine and renal vascular beds and is in part responsible for the non responsiveness of the vessel to vaso-active agents, plays a relevant role in smooth muscle relaxation, peripheral vasodilatation and lowering systemic blood pressure and improving blood supply to the feto-placental unit and maternal kidneys [16]. Impaired uterine perfusion due to deficient endogenous nitric oxide could play...
a central role in the pathogenesis, and is considered a causative factor in recurrent abortion [8].

The effect of NO donors - which are drugs capable of releasing nitric oxide - on the uterine artery blood flow in patients with RPL was tested in this study by application of 20 mg IMN vaginally during the mid luteal phase, and there was a significant decrease in the RI and PI of the uterine arteries (p<0.001) denoting increase of uterine blood flow. No similar studies were reported for comparison with our study, but our results add a new indication for treatment with NO donors and encourage its use in early pregnancy in patients with RPL. Previous studies used nitric oxide donors in different obstetric indication as intrapartum fetal distress related to uterine hyperactivity [17], to arrest premature labor [18], for pre-induction cervical ripening [19,20] and for treatment of pre eclampsia [21-23].

Isosorbid mononitrite is sheep and cost effective drug. The toxicology and teratogenicity studies proved its safety during pregnancy even at very high doses, and its use vaginally make most of its action locally, avoiding the undesirable systemic effects [19].

Conclusions:

Patients with unexplained RPL have decreased uterine arteries blood flow in the mid luteal phase compared to normal fertile women possibly due to impaired NO production. The NO donor IMN is a perfusion enhancer drug that causes vasodilatation and increase blood flow and may has a therapeutic role during pregnancy in patients with RPL.

Acknowledgement:

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