Original Article

The Effects of Nitric Oxide Donors on Uterine Artery and Sub-endometrial Blood Flow in Patients with Unexplained Recurrent Abortion

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Abstract

Background: Nitric oxide plays a major role in increasing uterine blood flow during the luteal phase and early pregnancy. This study was done to compare uterine artery and sub-endometrial blood flow indices during the luteal phase between patients with unexplained recurrent abortion and fertile women and also to evaluate the effects of nitric oxide donors on blood flow indices in the abortion group.

Methods: The study included a control group of 30 primiparae who had normal vaginal delivery and no history of abortion and an abortion group of 30 nulliparous women with history of two or more unexplained recurrent abortions. Transvaginal three-dimensional pulsed color Doppler ultrasound was done on days 21-23 of the cycle to measure uterine artery resistance and pulsation indices and the subendometrial vascular, flow and vascular-flow indices. Isosorbide mononitrate 20 *mg* tablet was administered vaginally in the abortion group and blood flow indices were measured again after two hours. The Student t-test and the paired t-test were used for analysis of results and a p-value of ≤ 0.05 was considered significant.

Results: Patients with unexplained recurrent abortion had significantly higher uterine artery resistance and pulsation indices and lower sub-endometrial vascular, flow and vascular-flow index ($p<0.01$-0.0001). Isosorbide mononitrite significantly decreased uterine artery and increased sub-endometrial blood flow indices ($p<0.001$).

Conclusion: Uterine artery and sub-endometrial blood flow decreased during the luteal phase in patients with unexplained recurrent abortion. Nitric oxide donors increased blood flow and may be of a therapeutic value.

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Received: Dec. 27, 2013
Accepted: Jun. 24, 2014

Keywords: Abortion, Habitual, Nitric oxide donors.
To cite this article: Abdel-Razik M, El-Berry S, Mostafa A. The Effects of Nitric Oxide Donors on Uterine Artery and Sub-endometrial Blood Flow in Patients with Unexplained Recurrent Abortion. J Reprod Infertil. 2014;15(3):142-146.

Introduction

Recurrent abortion (RA) is defined as three or more successive pregnancy losses before 20 weeks of gestation and affects about 0.5-3% of couples (1). Recently, it was defined as two or more (2) and this increases the incidence up to 5%. Recurrent abortion is frustrating for patient and clinician; a third of women are clinically depressed and 20% have levels of anxiety similar to psychiatric patients (3). Treatment of RA is a real challenge (4) and in most cases is unsuccessful as identifiable causes can be found only in 30-50% of women (5) and the rest remain unexplained. Scientists (6) showed that during the luteal phase of normal menstrual cycle, uterine artery impedance to blood flow decreases and there is an increase in uterine and sub-endometrial blood flow which reaches its maximum level during the period of implantation. Other studies (7) showed that uterine artery perfusion regulates uterine receptivity, influences the success of implantation, maintains early pregnancy and that impaired uterine perfusion plays a central role in the pathogenesis

of unexplained recurrent abortion (URA) (8). Past studies (9, 10) provide evidences that nitric oxide (NO) generated *in vivo* from the essential amino acid L-arginine by the vascular endothelium plays a major role in vascular smooth muscle relaxation. Subsequently, it results in a decrease in vascular resistance which leads to an increase in uterine artery blood flow observable in early pregnancy. Impaired L-arginine-nitric oxide pathway has been suggested to be a subtle cause of URA and treatment with nitric oxide donors is reported as a significant success in some studies (11-13). Based on these observations, an attempt was made to study uterine arteries and sub-endometrial blood flow during the luteal phase in normal fertile women and in patients with URA. Moreover, in this study, an investigation was carried out to find the effects of vaginal administration of isosorbide mononitrate (IMN) as a source of nitric oxide and a perfusion enhancer on uterine arteries and sub-endometrial blood flow indices in patients with URA.

Methods

The study was done during the period from January to November 2013 at Benha University Hospital after approval of the ethical committee (Australian New Zealand Clinical Trials Registry registration number: ACTRN 12614000069651) and informed consent was taken from every participant in two groups. The control group consisted of 30 primiparae who had normal vaginal delivery and no history of abortion. The abortion group consisted of 30 nulliparae with history of two or more successive (2) URA which entails (1) presence of a normal uterine cavity by hysterography, no luteal phase defect (progesterone level >10 *ng/ml*), normal thyroid function tests (TSH, T3 and T4), normal levels of lupus anticoagulant measured by the activated partial thromboplastin time (32-43 seconds), normal levels of anticardiolipin IgG (<20 GPL) and IgM (<15 MPL) measured by ELISA and normal karyotyping (done for 10 cases with more than three successive abortions). Women using hormonal contraception or intrauterine device or treated with vasodilators were excluded. Vaginal ultrasound (Voluson, PRO V, GE Health Care USA.) was done on cycle days 21-23 using 7.5 M. *Hz* probe; Transvaginal three-dimensional pulsed color Doppler ultrasound identified the uterine artery and three similar consecutive waveforms were displayed and the resistance index (RI) and the pulsation index (PI) were measured. An area within 5 *mm* of the echogenic endometrial border was identified and the histogram and the virtual organ computer aided analysis (VOCAL) were used to measure the sub-endometrial vascular index (VI), the flow index (FI) and the vascular-flow index (VFI). Isosorbide mononitrate (IMN) 20 *mg* tablet (Effox, Mina Pharma Co, Egypt; under license of Schwartz Pharma, Germany) was administered vaginally in the study group and blood flow indices were measured again after two hours.

Statistical design: Data were analyzed using SPSS software version 16. The mean, standard deviation and the Student t test were used to compare blood flow indices in the control and abortion groups and the paired t-test was used for comparison of blood flow before and after administration of IMN in the abortion group. The $p \leq 0.05$ was considered statistically significant.

Results

There was no significant difference between both groups regarding the mean age and the mean body mass index ($p>0.05$). However, there was a significantly higher uterine artery RI and PI and significantly lower sub-endometrial VI, FI and VFI in the abortion group as compared to the control group ($p<0.01$-0.0001). The results are shown in table 1.

After administration of IMN in the abortion group, there was a significant decrease of uterine artery RI and PI and a significant increase in sub-endometrial VI, FI and VFI ($p<0.001$) as shown in table 2.

Table 1. Comparison between bio data, uterine artery and sub-endometrial blood flow indices in the control and the abortion groups

Variables	Control (n=30) Mean±SD	Abortion (n=30) Mean±SD	t
Age	25.88±3.33	26.11±3.22	0.3
Body mass index	24.70±3.44	23.52±1.21	1.8
Resistance index	0.69±0.22	0.91±0.11	4.9 **
Pulsation index	2.44±0.11	2.88±0.66	3.6 **
Vascular index	1.05±0.32	0.56±0.22	6.9 **
Flow index	12.33±4.90	9.11±2.08	3.3 *
Vascular-flow index	0.21±0.01	0.17±0.03	6.9 **

* $p<0.01$, ** $p<0.001$

Table 2. Comparison between uterine artery and sub-endometrial blood flow indices before and after isosorbide mononitrite (IMN) administration in the abortion group

Variables	Before IMN (n=30) Mean±SD	After IMN (n=30) Mean±SD	paired t
Resistance index	0.91±0.11	0.72±0.44	4.51 *
Pulsation index	2.88±0.66	1.98±0.77	7.12 *
Vascular index	0.56±0.22	0.82±0.61	9.46 *
Flow index	9.11±2.08	10.22±2.11	6.73 *
Vascular-flow index	0.17±0.03	0.28±0.16	4.62 *

* $p<0.001$

Discussion

Decreased uterine and sub-endometrial artery blood flow is considered a causative factor in recurrent abortion (14). In the present study, the RI and PI of the uterine arteries were significantly higher during the luteal phase in patients with URA as compared to normal fertile women ($p< 0.001$). This result agreed with previous studies (15) which referred to the elevated uterine artery RI in women with unexplained recurrent pregnancy loss in the mid luteal phase of a non pregnant cycle. In another study (16), women with unexplained recurrent pregnancy loss had significantly higher uterine artery PI in the second half of the menstrual cycle than those in a control group. In the present study, patients with URA had significantly lower sub-endometrial VI, FI and VFI than the ones in the control group. Vaquero et al. (17) reported significant decrease of sub-endometrial blood flow indices with an increase in resistance to uterine artery blood flow in patients with unexplained recurrent miscarriage. Other studies (18) found that in IVF pregnancies with live birth, there was significantly higher sub-endometrial blood flow than those with miscarriage and that failure of implantation was associated with absence (19) or decrease (20) of sub-endometrial blood flow. El-mashad et al. (21) evaluated uterine PI and plasma adrenomedullin (AM) levels in women with unexplained recurrent pregnancy loss (RPL) compared to controls and found that uterine artery PI and AM levels were significantly higher in RPL group. However, Yildiz et al. (22) reported no statistically significant difference regarding uterine artery PI and RI values measured between the 18th and 23rd days of the menstrual cycle in patients with recurrent pregnancy loss and the control group. In the present study, IMN applied vaginally during the luteal phase in patients with history of URA caused significant decrease in uterine artery RI and PI and significant increase in sub-endometrial VI, FI and VFI. In a study by El Far et al., (11) intravaginal sildenafil citrate-a selective phosphodiesterase-5 (PDE-5) inhibitor that augments the action of nitric oxide by inhibiting cAmp degradation by the enzyme (PDE-5) successfully treated four patients with unexplained recurrent spontaneous miscarriage. Other authors (23) investigated the effect of sildenafil on uterine volumetric blood flow (UVF) and vascular impedance in nonpregnant, nulliparous women in the luteal phase and demonstrated a significant increase in UVF in response to sildenafil. In another study, Amin et al. (12) evaluated the effect of treatment with N-acetyl cysteine as a NO donor plus folic acid in patients with unexplained recurrent pregnancy loss and found a significant increase in the take-home baby rate as compared to treatment with folic acid alone. Amite et al. (13) studied the effect of NO donors on uterine blood flow velocity waveforms during the first trimester of pregnancy by administration of 5 *mg* IMN sublingual and found a fall in the RI of the uterine artery and concluded that NO donors may be of therapeutic value in cases where uterine circulation is impaired. The results of the present study supported Amite et al.'s conclusion. Nitric oxide has important physiological functions and its use in the luteal phase and during pregnancy is promising for treatment of URA. Nitric oxide increases uterine and sub-endometrial blood flow, increases utero-placental circulation (24), causes peripheral vasodilatation and lowers systemic blood pressure (25). Also, it has anti-thrombotic and anti-inflammatory effects by inhibiting platelet aggregation and adhesions of neutrophils to the vessel wall (26). Moreover, it regulates apoptosis (27) and causes relaxation of the myometrium (28).

Conclusion

Patients with URA had a decrease in uterine arteries and sub-endometrial blood flow during the luteal phase of the menstrual cycle. Nitric oxide donors act as perfusion enhancer drugs that increase blood flow and may be of value in improving endometrial receptivity and pregnancy outcome.

Conflict of Interest

The authors declare no conflict of interest.

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