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Effect of transcervical resection of adhesion combined with low−dose aspirin on uterine artery blood flow and Smad2/3 in endometrial tissue

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ABSTRACT

Objective: To study the effect of transcervical resection of adhesion combined with low-dose aspirin on uterine artery blood flow and Smad2/3 in endometrial tissue. **Methods:** A total of 78 patients with severe intrauterine adhesions who received transcervical resection of adhesion in our hospital between June 2012 and October 2014 were prospectively studied and randomly divided into two groups, observation group received postoperative estrogen-progestogen combined with low-dose aspirin therapy, and control group received postoperative estrogen-progestogen therapy. Ultrasound examination was conducted before and after treatment to determine uterine artery and endometrial blood flow parameters, intrauterine adhesion tissue was collected to detect the expression levels of Smad2 and Smad3 as well as downstream molecules, and serum was collected to determine the levels of cytokines. **Results:** On the ovulation day after 3 cycles of treatment, uterine artery RI and PI of observation group were significantly lower than those of control group, and endometrial VI, FI and VFI were significantly higher than those of control group; uPA expression level in intrauterine adhesion tissue of observation group was significantly higher than that of control group, Smad2, Smad3, PAI-1, ADAM15 and ADAM17 expression levels were significantly lower than those of control group, and serum TGF-β, VEGF, CTGF, IGF-I and TNF-α levels were significantly lower than those of control group. **Conclusions:** Transcervical resection of adhesion combined with low-dose aspirin therapy can improve the postoperative uterine artery and endometrial blood flow state, inhibit extracellular matrix deposition mediated by Smad2/3 signaling pathway and prevent intrauterine re-adhesion in patients with intrauterine adhesions.

1. Introduction

Intrauterine adhesions (IUA) are the endometrial basal layer damage caused by various factors and the resulting secondary endometrial fibrosis and adhesion between uterine wall and cervical canal. Abortion, mechanical injury, pathogen infection and so on are the common causes of the IUA, and will cause the abnormal menstruation, infertility, *etc*[1−3]. Transcervical resection and separation of adhesion is the main clinical treatment of intrauterine adhesions, but adhesion formation again after operation is a common complication. The causes of recurrent intrauterine adhesions are complex, and abnormal expression of a variety of cytokines and protease molecules in endometrial tissue will affect tissue repair, resulting in adhesion recurrence[4,5]. Providing estrogen-progestogen after transcervical resection of adhesion to establish artificial cycle is a common way to promote endometrial repair, but its preventive effect is not ideal on intrauterine re-adhesions, especially the re-adhesions after severe intrauterine adhesions surgery. Aspirin has inhibitory effect on the activity of cyclooxygenase, and can improve the endometrial blood flow state and promote tissue repair. In the following study, transcervical resection of adhesion combined with low-dose aspirin was used to treat intrauterine adhesions, and the uterine artery blood flow and the expression of Smad2/3 in endometrial tissue were analyzed.

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2. Materials and methods

2.1. Research subjects

A total of 78 women of childbearing age diagnosed with severe intrauterine adhesions in our hospital between June 2012 and October 2014 were selected for prospective study, all patients were diagnosed with severe intrauterine adhesions by hysteroscopy examination, and according to the diagnosis and treatment sequence, patients were included in observation group and control group in turn and were approved by the hospital ethics committee. The following patients were excluded: (1) with other endocrine system diseases; (2) taking hormonal drugs within the last three months. They received transcervical resection of adhesion from the same doctor after inclusion. General information of two groups of patients was as follows: (1) control group: received transcervical resection of adhesion combined with postoperative estrogen-progestogen intervention, were (32.5±5.6) years old, and were with BMI (23.1±4.6) kg/m^2 and course of disease (6.92±0.92) months; (2) observation group: accepted transcervical resection of adhesion combined with postoperative estrogen-progestogen and low-dose aspirin intervention, were (32.1±5.4) years old, and were with BMI (22.8±4.7) kg/m^2 and course of disease (6.36±0.98) months. The two groups of patients showed no significant difference in general data.

2.2. Treatment methods

Two groups received transcervical resection of adhesion at first, it was conducted 3-7 d after the menstruation was clean, and the method was as follows: they were fasting for solids and liquids for 8-12 h for regular bowel preparation and received propofol intravenous anesthesia, then 5% mannitol was selected as distending medium, the flow velocity was set at 250 mL/min, and the intraoperative pressure was monitored and maintained at 16 kPa; for electric resection, part of the adhesion tissue was cut open at first to observe the intrauterine adhesions, mild membranous adhesion was bluntly separated through the distending pressure, muscular adhesion was cut open through the electrode, trying to ensure that the adhesions were separated at the same cross line level. Both groups routinely took antibiotics for 3 d after operation and received estrogen-progestogen intervention according to the following methods: received oral administration of estradiol valerate 9 mg/d from the very day after operation for consecutive 21 d and oral administration of additional medroxyprogesterone 10 mg/d on the last 10 d, received the above medication again from the 5 d after the menses, and received total three cycles of treatment. On the basis of estrogen-progestogen intervention, observation group received low-dose aspirin intervention, and the method was as follows: aspirin enteric-coated tablets, 50 mg, oral administration, 1 time/day.

2.3. Uterine artery blood flow evaluation

After three cycles of treatment, follicular development was monitored from the 8th day of menstrual cycle, the day when ultrasound image showed the disappearance of mature follicle was selected as the day of ovulation, color Doppler ultrasonography was conducted on the day of ovulation to acquire uterine artery images and then determine resistance index (RI) and pulsatility index (PI), and then the endometrium was scanned to determine vascularization index (VI), blood flow index (FI) and vascularization flow index (VFI).

2.4. Determination of molecule expression levels in endometrial tissue

During operation and the day of ovulation after 3 cycles of treatment, a moderate amount of endometrial tissue was collected respectively, about 50 mg tissue was weighed, added in 0.3 mL of saline and fully grinded, the grinded suspension was centrifuged in centrifuge for 15-20 min at a speed of 12 000 r/min, the precipitation was abandoned, the supernatant was kept, and enzyme-linked immunosorbent assay kits were used to determine Smad2 and Smad3 as well as uPA, PAI-1, ADAM15 and ADAM17 levels.

2.5. Determination of serum Smad-related cytokine levels

Before operation and the day of ovulation after 3 cycles of treatment, 5ml of peripheral blood was collected respectively and centrifuged to get serum specimens, enzyme-linked immunosorbent assay was used to determine serum transforming growth factor- β (TGF- β), vascular endothelial growth factor (VEGF), connective tissue growth factor (CTGF) and insulin-like growth factor-I (IGF-I) levels.

2.6. Statistical methods

SPSS20.0 software was used to input and analyze data, measurement data analysis between two groups was performed by t test and P<0.05 indicated statistical significant differences.

3. Results

3.1. Uterine artery and endometrial blood flow parameters

On the day of ovulation after 3 cycles of treatment, uterine artery and endometrial blood flow signals of observation group were more abundant, and detailed parameter analysis was shown in Table 1. Uterine artery RI and PI of observation group after treatment were significantly lower than those of control group, and endometrial VI, FI and VFI were significantly higher than those of control group (P<0.05).

3.2. Smad2 and Smad3 expression levels in endometrial tissue

Table 1
Uterine artery and endometrial blood flow parameters of two groups.

Groups	Case No.	Uterine artery blood flow parameters		Endometrial blood flow parameters		
		RI	PI	VI	FI	VFI
Observation group	32	1.01±0.13	1.32±0.18	3.57±0.62	27.76±3.62	1.18±0.22
Control group	32	1.22±0.18	1.89±0.25	2.26±0.34	22.95±4.12	0.86±0.12
t		5.698	6.617	6.981	5.924	7.149
P		<0.05	<0.05	<0.05	<0.05	<0.05

Intraoperative Smad2 and Smad3 expression levels in endometrial tissue of two groups showed no significant difference (P>0.05); Smad2 and Smad3 expression levels in endometrial tissue of both groups after treatment were significantly lower than those in intraoperative tissue (P<0.05), and Smad2 and Smad3 expression levels in endometrial tissue of observation group after treatment were significantly lower than those of control group (P<0.05).

Table 2
Smad2 and Smad3 expression levels in endometrial tissue of two groups (ng/mL).

Groups (Case No.)	Treatment situation	Smad2	Smad3
Observation group (n=39)	Intraoperative tissue	67.12±9.33	3.14±0.52
	Tissue after treatment	22.35±3.59△▲	0.92±0.11△▲
Control group (n=39)	Intraoperative tissue	66.46±8.89	3.25±0.28
	Tissue after treatment	40.14±6.83△	1.67±0.23△

△: compared with intraoperative tissue within same group, differences were statistically significant, P<0.05; ▲: compared with control group of tissue at the same point in time, differences were statistically significant, P<0.05.

3.3. Smad downstream molecule expression levels in endometrial tissue

Intraoperative uPA, PAI-1, ADAM15 and ADAM17 expression levels in endometrial tissue of two groups showed no significant difference (P>0.05); uPA expression levels in endometrial tissue of both groups after treatment were significantly higher than those in intraoperative tissue while PAI-1, ADAM15 and ADAM17 expression levels were significantly lower than those in intraoperative tissue (P<0.05), and uPA expression level in endometrial tissue of observation group after treatment was significantly higher than that of control group while PAI-1, ADAM15 and ADAM17 expression levels were significantly lower than those of control group (P<0.05).

3.4. Serum Smad-related cytokine levels

Before operation, serum Smad-related cytokines TGF-β, VEGF, CTGF, IGF-I and TNF-α levels of two groups showed no significant difference (P>0.05); serum TGF-β, VEGF, CTGF, IGF-I and TNF-levels of both groups after treatment were significantly lower than those before operation (P<0.05), and serum TGF-β, VEGF, CTGF, IGF-I and TNF-α levels of observation group after treatment were significantly lower than those of control group (P<0.05).

4. Discussion

The pathogenesis of intrauterine adhesions has not been fully elucidated, the endometrial basal layer damage and difficulty in endometrial regeneration and repair are associated with the formation of adhesions, and excessive deposition of cellular matrix in the process of tissue repair is an important part causing cicatricial adhesion[6,7]. At present, surgical methods are mainly adopted for clinical treatment of intrauterine adhesions, transcervical resection

Table 3
Smad downstream molecule expression levels in endometrial tissue of two groups (pg/mL).

Groups (Case No.)	Treatment situation	uPA	PAI-1	ADAM15	ADAM17
Observation group (n=39)	Intraoperative tissue	103.52±15.52	25.86±3.52	9.22±1.02	17.84±2.68
	Tissue after treatment	234.94±35.86△▲	10.14±1.85△▲	3.04±0.54△▲	5.26±0.75△▲
Control group (n=39)	Intraoperative tissue	105.14±12.95	26.14±2.94	9.51±1.17	18.13±2.93
	Tissue after treatment	164.82±22.48△	17.89±2.19△	5.96±0.76△	9.51±1.18△

△: compared with intraoperative tissue within same group, differences were statistically significant, P<0.05; ▲: compared with control group of tissue at the same point in time, differences were statistically significant, P<0.05.

Table 4
Serum Smad-related cytokine levels of two groups (ng/mL).

Groups (Case No.)	Treatment situation	TGF-β	VEGF	CTGF	IGF-I	TNF-α
Observation group (n=39)	Before operation	9.32±1.05	47.66±6.74	25.42±4.26	17.96±3.15	72.32±9.64
	After treatment	3.62±0.56△▲	19.31±2.67△▲	9.35±1.18△▲	8.32±0.92△▲	24.62±4.67△▲
Control group (n=39)	Before operation	9.61±0.94	48.12±6.96	26.03±4.52	18.52±2.58	73.51±9.14
	After treatment	6.17±0.83△	28.45±4.18△	14.28±1.95△	13.31±1.86△	42.52±7.56△

△: compared with before operation within same group, differences were statistically significant, P<0.05; ▲: compared with control group at the same point in time, differences were statistically significant, P<0.05.

of adhesion can effectively release the adhered uterine tissue and improve adhesion-induced clinical symptoms, and postoperative adjuvant use of estrogen-progestogen can maintain the continuous endometrial proliferation state and promote endometrial repair to a certain extent, thus preventing postoperative intrauterine re-adhesion[8,9]. However, for patients with severe intrauterine adhesions, the incidence of postoperative re-adhesion is high. In recent years, studies have confirmed that low-dose aspirin can improve endometrial growth, and the drug inhibits the activity of cyclooxygenase to adjust the balance of TXA2/PGI2, thus improving the local endometrial blood circulation. In the study, low-dose aspirin was used after transcervical resection of adhesion, and the analysis of the uterine artery and endometrial blood flow parameters confirmed that uterine artery RI and PI of observation group after treatment were significantly lower than those of control group, and endometrial VI, FI and VFI were significantly higher than those of control group. This means that endometrial perfusion is more ideal after low-dose aspirin treatment and conducive to tissue repair after transcervical resection of adhesion.

In the process of endometrial tissue repair, good blood perfusion can create favorable conditions for tissue repair, and endometrial tissue constantly proliferates and completes the repair. The current studies believe that the recurrence of intrauterine adhesions after transcervical resection of adhesion is relate to the imbalance of the extracellular matrix synthesis and degradation, and fibroblast proliferation and excessive extracellular matrix deposition in local tissue can make connective tissue replace the normal endometrial tissue, resulting in adhesion between tissues[10,11]. Smad signaling pathway is an important pathway regulating the synthesis and degradation of extracellular matrix in the body. There are eight Smad signaling molecules in mammals, and it has been proved that Smad2 and Smad3 are closely related to intrauterine adhesions[12]. Existing research shows that Smad2 and Smad3 expression levels significantly increase in intrauterine adhesion tissue and can mediate the deposition of extracellular matrix[13]. In the study, analysis of the expression levels of above two Smad molecules in intrauterine adhesion tissue of two groups proved that Smad2 and Smad3 expression levels in endometrial tissue of observation group after treatment were significantly lower than those of control group. This means the application of low-dose aspirin after transcervical resection of adhesion can inhibit the expression of Smad2 and Smad3, then undermine the extracellular matrix deposition mediated by Smad2 and Smad3 signaling pathways and prevent intrauterine re-adhesion.

The signaling pathways mediated by Smad2 and Smad3 molecules are very complex, and the molecules that are associated with extracellular matrix deposition include a variety of proteases as well as their activated molecules and inhibitory molecules. uPA can activate plasminogen and degrade a variety of extracellular matrix components, and it can also activate a variety of matrix metalloproteinases (MMPs) and degrade extracellular matrix through MMP; PAI-1 is the inhibitory molecule of uPA, and has inhibitory effect on the degradation of extracellular matrix. Smad2 and Smad3 activation can activate the uPA and inhibit PAI-1, thereby promoting the extracellular matrix degradation and inhibiting intrauterine adhesion formation. In addition, Smad2 and Smad3 also have promoting effect on the function of ADAM15 and ADAM17 of ADAM family[14]. In the study, analysis of the expression levels of above Smad-related molecules in adhesion tissue proved that uPA expression level in endometrial tissue of observation group after treatment was significantly higher than that of control group while PAI-1, ADAM15 and ADAM17 expression levels were significantly lower than those of control group. In addition to the above proteases as well as their activated molecules and inhibitory molecules, Smad2 and Smad3 also regulate the synthesis and secretion of a variety of cytokines. TGF-β has promoting effect on fibroblast proliferation and fibronectin transcription, VEGF and CTGF have promoting effect on the growth and deposition of the connective tissue, IGF-I is the cytokine with growth-promoting effect and can increase the secretion of extracellular matrix, and TNF-α can affect endometrial repair through inflammatory response[15-17]. In the study, analysis of serum levels of above cytokines confirmed that serum TGF-β, VEGF, CTGF, IGF-I and TNF-α levels of observation group after treatment were significantly lower than those of control group. Above results indicate that the application of low-dose aspirin after transcervical resection of adhesion can not only directly regulate the expression of Smad2/3, but can also influence the expression of Smad2/3-related molecules.

To sum up, transcervical resection of adhesion combined with low-dose aspirin therapy can improve the postoperative uterine artery and endometrial blood flow state, inhibit extracellular matrix deposition mediated by Smad2/3 signaling pathway and prevent intrauterine re-adhesion in patients with intrauterine adhesions.

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