Effect of hysteroscopic adhesiolysis combined with growth hormone on endometrial blood flow and volume as well as Smad2/3 expression

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Objective: To study the effect of hysteroscopic adhesiolysis combined with growth hormone on endometrial blood flow and volume as well as Smad2/3 expression. Methods: A total of 64 patients with moderate or severe intrauterine adhesions who received hysteroscopic adhesiolysis in our hospital from May 2013 to October 2015 were selected as the research subjects and randomly divided into two groups who received different postoperative drug treatment, observation group received postoperative manual cycle intervention combined with growth hormone treatment and control group only received manual cycle intervention. Transvaginal ultrasonography was conducted after treatment to assess endometrial thickness, volume and blood flow, and endometrium was collected to determine Smad2, Smad3 and TGF-β 1 levels. Results: After treatment, endometrial blood flow signal of observation group was more abundant than that of control group, ultrasound parameters RI and PI were significantly lower than those of control group, and VI, FI and VFI as well as endometrial thickness and endometrial cavity volume were significantly higher than those of control group; Smad2, Smad3 and TGF-β 1 levels in endometrial tissue of observation group after treatment were significantly lower than those of control group. Conclusions: Hysteroscopic adhesiolysis combined with growth hormone therapy can promote endometrial repair and growth, increase endometrial blood flow and volume and also suppress the expression of Smad2/3 and TGF-β 1 in patients with intrauterine adhesions.

1. Introduction

Intrauterine adhesions (IUA), also known as Asherman syndrome, refers to endometrial fibrosis, intrauterine muscle wall and/or cervical canal adhesion caused by endometrial lesions, and can cause functional endometrial decrease as well as menstrual disorders, amenorrhea and infertility. Damage caused by uterine curettage during childbirth as well as myomectomy, polypectomy and other uterine operation is the common causes of IUA[1,2]. Adhesion separation or lysis under hysteroscope is the first choice for clinical treatment of IUA, and although it can effectively separate adhered intrauterine tissue, the postoperative recurrence rate is high and the non-ideal endometrial repair, menstrual disorders, infertility and other symptoms still exist[3,4]. Endometrial repair is the key to prevent adhesion recurrence and improve clinical symptoms after IUA. Growth hormone (GH) is the endocrine hormone that can widely promote growth and promote proliferation, and has promoting effect on endometrial growth[5,6]. In the following study, growth hormone and combined with hysteroscopic adhesiolysis was used to treat IUA, and the endometrial blood flow and volume as well as Smad2/3 expression levels were analyzed.

2. Materials and methods
2.1. Research subjects

A total of 64 patients with moderate or severe IUA who were treated in our hospital from May 2013 to October 2015 were selected as the research subjects, with the chief complaints such as secondary amenorrhea, menstrual blood volume decrease and infertility, and had received artificial abortion, curettage after drug abortion, inducing abortion in second trimester, placenta residue curettage after full-term pregnancy, transcervical resection of septum and other uterine cavity operation. According to the postoperative use of growth hormone, the included patients were divided into observation group and control group (n=32), and the general information was as follows: (1) control group: received hysteroscopic adhesiolysis combined with postoperative manual cycle intervention, were (33.2±5.3) years old, were with BMI (22.9±4.2) kg/m$^2$, and included 18 moderate cases and 14 severe cases; (2) observation group: received hysteroscopic adhesiolysis combined with postoperative manual cycle and growth hormone intervention, were (32.8±5.6) years old, were with BMI (22.5±4.1) kg/m$^2$, and included 19 moderate cases and 13 severe cases. The two groups of patients showed no significantly difference in general data.

2.2. Operation methods

Patients orally took polyethylene glycol electrolyte powder before operation for bowel preparation and were fasting for solids and liquids for 8-12 h, and those with decreased menstrual blood volume needed to receive surgery 3-7 d after menstruation was clean. The anesthesia method was intravenous anesthesia, distention medium was normal saline, flow rate was 250 mL/min, intraoperative distention was maintained in 16 kPa, partial adhesive band was incised at first to increase intrauterine volume and then observe the intrauterine shape and adhesion, mild membranous adhesion was bluntly separated through the distention pressure, muscular adhesion was incised by monopolar needle electrode, trying to ensure that the separation of adhesions was at the same cross line level, and after separation of adhesions, one No. 21 metal ring and one No. 8 Foley catheter were indwelled, and 4-6 mL of normal saline was injected in the sac.

2.3. Postoperative drug treatment

Control group conventionally received antibiotics for 2-3 d after operation, No. 8 Foley catheter was indwelled for 5 d, and manual cycle intervention was conducted according to the following methods: patients received oral administration of estradiol valerate 9 mg/d from that very day after operation for consecutive 21 d, received oral administration of medroxyprogesterone 10 mg/d on the last 10 d, received the medication again from the 5 d after menses according to the above methods, and received a total of three cycles of treatment. The usage of antibiotics, No. 8 Foley catheter indwelling and the manual cycle intervention of observation group were the same as those of control group, they also received growth hormone, and the method was as follows: they received subcutaneous injection of recombinant human growth hormone 4 U from that very day after operation for consecutive 5 d.

2.4. Ultrasonic evaluation of endometrial blood flow and volume

Transvaginal three-dimensional ultrasonography was conducted on the 10-15 d of the third menstrual cycle after treatment, probe frequency was 3.5-14.5 MHz, the endometrial ultrasound images were obtained at first to measure the endometrial thickness and volume, and then endometrial blood flow parameters were measured, including vascularization index (VI), flow index (FI) and vascularization flow index (VFI); the direction of probe was adjusted to obtained uterine artery images and measure blood flow resistance index (RI) and pulsatility index (PI).

2.5. Assessment of Smad2/3 expression levels

During operation as well as the 10-15 d of the third menstrual cycle after treatment, a moderate amount of endometrial tissue was obtained, added to PBS and grinded, the grinded suspension was centrifuged in the low-temperature centrifugal machine for 20 min at a speed of 12 000 r/min, and the supernatant was collected and stored in a -70°C refrigerator for test. After the specimen acquisition from all cases, enzyme-linked immunosorbent assay kits were used to determine Smad2, Smad3 and transforming growth factor-β (TGF-β) 1 in all supernatant samples, and all the steps were conducted according to the kit instruction.

2.6. Statistical methods

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

3. Results

3.1. Ultrasonic evaluation results of endometrial blood flow and volume

RI and PI of observation group after treatment were significantly
lower than those of control group, and VI, FI and VFI were significantly higher than those of control group, shown in Table 1. Analysis of endometrial volume parameters was as follows: endometrial thickness and endometrial cavity volume of observation group after treatment were significantly higher than those of control group, shown in Table 2.

### Table 2
Endometrial volume parameters of two groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>Endometrial thickness (mm)</th>
<th>Endometrial cavity volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observation</td>
<td>32</td>
<td>6.71±0.89</td>
<td>3.77±0.65</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>5.98±0.77</td>
<td>2.54±0.41</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>5.582</td>
<td>7.548</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

### 3.2. Smad2/3 expression levels

There were no significant differences in Smad2, Smad3 and TGF-β1 levels in intraoperatively removed endometrial tissue of observation group and control group; Smad2, Smad3 and TGF-β1 levels in endometrial tissue of observation group after treatment were significantly lower than those of control group, shown in Table 3.

### Table 3
Smad2, Smad3 and TGF-β1 expression levels of two groups (ng/mL).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Case No.</th>
<th>Intraoperative tissue</th>
<th>Postoperative tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Smad2</td>
<td>Smad3</td>
</tr>
<tr>
<td>Observation</td>
<td>32</td>
<td>52.34±8.41</td>
<td>1.96±0.22</td>
</tr>
<tr>
<td>Control</td>
<td>32</td>
<td>54.12±7.81</td>
<td>2.04±0.28</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>0.582</td>
<td>0.274</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&gt;0.05</td>
<td>&gt;0.05</td>
</tr>
</tbody>
</table>

### 4. Discussion

Endometrial repair is the key to prevent the recurrence of IUA after hysteroscopic adhesiolysis[7]. At present, the common clinical therapeutic regimen after hysteroscopic adhesiolysis is exogenous supplementation of estrogen and progestogen, and its purpose is to promote endometrial hyperplasia and adjust menstrual cycle. The continuous use of high doses of estrogen the first 10 days after operation can promote endometrial hyperplasia and suppress endometrial transition to secretory stage, which ensures that the endometrium is continuously in hyperplasia period, promotes endometrial repair and prevents the happening of intrauterine adhesion again[8,9]. Even so, the postoperative recurrence rate is still high in patients with moderate-severe intrauterine adhesions, which causes adverse effects on both quality of life and life health of the patients. Growth hormone is a kind of endocrine hormone that can extensively promote growth and proliferation, and it can act on endometrial tissue and then induce granulosa cells to secrete estrogen and progestogen and promote endometrial growth[10,11]. At present, the reports about the application of growth hormone after hysteroscopic adhesiolysis are not much, and the value of growth hormone for preventing the recurrence of IUA is not very clear. Hu et al[12] reports the preventive effect of postoperative growth hormone application on postoperative recurrence of hysteroscopic adhesiolysis, and it is believed that the growth hormone has promoting effect on endometrial repair of hysteroscopic adhesiolysis, and can increase the endometrial thickness. The analysis results of the endometrial thickness in the above study were consistent with the research of Hu et al[12], and the postoperative endometrial thickness of observation group who received growth hormone therapy increased significantly. In addition to endometrial thinning, there are also the changes of overall intrauterine shape and the decrease of endometrial cavity volume in IUA patients, and the wider the adhesion range and the more severe the degree in patients, the more significant the decrease of endometrial cavity volume[13,14]. In the study, endometrial cavity volume of two groups was analyzed after treatment, and the endometrial cavity volume of observation group after treatment was significantly higher than that of control group. This means that application of growth hormone after hysteroscopic adhesiolysis can effectively promote the endometrial repair, characterized by the increase of endometrial thickness and enlargement of endometrial cavity volume. There are also the local blood flow features of reduced blood flow, increased vascular resistance and insufficient vascularization degree in patients with intrauterine adhesions and repeated failure of assisted reproduction[15]. In the study, the analysis of blood flow parameters confirmed that RI and PI of observation group after treatment were...
significantly lower than those of control group, and VI, FI and VFI were significantly higher than those of control group. This means that the application of growth hormone after hysteroscopic adhesiolysis can effectively improve the endometrial blood flow state and promote the endometrial growth of and repair.

Studies about the IUA-related molecular mechanisms in recent years have confirmed that endometrial fibrosis under the action of a variety of molecules is an important part causing intrauterine adhesions. TGF-β is the promoter of tissue fibrosis in the body and includes three isomers: TGF-β1, TGF-β2 and TGF-β3, and the TGF-β1 activity is the strongest[16,17]. TGF-β1 can achieve the chemotaxis of fibroblasts and act on receptors on the cell membrane, and then conduct signal transduction through the downstream molecules Smad2 and Smad3, stimulate fibroblasts to secrete matrix proteins such as collagen and laminin, increase the deposition of extracellular matrix and eventually form tissue fibrosis. Study has shown that the expression levels of TGF-β1 as well as Smad2 and Smad3 increase significantly in endometrial tissue of patients with IUA[18]. In the study, analysis of the expression levels of TGF-β1 as well as Smad2 and Smad3 in endometrial tissue after drug treatment proved that Smad2, Smad3 and TGF-β1 levels in endometrial tissue of observation group after treatment were significantly lower than those of control group. This means that the application of growth hormone after hysteroscopic adhesiolysis can inhibit TGF-β1-mediated Smad2/3 pathway, thereby inhibiting extracellular matrix deposition and tissue fibrosis.

To sum up, hysteroscopic adhesiolysis combined with growth hormone therapy can promote endometrial repair and growth, increase endometrial blood flow and volume and also suppress the expression of Smad2/3 and TGF-β1 in patients with IUA.

References


