

Impact and clinical significance of Embosphere microsphere artery embolization therapy in serum VEGF expression level of women patients with uterine fibroids

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Abstract. – OBJECTIVE: We aimed to investigate the effect of Embosphere microsphere artery embolization on the serum level of vascular endothelial growth factor (VEGF) in patients with uterine fibroid.

PATIENTS AND METHODS: From March 2014 to December 2015, 128 women in child-bearing age with uterine intramural fibroids were enrolled in the patient group. At the same time, 128 healthy cases in child-bearing age were randomly selected and enrolled in the control group. Enzyme-linked immunosorbent assay was used to measure the serum level of VEGF, and immunohistochemical staining method was used to study the expression of VEGF in the uterine fibroids. Embosphere microsphere artery embolization surgery was performed on cases in the patient group.

RESULTS: The serum level of VEGF in the patient group was significantly higher than that of the control group. Immunohistochemical staining results showed that in the control group, VEGF expression level in uterine fibroid tissue was significantly higher. Compared with before the treatment, tumor diameter in the patient group reduced significantly 3 months after the treatment. Erythrocyte count, hemoglobin, and menstrual blood volume increased significantly 6 months after treatment.

CONCLUSIONS: Serum VEGF level can be considered as a marker for uterine fibroid, and by using VEGF as a marker we can increase the probability of early diagnosis. We showed that, compared with hysterectomy, Embosphere microsphere embolization had an evident advantage and might be an excellent candidate to replace hysterectomy.

Key Words:

Embosphere microsphere artery embolization, Uterine fibroids, Uterine artery embolization, VEGF.

Introduction

The uterine fibroids are common tumors in female reproductive system. In China, the incidence rate of uterine fibroids in women in child-bearing age is about 20%^{1,2}. Prior studies showed that in 40 % to 60 % of cases, problems such as anemia and menstrual pain associated with uterine fibroid disappeared after complete hysterectomy^{3,4}. However, with the improvement of people's life quality, more and more uterine fibroid patients are eager to reserve their uterus. The early-stage uterine fibroids may be cured by arterial embolization. Vascular endothelial growth factor (VEGF) is one of the most important regulatory factors involve in promoting tumor growth and transfer^{5,6}. Results obtained from previous studies showed that VEGF expression levels were different in various tumors⁷⁻⁹. In the past, Embosphere microsphere artery embolization was usually used for liver cancer treatment and especially for cutting large tissue volumes¹⁰. The artificial embolus made by microsphere technique is considered a proper method to cut the blood flow to the tumor^{11,12}. We, in this study, investigated the effect of Embosphere microsphere artery embolization on the expression level of VEGF in uterine fibroid patients.

Patients and Methods

Patients

From March 2014 to December 2015, 128 women in child-bearing age who were treated in the Gynecology Department of our Hospital for uterine intramural fibroids were enrolled in the

patient group. At the same time, 128 healthy cases in child-bearing age were randomly selected and enrolled in the control group. The average age in the patient group was (37.3 ± 2.5) years. Average tumor diameter in the patient group was (45.8 ± 12.4) mm. Differences between two groups' baseline data had no statistical significance ($p > 0.05$). (Table I). This study was approved by the ethics committee of Women and Infants Hospital of Zhengzhou. Signed written informed consents were obtained from all participants before the study.

Inclusion Criteria

(1) All patients had clear clinical symptoms, such as abdominal pain, menostaxis, increased menstrual volume and anemia; (2) Diameter of solitary tumor was above 3 cm; (3) All tumors were uterine intramural fibroids; (4) Patients' general condition was good, and they did not have any contraindications to interventional therapy; (5) Patients agreed on interventional therapy personally.

Exclusion Criteria

(1) Pregnant patients; (2) Patients with bleeding and those with abnormal blood coagulation mechanism; (3) Patients who were in the period of menstruation; (4) Patients with acute or chronic bacterial and/or viral infections and those with autoimmune disease; (5) Patients with connective tissue diseases; (6) Patients with malignant reproductive system tumor; (7) Patients with hepatic function insufficiency; (8) Patients with chronic muscle diseases; (9) Patients allergic to imaging reagents; (10) Patients with peripheral vascular disease, thyroid disease and those suffered from major trauma or underwent surgery within 6 months prior to their enrollment; (11) Patients who underwent myocardial infarction surgeries, percutaneous transluminal coronary angioplasty, coronary artery bypass grafting within 6 months prior to their enrollment; (12) Patients who were taking adrenocortical hormone or other

immunomodulator medications; (13) Patients with psychiatric history and those who were not cooperative.

Reagents and Materials

We used VEGF ELISA kit (Elisa Biotech Company, Shanghai, China) and 0.9 % of sterile saline solution (Otsuka Pharmaceutical, Tokyo, Japan). The diameter of microsphere made by Embosphere Company was 300-500 pm, and 5-10 mL of microsphere was used according to the tumor size. Developing reagent iopromide (Ultravist 300). Taq Master Mix (Sino Biological, Beijing, China), agarose (Bio-west, Nuaille, France), sterile double distilled water, anti-phosphorylated VEGF (p-VEGF) (1:1.000; Cell Signaling Technology, Danvers, MA, USA), β -actin antibody (1:5.000; Invitrogen, Carlsbad, CA, USA), phosphorylated VEGF (p-VEGF1 antibody 1:1.000; Cell Signaling Technology, Danvers, MA, USA). 0.9% of sterile saline solution (Otsuka Pharmaceutical, Tokyo, Japan), Trizol (Invitrogen, Carlsbad, CA, USA). We also used PCR amplification instrument (Bio-Rad, Hercules, CA, USA), vilber lourmat (Bio-Rad, Hercules, CA, USA), electrophoresis apparatus (Beijing Liuyi Instrument Factory, Beijing, China), centrifugal machine (Eppendorf, Hamburg, Germany), micropipette (Eppendorf, Hamburg, Germany), Haier ice machine, Western blot electrophoresis apparatus (Bio-Rad, Hercules, CA, USA), -80°C refrigerator (Thermo-Fisher Scientific, Waltham, MA, USA), 10 ml injector, 5 ml injector (Hanaco, Tianjin), NanoDrop 2000 photometric analyzer (Thermo-Fisher Scientific, Waltham, MA, USA), EP tube (Eppendorf, Hamburg, Germany). Digital subtraction apparatus made by General Electric Company (GE, Little Chalfont, Buckinghamshire, UK); Pathological microtome (Leica, Wetzlar, Germany).

Enzyme-linked Immunosorbent Assay

Enzyme-linked immunosorbent assay was used to measure the serum level of VEGF.

Table I. General clinical data of enrolled patients.

	Age (year)	Disease course (year)	Tumor diameter (cm)	BMI (kg/m^2)
Patient group	39.3 ± 5.5	1.29 ± 0.14	8.8 ± 2.4	18.8 ± 1.2
Healthy control group	38.8 ± 4.7	–	–	19.27 ± 0.87
<i>t</i> -value	0.98	–	–	0.87
<i>p</i> -value	0.192	–	–	0.382

EDTA (Bio-Sharp, Hefei, China) was used as the anticoagulant, and blood samples were centrifuged at 2000 rpm for 30 min at 2-8°C. For ELISA assay we closely followed that instruction provided by kits' manufacturer (Beijing Kang Century Co., Ltd., Beijing, China). A detailed protocol for Enzyme-linked immunosorbent assay was according to a previous report¹³.

Immunohistochemical Staining Method

To study the expression of VEGF in uterine fibroid cells, we used immunohistochemical staining. Paraffin uterine fibroids sections were prepared, and 50 µl I-anti rabbit anti-human VEGF-antibody (Abcam, Cambridge, MA, USA) were added to each sample. Samples were placed at room temperature for 1 h followed by addition of DAB coloration (Invitrogen, Carlsbad, CA, USA). After 5 to 10 min, samples were studied under a microscope and, if the cytoplasm was brown, we stopped the reaction

and washed the sample under the running water for 10 min. Next, we added the hematoxylin for 2 min. A detailed protocol for immunohistochemical staining of VEGF was according to a previous report¹⁴.

Embosphere Microsphere Artery Embolization

Patients underwent surgery within 5 to 12 days after the end of their menstruation. Sindinger was used to puncture the right femoral artery, and 4F or 5F Cobra tube was put into the uterine artery under X-ray angiography guidance. The angiography was conducted to confirm the position, size and the blood supply of uterine fibroid. The embolization agent was slowly injected, and the injection was stopped when the contrast agent stranded in the tumor. Embolization agent was injected again until the disappearance of stain in the tumor. The tube was removed after surgery, and local compres-

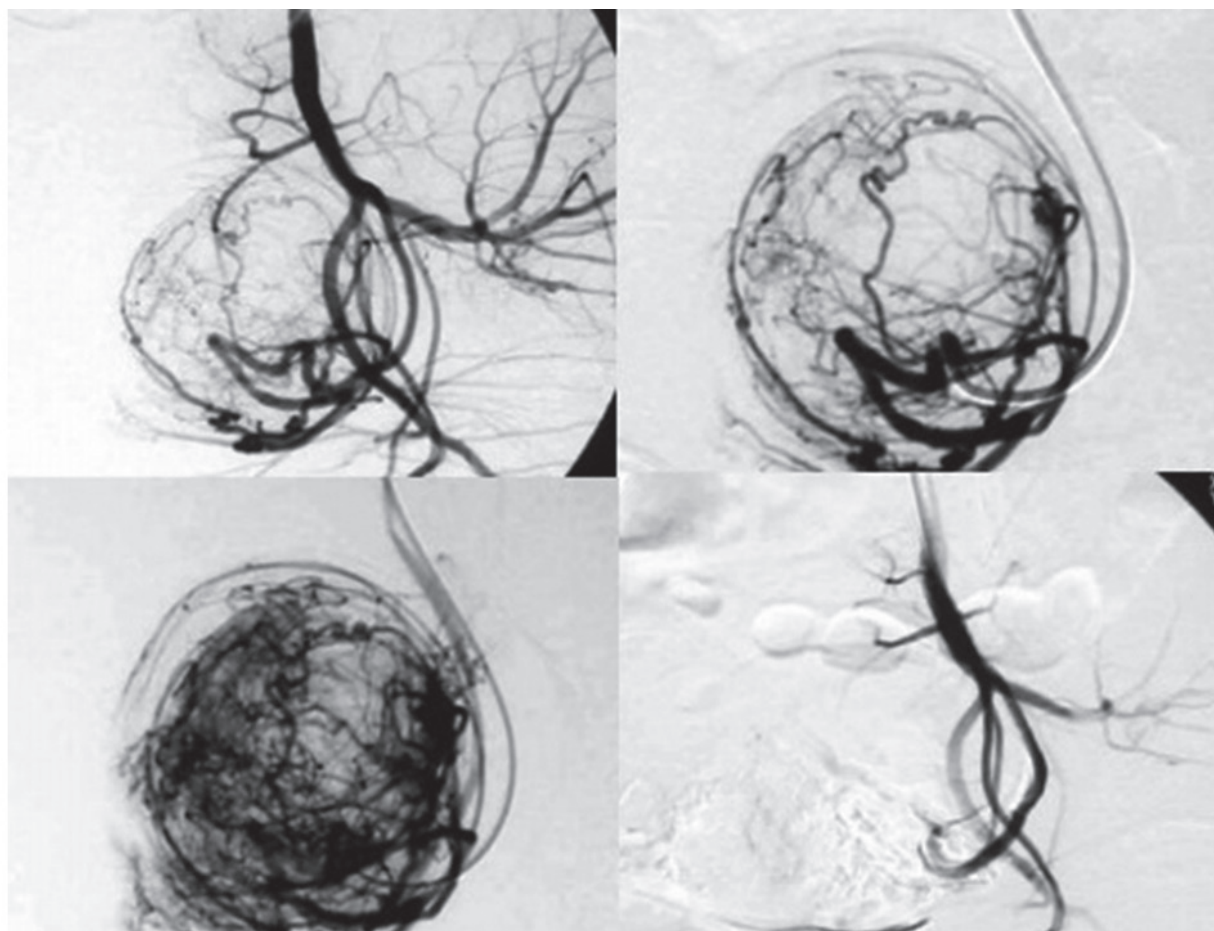


Figure 1. A-C, Uterine artery angiography. D, Uterine artery embolization.

sion was adopted for hemostasis. Patients received 20 min of pressure dressing, and stayed in bed for 24 h, and the symptomatic treatment was provided (Figure 1). Detailed steps for Embosphere microsphere artery embolization was according to a previous report¹⁴.

Complete Hysterectomy

Median incision of lower abdomen was conducted, and using hemostatic forceps we clamped two horns of the uterus. Line 7 was used to stitch round ligament about 2 two 3 cm away from the uterine horn, to stitch pelvic ligament doubly in the position a little far away from the pelvic wall. There were ovarian artery and venous plexus going through the pelvic ligament. The ligament and uterine bladder peritoneal were cut and reversed. Uterus and suture line were lifted up, and the infundibulopelvic ligament and round ligament were cut to block uterus blood flow in uterine horn. The anterior lobe of broad ligament between the infundibulopelvic ligament and round ligament was cut and reversed to the opposite sides. We used a finger to slowly separate the bladder downward along the surface of loose connective tissue between uterus and bladder to make part of the cervix visible. Next, we separated tissues on both sides of the bladder to make the vein and the artery visible. The posterior lobe of broad ligament on both sides of the uterine body was cut to the topside of the uterine artery. Uterus was lifted up, and the anterior fornix of the vagina was cut. Forceps was used to clamp and lift the anterior vaginal wall up, and a piece of gauze was stuffed in the incision to prevent the vaginal fluid flowing into the pelvic cavity. Forceps was used to clamp and lift the labium anterius, and the labium anterius was cut along the vaginal vault, and the uterus was removed. Vaginal cuff and pelvic peritoneum were stitched. Detailed surgical steps for complete hysterectomy was according to a previous report¹⁵.

Statistical Analysis

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Variance analysis (ANOVA) test followed by the post-hoc test (Least Significant Difference) and χ^2 -test, were used for normally distributed data. We used Fisher exact probability test for fourfold table data of unqualified items. Paired t -test or χ^2 -test was used for comparison between non-normally distributed data. $p < 0.05$ meant that the difference was statistically significant.

Table II. Serum VEGF level in both groups.

Groups	Cases	VEGF (ng/ml)
Patient group	128	28.87
Healthy control group	128	2.35
<i>F</i> -value	–	22.48
<i>p</i> -value	–	0.005

Results

Serum VEGF Expression Levels

Results obtained from ELISA tests showed that the serum level of VEGF in the patient group was significantly higher than that of the healthy control group. The difference was statistically significant ($p < 0.05$) (Table II and Figure 2).

Immunohistochemical staining results showed that in the control group, VEGF expression level in uterine fibroid tissue was significantly higher, and the difference was statistically significant ($p < 0.05$) (Figure 3A-B and Figure 4 C).

Changes in Uterine Fibroid Volume and Serum VEGF Expression Level

Compared with before the treatment, tumor diameter in the patient group reduced significantly 3 months after the treatment. The difference was statistically significant ($p < 0.05$) (Table III and Figure 4). We followed up the cases in the patient group for 3 to 6 months in order to verify and study their VEGF expression levels. We dis-

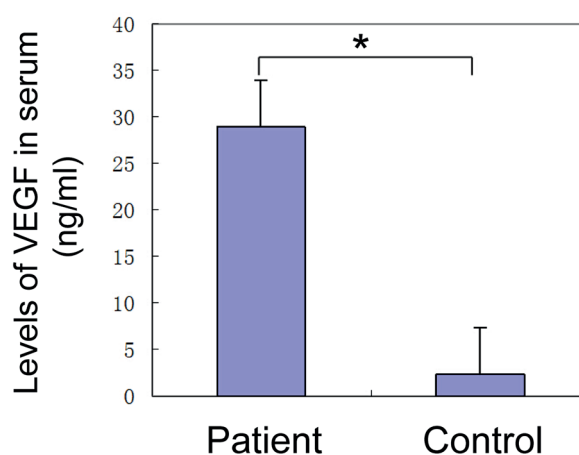


Figure 2. The serum level of VEGF in the healthy control group was significantly lower than the patient group.

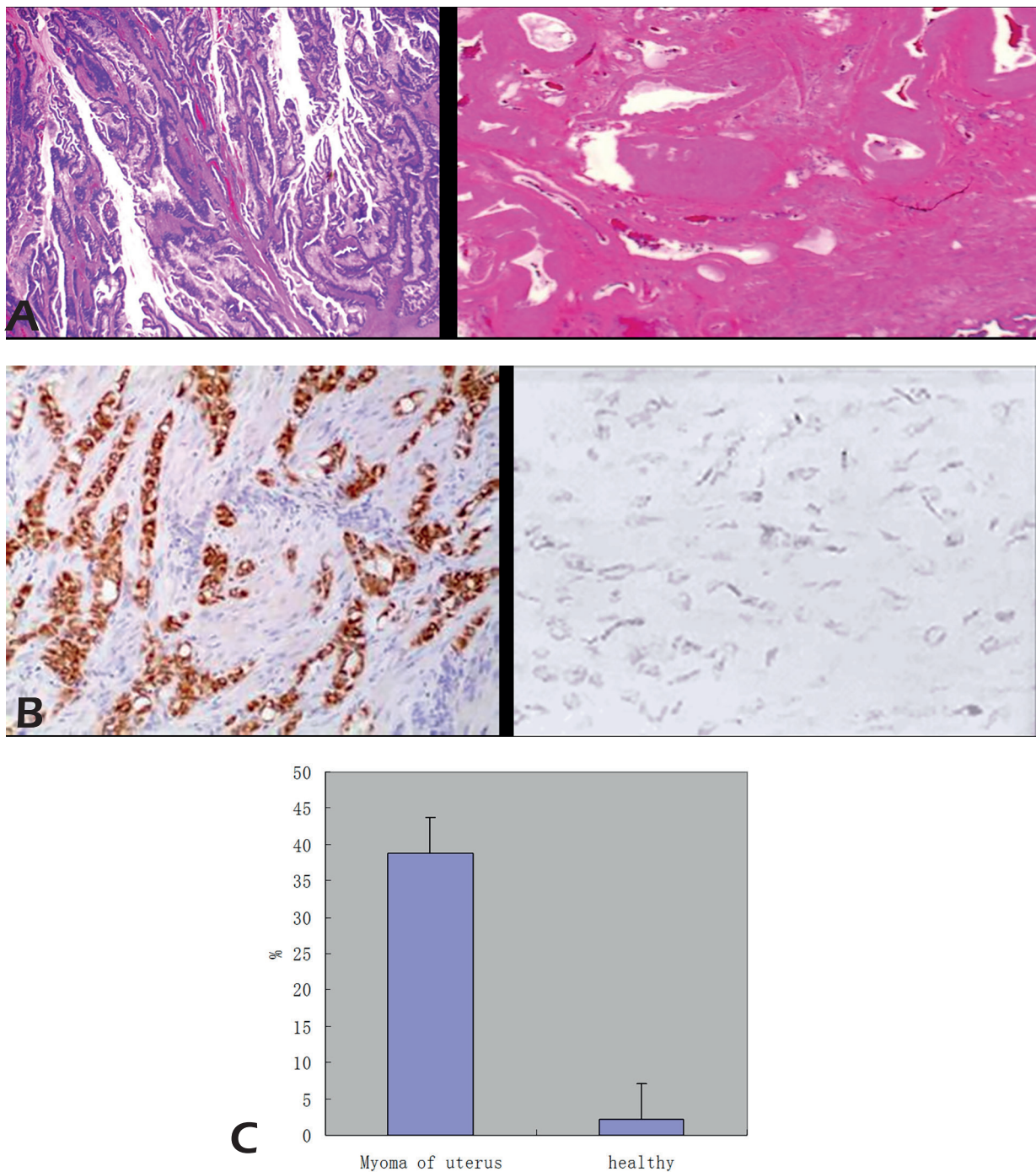


Figure 3. *A*, HE staining (200X). Left: uterine fibroid tissue. Right: peritumor normal tissue. *B*, HE staining (200X). Left: uterine fibroid tissue VEGF immunohistochemical staining. Right: peritumor normal tissue TGF immunohistochemical staining. *C*, VEGF expression level in uterine fibroid tissue and peritumor normal tissue.

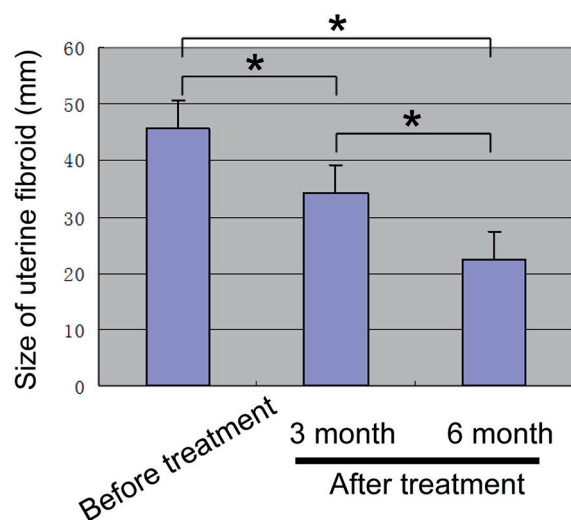
covered that compared with before the treatment, VEGF expression level declined significantly 3 months after treatment. The difference was statistically significant ($p < 0.05$) (Table IV and Figure 5).

Comparison of Patients' Postoperative Clinical Effects

General indexes were compared before and after the operation. These indexes included erythrocyte count, hemoglobin, menstrual blood volume, ane-

Table III. Changes in uterine fibroid diameter diagnosed by ultrasonic before and after the treatment.

Groups	Cases	Before the treatment (mm)	Within 3 months after the treatment (mm)	Within 6 months after the treatment (mm)	F-value	p-value
Patient group	312	45.8 ± 12.4	34.28 ± 7.87	22.49 ± 4.36	22.87	< 0.001

**Figure 4.** Changes in uterine fibroid volume of patients upon ultrasonic diagnosis before and after the treatment. The diameter of tumor significantly decreased within three months after the treatment.

mia, and dysmenorrhea. Erythrocyte count, hemoglobin, and menstrual blood volume increased significantly 6 months after treatment, and the difference was statistically significant ($p < 0.05$). The population of dysmenorrhea was significantly lower than that before the treatment, and the difference was statistically significant (Tables V and VI).

Discussion

Currently, the uterine fibroids are mainly treated by complete hysterectomy and hysteromyomectomy^{1,3,16,17}. Hormonal regulation and mini-

mally invasive surgeries can be used to suppress tumor growth and to reach the goal for uterine fibroids palliative treatment. Serum level of VEGF can reflect tumor's growth and metastasis¹⁶. Prior studies¹⁸ showed that cucurbitacin B suppressed breast cancer metastasis and angiogenesis by inhibiting FAK/MMP-9 signal path induced by having an effect on endothelial growth factor. In patients with local advanced rectal cancer, KRAS mutation and high expression of VEGF are usually happen at the same time¹⁸. Some scholars¹⁹ believe that VEGF may have a dual function in the tumor, where on the one hand, it promotes the tumor cells proliferation, and on the other hand, suppresses tumor cells proliferation. In the present study, we discovered that the serum level of VEGF in uterine fibroid patients was significantly higher than the control group. This suggested that the increase in serum VEGF level is one of the main sources of uterine fibroid continuous growth, menometrorrhagia, anemia, menostaxis and vaginal irregular bleeding. Embosphere microsphere artery embolization therapy is a minimally invasive method of therapy for these patients that can preserve the uterus. Embosphere microsphere artery embolization's embolus is usually controllable²⁰. We showed that Embosphere microsphere artery embolization generated satisfactory results for larger tumors. However, there was no significant difference between the clinical effect of Embosphere microsphere artery embolization therapy and hysterectomy. Nevertheless, anemia and dysmenorrhea improved after the operation. Embosphere microsphere artery embolization demonstrated

Table IV. Changes in uterine fibroid diameter diagnosed by ultrasonic before and after the treatment.

Groups	Cases	Before the treatment	Within 3 months after the treatment	Within 6 months after the treatment	F-value	p-value
Experimental group	64	28.87	17.63	11.26	13.41	< 0.01
Control group	64	26.27	16.35	10.49	12.24	< 0.01
t-value	-	0.33	0.29	0.35	-	-
p-value	-	0.68	0.77	0.62	-	-

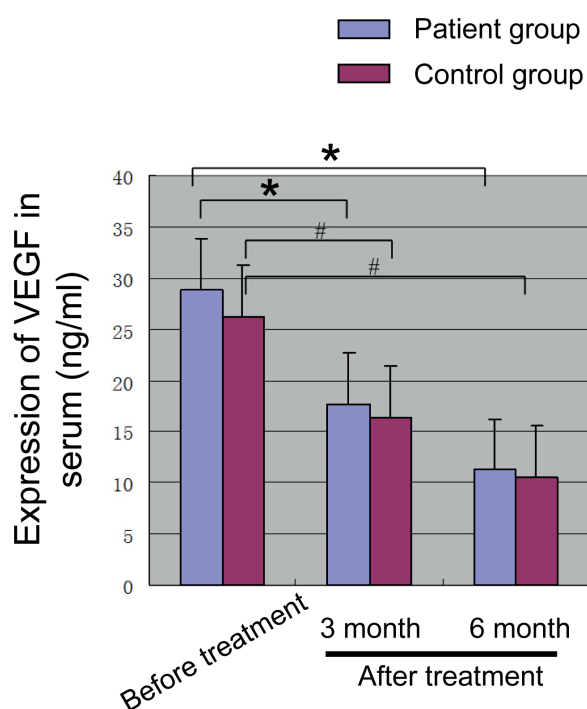


Figure 5. Changes in patients' serum VEGF expression level before and after treatment (ng/ml).

a better clinical effect in curing uterine fibroids. Our results showed that 3 months after the operation, uterine fibroid diameter shrank considerably. Probably, Embosphere microsphere artery embolization, cut uterine fibroid's main blood supply, and negatively affect fibroids' growth rate. Some parts of uterine fibroids might even suffer from necrosis and atrophy. However, this needs to be studied further. Our findings also demonstrated that, compared with before treatment, serum VEGF level declined significantly after treatment. However, compared with the VEGF level in the control group, the difference had no statistical significance ($p > 0.05$). We discovered that VEGF expression level increased in uterine fibroid tissue, which was closely related to the vigorous growth of the tumor. Most of the cancer cells can proliferate by increasing the glycolysis rate and lactic acid fermentation rate under the anaerobic condition (Warburg effect). Glycolysis rate and lactic acid fermentation rate of tumor cells may also increase under aerobic condition (aerobic glycolysis)¹⁹. Anionic metabolism can activate the HIF-1-VEGF signal pathway

Table V. Comparison of patients' postoperative clinical effects.

Items	Groups	Before the treatment	Within 3 months after the treatment	Within 6 months after the treatment	F-value	p-value
Erythrocyte number ($\times 10^9$)	Experimental group	3.18 \pm 1.21	3.72 \pm 0.89	4.31 \pm 0.27	1.28	0.031
	Control group	3.28 \pm 0.87	3.92 \pm 0.74	4.68 \pm 0.39	2.19	0.028
	t-value	1.20	1.33	0.27	—	—
	p-value	0.32	0.48	0.72	—	—
Hemoglobin (g/L)	Experimental group	85.6 \pm 7.12	91.7 \pm 9.04	111.4 \pm 3.81	3.31	0.018
	Control group	56.3 \pm 7.21	93.2 \pm 3.21	113.2 \pm 1.82	1.25	0.024
	t-value	0.38	0.22	0.18	—	—
	p-value	0.32	0.55	0.81	—	—
Menstrual blood volume (ml)	Experimental group	214.3 \pm 38.12	187.3 \pm 12.6	122.7 \pm 2.9	2.81	0.021
	Control group	238.4 \pm 12.6	186.2 \pm 18.7	129.3 \pm 3.8	1.92	0.042
	t-value	0.22	0.18	0.91	—	—
	p-value	0.59	0.93	0.17	—	—

Table VI. Improvement condition of enrolled patients' anemia and dysmenorrhea.

Items		Experimental group	Control group
Dysmenorrhea	Improved	55	52
	Unimproved	9	12
	χ^2 -value	0.36	
	p-value	0.281	
Anemia	Improved	50	58
	Unimproved	14	6
	χ^2 -value	0.72	
	p-value	0.483	

in healthy people, causing an increase in VEGF expression level, thus promoting the angiogenesis around the tumor tissues^{5,12,20-22}. With the growth of uterine fibroids, the number of estrogen and progesterone receptors increases, and as a result estrogen and androgen balance can be disturbed and the patient may suffer from different degrees of irregular menstruation. Embosphere microsphere artery embolization therapy significantly reduces the blood supply of the whole uterus, and in this way, a part of tumor cells dies, and VEGF level drops significantly. Embosphere microsphere artery embolization therapy not only improved clinical symptoms, but also adjusted endocrine disorders caused by the tumor. When compared with the traditional hysterectomy, it has a more evident advantage. However, this method of therapy has some limitations. There is evidence showing that the use of Embosphere microsphere artery embolization for the treatment of liver cancer with colon metastasis, can increase the risk of tumor lysis and the release of cancer cells to enterocoelia¹¹.

Conclusions

Serum VEGF level can be considered a valid marker for uterine fibroid, and by using VEGF as a marker, we can increase the chance of early diagnosis. We showed that, compared with traditional hysterectomy, Embosphere microsphere embolization had a clear advantage and had the potential to replace hysterectomy.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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