The expression of plasma lysophosphatidic acid in patients with epithelial ovarian cancer at advanced stage before and after interventional therapy

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Abstract: The objective is to evaluate the clinical effect of artery chemotherapy infusion and embolism in patients with epithelial ovarian cancer at an advanced stage by investigating the expression of plasma lysophosphatidic acid(LPA) and the tumor volume before and after the interventional therapy. Uterine artery and ovarian artery chemotherapy infusion and embolism were performed on 22 patients with epithelial ovarian cancer at an advanced stage. The level of plasma LPA in patients with benign epithelial ovarian tumor (22 cases) and epithelial ovarian cancer (22 cases, before and after interventional therapy)was measured by biochemical method. At the same time, the tumor volume of patients with epithelial ovarian cancer was observed both before and after interventional therapy. The Results were that the average levels of plasma LPA of 20 benign cases were within the normal range. The average levels of plasma LPA in patients with epithelial ovarian cancer at an advanced stage were significantly higher than the levels in the benign cases($P<0.05$). Following interventional therapy, the levels of plasma LPA and the tumor volume both declined, albeit at different degrees. There was also a significant difference between the levels before and after interventional therapy($P<0.05$). So we found that the application of artery chemotherapy infusion and embolism can decrease the levels of plasma LPA, reduce the volume of the tumor, and improve the resection rate for the patients with epithelial ovarian cancer at an advanced stage. Interventional therapy is an effective method to treat epithelial ovarian cancer at an advanced stage.

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【**Key words**】interventional chemotherapy infusion and embolism; advanced stage epithelial ovarian cancer; lysophosphatidic acid; tumor volume

1. Introduction

Ovarian cancer is a common type of gynecologic malignant tumor. Because of the position of the ovaries deep within the pelvis and because of a lack of early symptoms, ovarian cancer has the highest mortality rates of any gynecological malignancies. Approximately 75-80% of patients receive a late diagnosis, and the 5-year survival rate is below 30%. The related antigen CA125 has been used clinically to detect the biological index of ovarian cancer for many years. However, its sensitivity and specificity are low (72% and 73%, respectively) [1]. Lysophosphatidic acid (LPA) is a cell membrane lipid derivative, which is significantly elevated in the plasma and ascites of patients with ovarian cancer, This rise in LPA levels seems to be specific for ovarian cancer since a similar increase is not seen in patients with other cancer types, such as leukemia and breast cancer [2]. Eder et al reported that LPA levels produced by normal ovarian epithelial cells is far below the levels produced by ovarian epithelial cancer cells [3]. Additionally, LPA can stimulate the cancer cells to produce more LPA, thus forming an autocrine loop[4].The use of LPA levels to diagnose ovarian cancer provides greater sensitivity and specificity compared to the common clinical tumor marker CA125.In vitro studies show that LPA can restrain ovarian tumor cell proliferation and promote apoptosis [5]. Therefore, it is inferred that LPA is related to the occurrence, development, infiltration, and transfer of ovarian cancer [6]. This study explores the plasma LPA levels and the tumor volume changes of late stage epithelial ovarian cancer patients before and after interventional treatments. The goal is to understand the therapeutic value of interventional therapy for late stage epithelial ovarian cancer.

2.Materials and methods

1. Case choice: We selected 42 patients with epithelial ovarian cancer for our study. Patients were recruited between October 2008 and December 2011 from the first affiliated hospital of Zhengzhou

University. 22 patients had malignant disease(15 cases of serous cystadenocarcinoma and 7 cases of mucinous adenocarcinoma of the capsule) and were classified as Ⅲ ~ Ⅳ period patients. 20 patients had benign cases. These included 14 cases of serous cystadenoma, 5 cases of mucinous cystadenoma, and 1 case of an ovaries endomembrane tumor. All patients between the ages of 38 and 70 years old were seeing the doctor for the first time. Benign cases were separated from both ovarian tumors and from the one disaster side ovariectomy. Malignant cases were characterized by adhesion to surrounding organs. Thus, there was poor mobility within the pelvic cavity, and abdominal transfer was also exist. All cases underwent CT or ultrasound examination, which was better able to identify cases of ovarian cancer. Because of the specific condition of these patients, doctors were unable to perform surgery. Instead, they tried to reduce and destroy tumor cells via intervention treatment. This helped to cure epithelial ovarian cancer cases.

2. Methods

2.1 LPA determination: We drew approximately 4ml of venous blood from fasting patients with benign ovarian epithelial neoplasms. Blood was drawn from patients both before and after intervention treatment and also 15-20 days before surgery. According to the LPA determination protocol, we placed blood samples in anticoagulation tubes. Samples were centrifuged for 10 min at 8000 r/min. From 1ml of the supernatant, we extracted phospholipids. The phospholipid composition was also enriched and separated. As a final step, samples were placed in a 90℃ water bath for 5min after adding a specific color-developing agent. After 35min from removing samples from the greenhouse, we measured the samples colors at a wavelength of 636nm, The LPA reagent was produced by Beijing's technology development corporation by following the biochemical method. All readings were taken on the 722 spectrophotometer instrument. Reference range: normal < 2.9 u mol/L, critical value 2.9 ~ 3.2 u mol/L, abnormal > 3.2 u mol/L.

2. 2 Malignant cases were performed right femoral artery puncturing. A 5F Pigtail catheter was placed by using Seldingers technology. We then performed abdominal aortography at the 12 thoracic vertebrae level in order to observe the blood supply of the arteria ovarica or inferior mesenteric artery. We also determined the position of the bilateral iliac artery bifurcate and the distribution of blood supply of the internal iliac artery. We performed the bilateral internal iliac artery angiography. After exchanging, the 5 F Cobra catheter is placed in the internal iliac artery to observe the blood supply of the internal iliac artery. The catheter was placed in the uterine artery, and we used the micro-catheter to choose the ovarian artery. We performed infusion chemotherapy after diluting the anticarcinogen cisplatin with physiological saline. We used gelatin sponge particles to embolize until the mainblood flow was blocked. The 5 FCobra catheter was used for inferior mesenteric artery radiography. Five cases can be seen with tumor stain. For these cases, it is considered that the intestinal tract has been infringed upon, thus, cisplatin chemotherapy perfusion is adopted. The total cisplatin given is 80 mg/m2 according to the blood supply. The ovary tumor rebulking operation was adopted after 15 to 20 days.

2.3 tumor size: We ultrasonically determined the tumor diameter line before and after intervention treatment and calculated the tumor size according to the formula (4 π abc / 3) cm3 tumor.

2.4 efficacy judgment standard: It is the change of LPA and the tumor size of the epithelial ovarian cancer before and after the intervention treatment (two weeks after the vaginal B ultrasound to check).

2.5 statistics management: We adopted the count material related analysis method and, managed the statistical data with the statistical software SPSS 17.0. Data is represented as mean ± standard deviation. We compared differences between groups, and took $P < 0.05$ to be statistically significant.

3.Results

3.1 interventional treatment results: Only 2 out of 20 patients in the benign ovarian epithelial neoplasm group had levels of plasma LPA greater than 3.2umol/L. All others were in the normal range. The 20 cases of late stage epithelial ovarian cancer displayed plasma LPA levels that were significantly higher than normal before interventional therapy. After interventional treatment, the levels dropped (Table 1) and tumor size shrank to a significant degree($P<0.05$) (Table 2).

22 patients with cases of late epithelial ovarian cancer had DSA radiography that showed that the bilateral iliac artery (mainly for uterine artery) and the ovarian artery were thick. The pelvic tumor was stained with dye. The intestinal tract of some cases were violated, so tumor color dyeing was also seen here. After two weeks of performing gynecologic B ultrasound examinations, we detected mass reduction, a softer mass, and improved mobility. These effects can be seen in table 1 after two weeks. After interventional treatment, the 20 patients displayed reduced levels of ovarian cancer cells after 20 days. However, for 2 cases, we could not perform surgery because of extensive abdominal transfer. It can be seen from the operation that the tumor size decreases, the mass becomes softer, the mobility is improved, it is easier to strip, the lymph nodes undergo necrosis, and there is less bleeding among the 19 patients.

3.2 adverse reactions and complications

Common adverse reactions include embolization reaction after interventional treatment, such as fever, pain, nausea, and vomiting. Additionally, there is a decrease in white blood cells down and liver function. 5 cases displayed intestinal invasion. One had intestinal obstruction after the operation and was fully recovered after being disposed of for one week. All patients experienced ventosity to varying degrees because the tumor tissue undergoes ischemia, anoxia, and necrosis after chemotherapy embolization. The patients were advised to breathe gradually to alleviate symptoms, and this does not appear to be a serious complication.

Table 1 The plasma LPA test results of the benign ovarian epithelial neoplasm, epithelial ovarian cancer group before and after interventional treatment

Tissue types	numbers (n)	lysophospholipids acid (μ mol/L)
Benign epithelial ovarian tumor	20	2.877±0.354
Epithelial ovarian cancer group		
before intervention	22	6.286 ±1.033 *
after intervention	22	4.919 ±1.404 #

* : benign group, epithelial ovarian cancer before the intervention group plasma LPA results show: $t = 14.435$, $p < 0.05$, a statistically significant difference.

: epithelial ovarian cancer groups before and after interventional treatment plasma LPA results show: $t = 4.437$, $p < 0.05$, a statistically significant difference.

Table 2 the ovarian epithelial cancer tumor size changes before and after interventional treatment

Before and after intervention treatment	numbers	tumor size (cm3)
before interventional treatment	22	1463.617 ±1408.069
after interventional treatment	22	807.362 ±759.195

The epithelial ovarian cancer groups' tumor size before and after intervention treatment : $t = 4.437$, $p < 0.05$, a statistically significant difference.

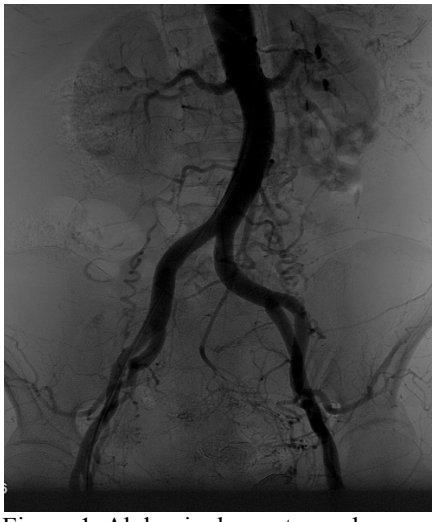

Figure 1. Abdominal aortography

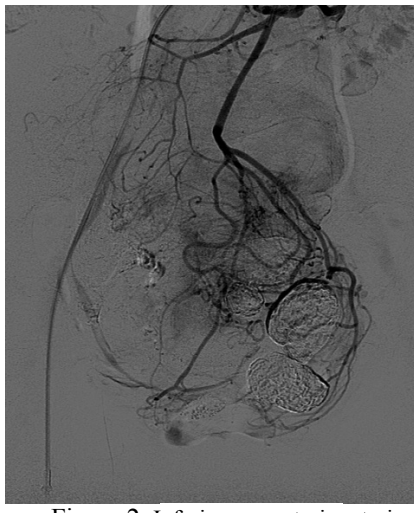

Figure 2. Inferior mesenteric arteriography

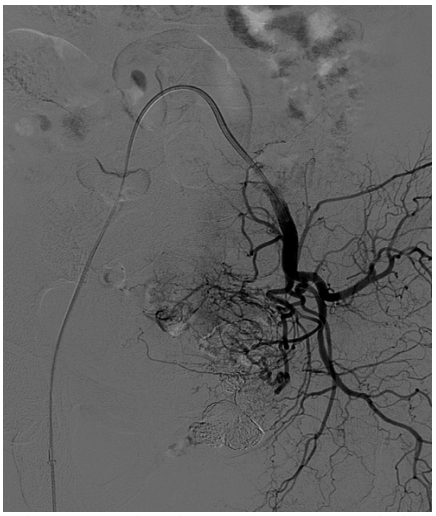

Figure 3. Before left uterine artery embolism

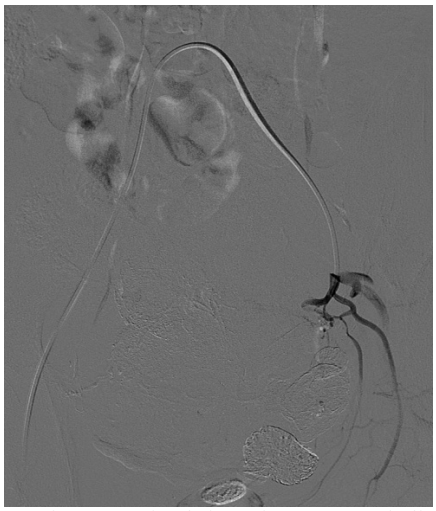

Figure 4. After left uterine artery embolism

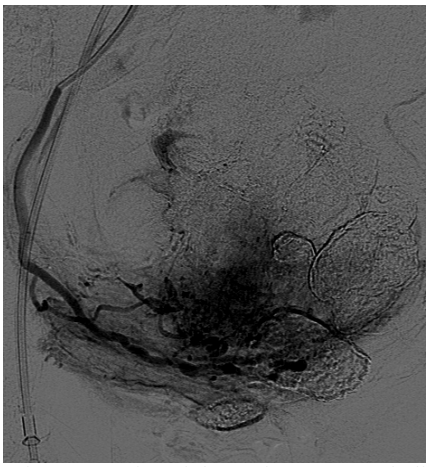

Figure 5. Before right uterine artery embolism

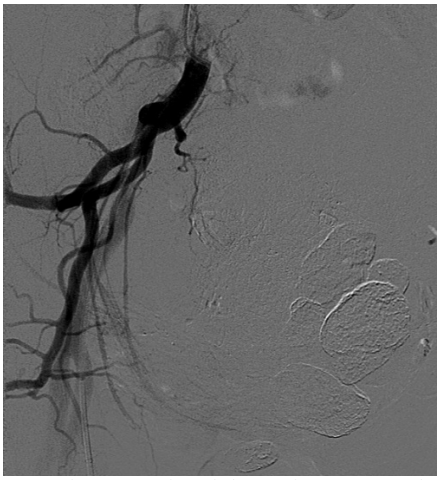

Figure 6. After right uterine artery embolism

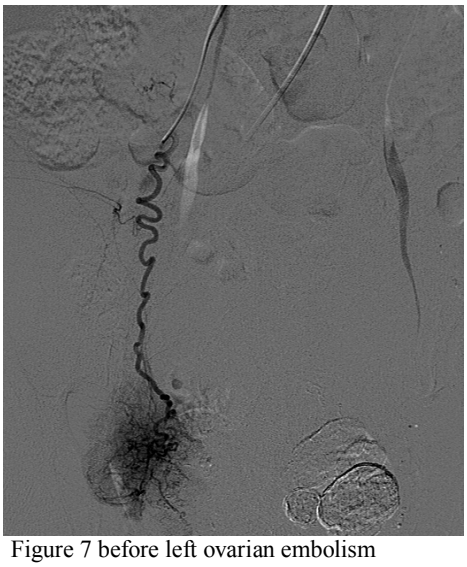

Figure 7 before left ovarian embolism

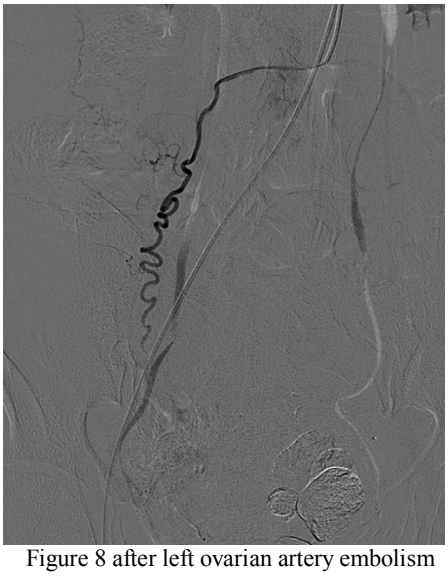

Figure 8 after left ovarian artery embolism

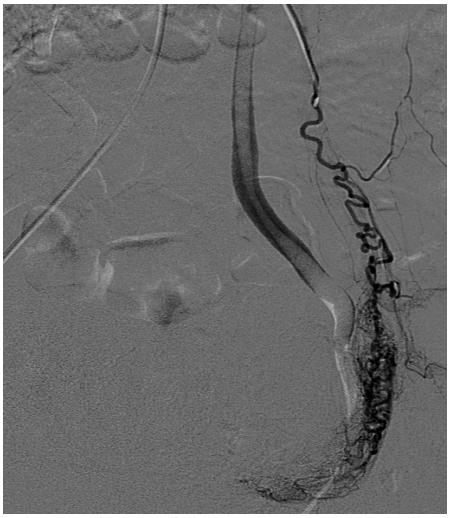
Figure 9 before right ovarian artery embolism

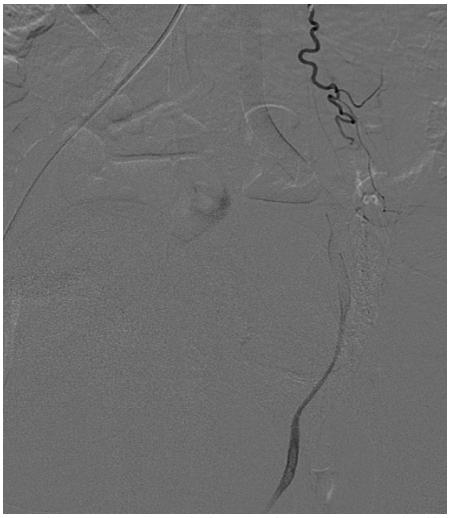
Figure 10 after right ovarian artery embolism

4. Discussions

LPA is a multi-functional phospholipid that induces intercellular signal transduction and elicits a number of biological effects via signaling through G protein coupled receptors [7]. Xu and Sutphen [8] found that nearly 90% of patients with stage I ovarian cancer have plasma LPA levels that are increased. Furthermore, these levels increase with increasing tumor grade. Thus, plasma LPA levels can be used as a biomarker for ovarian cancer diagnosis. LPA is related to the cancer occurrence, progression, infiltration, and transfer. LPA can promote ovarian cancer cell proliferation and survival. It can also promote production of proteases and other factors that promote angiogenesis. In addition, LPA can inhibit cancer cell apoptosis through inducing expression of vascular endothelial growth factor (VEGF) [9].

Presently, the most common treatment options for ovarian cancer are surgery and chemotherapy. However, surgical resection for advanced patients (stage III and above) is difficult and accompanied by a poor prognosis. For advanced patients, primary cytoreductive surgery and postoperative platinum chemotherapy are the most common treatment methods. With the development of interventional therapy technology, arterial infusion chemotherapy and embolism have gradually become part of the treatment regimen for gynecologic malignancies. The combination of chemotherapy and embolism can significantly enhance the clinical effect. Many studies show that artery infusion chemotherapy can shrink the tumor volume, reduce tumor stage, and create the opportunities for successful surgery [10, 11].

The blood supply to the ovary mainly comes from the ovarian branch of uterine arteries and the ovarian artery. The interventional therapies for ovarian cancer include intra-ovarian arterial chemotherapy perfusion and uterine arterial embolization. Research shows that even a single increase in local drug concentration results in an increased number of the cancer cells being killed by almost 10-fold. Selective iliac artery intubation can deliver chemotherapy drugs directly into the tumor blood supply artery to increase the local drug concentration to a greater extent than systemic venous chemotherapy. This can improve the effect of the treatment. In patients with advanced ovarian cancer, the tumor cells are widely transferred in the pelvic cavity and the peritoneal cavity. As a result, operation is not possible. In this case, the intervention therapies include laparotomy and cytoredutive surgery. They are effective at increasing patient life span. The role of LPA and its receptor in tumor formation and transfer has been confirmed. In this study, 22 cases showed high plasma LPA levels that decreased after the intervention. Focal cancers decreased and the disease was controlled to some extent. 2 cases did not undergo cytoreductive surgery because tumor cells were too widely transferred. Artery perfusion chemotherapy and embolism are effective treatments for ovarian cancer, especially in the case of advanced disease. Monitoring plasma LPA levels in ovarian cancer has clinical value.

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