# Curative effect of paclitaxel and cisplatin combined chemotherapy on cervical cancer and its relation with tissue micro vascular and lymphatic vessels density

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**Abstract**: This study was to discuss the curative effect of paclitaxel and cisplatin combined chemotherapy on cerical cancer and its relation with tissue micro vascular and lymphatic vessels density. The combined chemotherapy of paclitaxel 135mg/$m^2$ and cisplatin 25mg/$m^2$ were taken to observe the clinical curative effect. The postoperative paraffin tissue had been collected, had performed the LYVE-1 (lymphatic endothelium specific hyaluronan receptor-1) and CD31 immunohistochemical staining. The complete remission rate of high micro lymphatic vessels density group (was or more 6.0) and high micro vascular density group were obviously higher than in low micro lymphatic vessels density group and low micro vascular density group, the difference was statistically significance ($P<0.05$). This study further analyzed the relation of MVD and LVD with clinical pathological parameters. The difference was statistically significant ($P<0.01$). The curative effect of paclitaxel and cisplatin combined therapy was promising, positive and was closely related with cervical cancer tissue LVD and MVD. The LVD and MVD could be one of the predictors of early cervical CIN and early cervical cancer development.

**Keywords**: Paclitaxel, cisplatin, cerical cancer, micro vascular, micro-lymphatic vessels, chemotherapy.

## INTRODUCTION

Cervical cancer was one of the common malignant tumor in female reproductive system. In recent years, the incidence of young patients increased year by year. With the popularity of cervical cytology screening and application of Liquid-based cytology, the CIN (cervical intraepithelial neoplasia) and cervical cancer could be detected and treated in the early stage of disease (Liao *et al*, 2014). With further the development of disease, tumor cells further transferred to tissue adjacent to the uterus. The main transfer mode was lymphatic metastasis, hematogenous metastasis and direct infiltration. Chemotherapy was the main means to treat the cervical cancer. In recent years, the function of capillaries and micro lymphatics in early cervical cancer had became the hot spot in the domestic and foreign oncology research (Zhao *et al*, 2013).

## INFORMATION AND METHODS

### *General information*

This study had selected 55 cases of patients with CIN (cervical intraepithelial neoplasia) and early cervical cancer patients in Xinxiang Central Hospital from May of 2012 to December of 2013. Approved by the ethics committee, patients were with age from 28~51 years old, average 39.5±4.1 years of age. FIGO staging: 5 cases for Ib stage, 15 cases for IIa stage, 5 cases for IIb stage. This study then selected the specimens with typical lesions and with normal tissue ranging 5mm or more distance.

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### *Chemotherapy regimens method*

All the patients were preoperative patients and signed the informed consent files. These patents had been injected dexamethasone 10mg before chemotherapy, then given paclitaxel 135mg/$m^2$ for the first day and then were injected cisplatin combined chemotherapy 25mg/$m^2$ from the first day to the third day. The patients were adopt intravenous chemotherapy, with interval of 2~3 weeks, total of three courses.

### *Immunohistochemical method*

The specimens were paraffin serial section of slice with thickness of 3μm. This study had applied the immunohistochemical staining for slicing lymphatic endothelial hyaluronan receptor1 (LYVE-1) and CD31. Both respectively were the specific molecular markers of micro lymphatic vessel endothelial cells and micro vascular endothelial cells. Immunohistochemical staining steps were below: paraffin sections were dewaxed by dimethylbenzene, then for ethanol gradient dehydration, then for distilled water rinse, then applied 3% of hydrogen peroxide to eliminate the endogenous peroxicdase activity, then again for distilled water rinse, then applied phosphate buffer solution to clean, then added the 1 antigen (LYVE-1 with ratio of 1 to 80 and CD31 with ratio of 1 to 100), then kept in incubation for one hour, then added II antigen, then kept in incubation for 30 minutes, then washed by phosphate buffer solution, then DAB coloration, then for distilled water rinse, then for hematoxylin re-coloration, then for distilled water rinse, then for ethanol gradient dehydration, then for dimethylbenzene transparence, then for neutral gum

sealing, finally, detected it under light microscope. Each case had been took 3 pieces of positive dense staining region under low power microscope, then calculated the quantity in high power microscope, then figured out the average as the LVD and MVD of the case.

## STATISTICAL ANALYSIS

The SPSS 13.0 statistical software were applied to analyze the data and the mean of two groups was compared by *t* test, normal measurement data was measured by x±s, the $P<0.05$ was taken as difference with statistically significant.

## RESULTS

### *Curative effect of cervical cancer chemotherapy and the relations with micro vascular and mircro lymphatic vessels density*

In the 55 cases of patients, there were 44 cases of patient had completely remission and 8 cases of partially remission, complete response rate was 94.54%. The chemotherapy complete remission rate of high micro lymphatic vessel density (not less than 6.0 cases) and high micro vascular density (not less than 20.0 cases) were respectively 90.65% and 84.21%. These were obviously higher than that in low micro lymphatic vessel density and low micro vascular density, the difference was statistically significant (P<0.05, see table 1).

### *Positive staining condition of micro lymphatic vessel and micro-vascular for cervical cancer*

In cervical cancer cases and positive staining of lymphatic drainage, the quantity was more and with various of shapes, such as circular, elliptical and irregular shape (see fig. 1, left); In the cancer lesion, the tube cavity structure was complete and with tan positive staining; carcinoma capillary in the lesion surrounding tissue was small and with uneven thickness of cell membrane (see fig. 1, right)

### *Analysis on micro lymphatic vessel density related factors*

Micro lymphatic vessel density was related with whether patients was in postmenopausal or not, and histological grading, and FIGO grading. Micro lymphatic vessel density of lesion and lesion around tissue of postmenopausal patients was significantly higher than that of premenopausal patients (P<0.01). The higher histological grade and FIGO staging for cases, the higher of micro-vascular density in lesion surrounding normal tissue (P<0.01, see table 2).

### *Analysis on micro-vascular density associated factors*

Capillary density was closely related to whether patients was in postmenopausal or not and FIGO staging. Micro-vascular density in lesion and lesion around normal tissue for postmenopausal patients was higher than that of premenopausal patients (P<0.01); The higher of the FIGO staging for patients, the higher micro vascular density of lesion and lesion around normal tissue (P<0.05, see table 3).

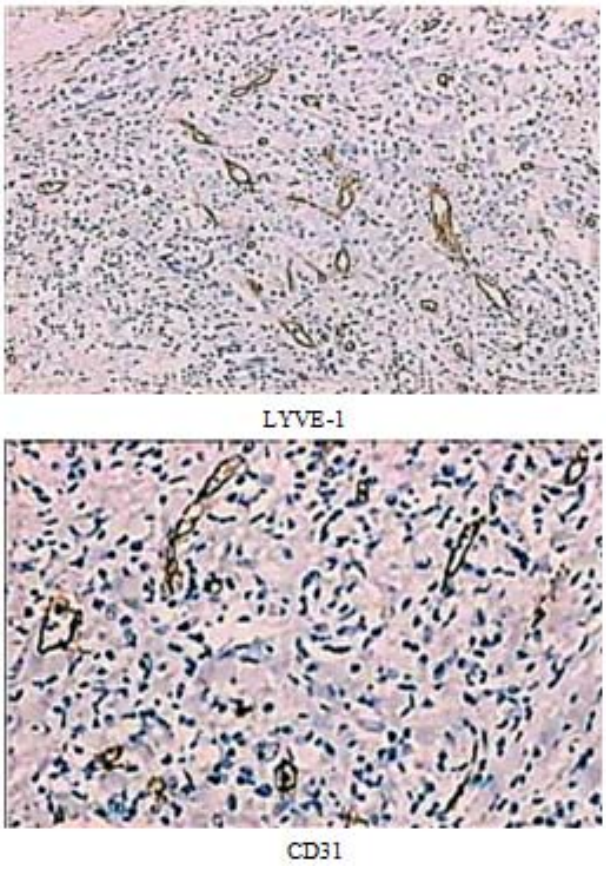


**Fig. 1**: The expression of LYVE-1 and CD31 in cervical cancer tissue detected by IHC (×400)

## DISCUSSION

At present, there were so many regimens for cervical cancer chemotherapy and with some adverse reactions. The majority of patients cannot tolerate the chemotherapy, and different patients had different drug-resistant on different drugs. Some patients were sensitive to drugs, while, some could tolerate the chemotherapy drugs. After the chemotherapy treatment, the tumor size did not decrease and clinical remission rate did not increase, of which the reason might be associated with the individual differences of patients with tumor. Tumor biological behavior would directly affect tumor chemotherapy sensitivity. Paclitaxel and cisplatin were the most common tumor chemotherapy drugs and had been widely applied in women ovarian cancer. It could inhibit tumor cell mitosis to promote tumor cell necrosis and reach the effect of chemotherapy. Numerous studies have confirmed that the combined chemotherapy effects of the two medicine, this study also confirmed the cervical cancer clinical effectiveness reached 94.54% after paclitaxel and

**Table 1**: The relationship between chemotherapy and LVD, MVD

| Group | Case | Complete remission | Partial remission | Progress | Complete remission rate |
|---|---|---|---|---|---|
| High micro lymphatic vessel density group | 32 | 29 | 3 | 0 | 29(90.65) |
| Low micro lymphatic vessel density | 23 | 15 | 5 | 3 | 15(65.22)* |
| High micro vascular density group | 38 | 32 | 5 | 1 | 32(84.21) |
| Low micro vascular density group | 17 | 12 | 3 | 2 | 12(70.58)$^{\#}$ |

*P<0.05, compared with high LVD group; $^{\#}P$<0.05, compared with high MVD group

**Table 2**: Micro lymphatic density related factors analysis (per high power field)

| Item | | Case | Lesions tissue | P | Lesion surrounding tissue | P |
|---|---|---|---|---|---|---|
| Postmenopausal or not (cervical cancer group and Precancerous lesions) | Premenopausal cases | 37 | 0 | <0.01 | 3.85±0.05 | <0.01 |
| | Postmenopausal cases | 13 | 1.01±0.21 | | 4.84±0.43 | |
| FIGO staging (cervical cancer group) | Ⅰa | 5 | 1.05±0.25 | <0.01 | 4.67±0.56 | <0.01 |
| | Ⅰb | 15 | 0 | | 5.68±0.60 | |
| | Ⅱa | 5 | 0 | | 6.68±0.62 | |

**Table 3**: Analysis on micro vascular density associated factor (per high power field)

| Item | | Case | Lesions tissue | P | Lesion surrounding tissue | P |
|---|---|---|---|---|---|---|
| Postmenopausal or not (cervical cancer group and Precancerous lesions) | Premenopausal cases | 37 | 14.33±1.45 | <0.01 | 13.15±1.50 | <0.01 |
| | Postmenopausal cases | 13 | 18.66±1.21 | | 16.48±1.66 | |
| FIGO staging (cervical cancer group) | Ⅰa | 5 | 14.01±1.21 | <0.01 | 16.67±1.06 | <0.01 |
| | Ⅰb | 15 | 16.98±1.54 | | 20.68±2.30 | |
| | Ⅱa | 5 | 18.36±2.07 | | 22.68±2.62 | |

cisplatin chemotherapy in patients. Further immunohistochemical calculation tested the micro vascular density and micro lymphatic vessel density. Statistics found that the complete response rates of chemotherapy for micro lymphatic vessel density and high microcascular density were respectively 90.65% and 84.21%. These were significantly higher than the low density of lymphatic vessels group and low micro vascular density, the difference was statistically significant (P<0.01). It preliminary showed that patients with different vascular density had different sensitivity against chemotherapy drugs.

In recent years, the function of micro lymphatics in the early occurrence and development of tumor had became the hot sport in clinical and basic research, which had been proved by the existed research. The quantity of lymphatic vessels was closely related to tumor staging and pathological grading. With the further study of lymphatic endothelial specific markers, the mutual interaction of the occurrence, development and transfer of new microlymphatics and tumor had already became the hot spot of clinical research. Most scholars believed that (Ouldamer *et al*, 2012; Ferrandina *et al*, 2008), there was no lymphatic existing in solid tumors and its only found the tissue adjacent to carcinoma. It had been analyzed that it may due to the rapid growth of cancer cells leading to the increase of tumor inner pressure, namely blocked the formation of lymphatic vessels. Micro lymphatics often measured by micro lymphatic density. Clinical research proved (Meng and Luo, 2014; Duenas *et al*, 2002; Liu *et al*, 2003), micro lymphatic vessel density of tissue adjacent to carcinoma was highly expressed in rectal cancer, laryngeal cancer, esophageal squamous carcinoma. There were so many specificity of the lymphatic endothelial markers. LYVE-1 was one of the most commonly applied and was also the most stable markers expression. LYVE-1 was the homologous compounds CD44 glycoprotein and the lymphatic endothelial hyaluronan receptor. Studies had shown that (Zheng *et al*, 2014; Patrick, 2004), LYVE-1 was a new micro marker of lymphatic tumors. Foreign scholars had researched the positive expression of LYVE-1 in the specimens of melanoma and breast cancer. While, there were few research of it LYVE-1 on cervical cancer. There were scholars applied carcinoembryonic antigen M2A

monoclonal antibody to mark the lymphatic endothelium. The results had shown that the lymphatic vessels density was related with pathological types in cervical cancer. It needed to research the function of lymphatic vessel density on cervical cancer. In 1971, the tumor angiogenesis theories had been proposed and suggested the growth and transfer of tumor depended on the survival of tumor blood vessels (Lu and Yang, 2009; Huh *et al*, 2001). Studies had confirmed that (Wang *et al*, 2014), during the CIN developing into infiltrating carcinoma, it was still existed the formation of tumor blood vessels, micro vascular density was the gold standard for evaluation of tumor angiogenesis. CD31 was the marker for micro vascular in good condition and could be used for an independent prognostic indicators for cervical cancer survival rate without disease progression and overall survival rate. At present, in domestic and foreign research, there was no specific definition for the scope of micro lymphatic density and micro vascular density of cervical cancer. While, scholars only did a rough division of lesion and lesion around tissue. This study observed micro lymphatic vessel density and micro vascular density of the cervical lesions and lesions around lesion. The results showed that, micro lymphatic vessel density within lesion and lesion around tissue of postmenopausal cases was higher than that in premenopausal cases; The higher of FIGO staging, the higher of micro lymphatic vessel density in lesion around tissue. Micro vascular density in the lesion was significantly higher than micro lymphatic vessel density in patients with cervical cancers. Micro vascular density was closely related with whether patients had postmenopausal or not and FIGO staging. The higher of FIGO staging for patients, the higher of the micro vascular density in lesion and normal tissue surrounding lesion.

In a conclusion, it has a positive effect for the curative effect of the combined themotherapy of paclitaxel and cisplatin. And it was closely related with cervical cancer tissue LVD and MVD. LVD and MVD could be predictors of the development of CIN and early cervical cancer.

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