



# Effects of lentinan and thymopentin combined with adjuvant chemotherapy on immunity, oxidative stress, matrix metalloproteinases and related factors in patients with tongue squamous cell carcinoma 舌鳞癌

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## ABSTRACT

**Objective:** To investigate the effects of lentinan and thymopentin combined with adjuvant chemotherapy on immunity, oxidative stress, matrix metalloproteinases and related factors in patients with tongue squamous cell carcinoma. **Methods:** Retrospective analysis of 78 patients with tongue squamous cell carcinoma admitted to our hospital from February 2013 to November 2017, according to the postoperative chemotherapy scheme, the patients were divided into control group ( $n=42$ ) and observation group ( $n=36$ ). The patients in control group were given PF chemotherapy scheme (cisplatin + compound fluorouracil injection), on this basis, the patients in observation group were given lentinan and thymopentin injection combined treatment. The immune index, oxidative stress, matrix metalloproteinase, cyclin D1 (CyclinD1) and B lymphocyte tumor-2 (BCL-2) were detected and analyzed before and after treatment. **Results:** After treatment, the immunoglobulin level in the control group was not statistically significant compared with that before treatment ( $P>0.05$ ); the levels of IgA, IgG and IgM in the observation group were significantly higher than those before treatment ( $P<0.05$ ), and the levels of IgA, IgG and IgM in the observation group were significantly higher than those in the control group ( $P<0.05$ ); the level of SOD in control group was significantly lower than that before treatment ( $P<0.05$ ), and the level of MDA was significantly higher than that before treatment ( $P<0.05$ ); the observation group was the opposite, and its SOD level was significantly higher than that of the control group ( $P<0.05$ ), and the MDA level was significantly lower than the control group ( $P<0.05$ ); the levels of MMP-2 and MMP-9 in the two groups were significantly lower than those before treatment ( $P<0.05$ ), and the levels of MMP-2 and MMP-9 in the observation group were significantly lower than those in the control group ( $P<0.05$ ); the levels of CyclinD1 and BCL-2 in the two groups were significantly lower than those before treatment ( $P<0.05$ ), and the levels of CyclinD1 and BCL-2 in the observation group were significantly lower than those in the control group ( $P<0.05$ ). **Conclusions:** Lentinan and thymopentin assisted PF chemotherapy regimen can enhance the immune function of patients with tongue squamous cell carcinoma after radical surgery, improve oxidative stress response, inhibit tumor cell proliferation and metastasis. It has the significance of clinical popularization.

## 1. Introduction

Tongue squamous cell carcinoma is a common malignant tumor in the oral and maxillofacial region, which seriously affects the facial function of chewing, language and swallowing, and endangers

life safety and quality of life[1,2]. At present, tongue squamous cell carcinoma is mainly treated with surgery, chemotherapy or radiotherapy was given after operation, PF chemotherapy can improve the degree of tumor shrinkage and deterioration, but it will also damage the body while killing tumor cells and there are adverse effects such as multidrug resistance and increased toxicity[3,4]. Lentinan and thymopentin have antiviral, anti-tumor and immune enhancement effects, and have high safety[5–7]. However, there are few reports on the study of tongue squamous cell carcinoma between the two drugs. In view of this, this study explored the effects of lentinan and thymopentin-assisted PF chemotherapy on various serum indicators of the disease. It is reported as follows.

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## 2. Materials and methods

### 2.1 General information

A retrospective analysis of 78 patients with tongue squamous cell carcinoma admitted to our department from February 2013 to November 2017, they were divided into control group ( $n=42$ ) and observation group ( $n=36$ ) according to postoperative chemotherapy. There were 25 males and 17 females in the control group, aged 32-70 years old. According to the 8th edition of TNM staging criteria for oral cancer by American Joint Committee on Cancer in 2016, 22 cases of stage II, 13 cases of stage III, 7 cases of stage IV; The tumor sites: there were 23 cases at tongue margin, 12 cases at tongue abdomen, and 7 cases at tongue back. The degree of tumor differentiation: 18 cases with high differentiation and 24 cases with poor differentiation. There were 21 males and 15 females in the observation group, aged 34-68 years old. TNM staging: 19 cases in stage II, 11 cases in stage III, and 6 cases in stage IV. Tumor site: 19 cases at tongue margin, 9 cases at tongue abdomen, 88 cases at tongue back, the degree of tumor differentiation: 15 cases with high differentiation and 21 cases with poor differentiation. There were no significant differences in gender, age, TNM stage, tumor location and tumor differentiation between the two groups of tongue squamous cell carcinoma ( $P>0.05$ ).

### 2.2 Inclusion and exclusion criteria

Inclusion criteria: All subjects had palpable neck and swollen lymph nodes, which were rejected by radical surgery and were diagnosed as tongue squamous cell carcinoma by imaging examination and pathological analysis[8]; TNM stage was stage II~IV; No tumor-related treatment was received before admission. Exclusion criteria: those with chemotherapy contraindications; associated with diseases affecting the postgraduate index, such as hypertension, diabetes and other tumors; those with a predicted survival of less than 3 months; those with a lower degree of cooperation in this study.

### 2.3 Treatment method

According to the patients' voluntary choice of postoperative chemotherapy, the patients were divided into the control group and the observation group. The control group received PF chemotherapy. On the first day, cisplatin (Yunnan Botanical Pharmaceutical Co., Ltd., Approval number: H53021678) was given intravenously, 20 mg/m<sup>2</sup>; compound fluorouracil injection was given on days from the 1st to 5th day (Jiangsu Zhenguo Pharmaceutical Co., Ltd., Approval number: H32025827) intravenous drip, 750 mg/m<sup>2</sup>.

On the basis of the PF chemotherapy regimen, the observation group received 2 mg of lentinan injection (Jinling Pharmaceutical Co., Ltd. Fuzhou Meifeng Pharmaceutical Factory, Approval number: H20030131) and thymopentin injection 2 d before chemotherapy.

Pharmaceutical Co., Ltd., Approval number: H20052528) 20 mg diluted in 5%, 250 mL glucose solution, intravenous drip for 14 d. One course of treatment for 3 weeks, both groups were treated for 6 courses.

### 2.4 Observation indicators

Venous blood was collected before and after treatment, immunoglobulin A (IgA), immunoglobulin M (IgM), immunoglobulin G (IgG) were determined by nephelometry; thiobarbituric acid colorimetric method and xanthine oxidase colorimetric method were used for determination of superoxide dismutase (SOD) and malondialdehyde (MDA); detection of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) by radioimmunoassay, Cyclin D1 and B lymphocyte tumor-2 (BCL-2) were determined by enzyme-linked immunosorbent assay. The above kits are all produced by Merck.

### 2.5 Statistical analysis

The data of this study were analyzed by SPSS 22.0, and the chi-square test of counting data; immunoglobulin, oxidative stress index, matrix metalloproteinase, CyclinD1 and other indicators were compared using t test, indicated by ( $\bar{x}\pm s$ ).  $P<0.05$  indicated that the difference was significant and statistically significant.

## 3. Results

### 3.1 Comparison of immunoglobulin levels before and after treatment in both groups

Before treatment, there was no significant difference in immunoglobulin levels between the two groups ( $P>0.05$ ). After treatment, the immunoglobulin level of the control group was not significantly different from that before treatment ( $P>0.05$ ). The levels of IgA, IgG and IgM in the observation group were significantly higher than those before treatment ( $P<0.05$ ), and the IgA, IgG and IgM levels in observation group [(1.39±0.25) g/L, (20.33±4.86) g/L, (1.75±0.42) g/L] were significantly higher than the control group [(1.19±0.22) g/L, (11.09± 3.08) g/L, (1.33 ± 0.37) g/L] ( $P<0.05$ ). See Table 1.

**Table 1.**

Comparison of immunoglobulin levels before and after treatment in both groups.

| Group                        | Treatment time   | IgA(g/L)               | IgG(g/L)                | IgM(g/L)               |
|------------------------------|------------------|------------------------|-------------------------|------------------------|
| Observation group ( $n=36$ ) | Before treatment | 1.19±0.18              | 10.59±2.60              | 1.25±0.31              |
|                              | After treatment  | 1.39±0.25 <sup>#</sup> | 20.33±4.86 <sup>#</sup> | 1.75±0.42 <sup>#</sup> |
| Control group ( $n=42$ )     | Before treatment | 1.21±0.20              | 10.28±2.19              | 1.27±0.35              |
|                              | After treatment  | 1.19±0.22              | 11.09±3.08              | 1.33±0.37              |

Note: Compared with before treatment, <sup>#</sup> $P<0.05$ ; compared with the control group after treatment, <sup>#</sup> $P<0.05$

### 3.2 Comparison of oxidative stress indicators before and after treatment in both groups

Before treatment, there was no significant difference in SOD and MDA levels between the two groups ( $P>0.05$ ). After treatment, the SOD level of the control group was significantly lower than that before treatment ( $P<0.05$ ), and the MDA level was significantly higher than that before treatment ( $P<0.05$ ). The observation group was the opposite, and its SOD level [(92.77±17.85) U/L] significantly higher than the control group [(65.29 ± 7.22) U/L] ( $P<0.05$ ), MDA [(10.08 ± 1.95) mol/L] was significantly lower than the control group [(15.73 ± 3.56) mol/L] ( $P<0.05$ ). See Table 2.

**Table 2.**

Comparison of oxidative stress indicators before and after treatment in both groups.

| Group                    | Treatment time   | SOD(U/L)                 | MDA(mol/L)              |
|--------------------------|------------------|--------------------------|-------------------------|
| Observation group (n=36) | Before treatment | 78.63±10.11              | 12.26±2.03              |
|                          | After treatment  | 92.77±17.85 <sup>#</sup> | 10.08±1.95 <sup>#</sup> |
| Control group (n=42)     | Before treatment | 76.24±9.80               | 12.05±1.97              |
|                          | After treatment  | 65.29±7.22 <sup>*</sup>  | 15.73±3.56 <sup>*</sup> |

Note: Compared with before treatment, <sup>\*</sup> $P<0.05$ ; compared with the control group after treatment, <sup>#</sup> $P<0.05$

### 3.3 Comparison of matrix metalloproteinase levels before and after treatment in both groups

Before treatment, there was no significant difference in the levels of MMP-2 and MMP-9 between the two groups ( $P>0.05$ ). After treatment, the levels of MMP-2 and MMP-9 in the two groups were significantly decreased when compared with those before treatment ( $P<0.05$ ). MMP-2 and MMP-9 levels in the observation group [(311.28±70.38) ng/mL, (95.34±18.53) ng/mL] were significantly lower than the control group [(487.60±130.82) ng/mL, (157.56±40.11) ng/mL] ( $P<0.05$ ). See Table 3.

**Table 3.**

Comparison of matrix metalloproteinase levels before and after treatment in both groups.

| Group                    | Treatment time   | MMP-2(ng/mL)               | MMP-9(ng/mL)              |
|--------------------------|------------------|----------------------------|---------------------------|
| Observation group (n=36) | Before treatment | 657.36±125.30              | 258.22±49.63              |
|                          | After treatment  | 311.28±70.38 <sup>#</sup>  | 95.34±18.53 <sup>#</sup>  |
| Control group (n=42)     | Before treatment | 671.06±146.90              | 266.79±60.74              |
|                          | After treatment  | 487.60±130.82 <sup>*</sup> | 157.56±40.11 <sup>*</sup> |

Note: Compared with before treatment, <sup>\*</sup> $P<0.05$ ; compared with the control group after treatment, <sup>#</sup> $P<0.05$

### 3.4 Comparison of CyclinD1 and BCL-2 levels before and after treatment in both groups

Before treatment, the levels of CyclinD1 and BCL-2 in the two groups were not significantly different ( $P>0.05$ ). After treatment, the levels of CyclinD1 and BCL-2 in the two groups were significantly

decreased than those before treatment ( $P<0.05$ ), and CyclinD1 and BCL-2 levels in the observation group [(4.80±1.36) ng/mL, (30.47±4.02) U/mL] were significantly lower than the control group [(6.85±1.83) ng/mL, (39.50±5.33) U/mL] ( $P<0.05$ ). See Table 4.

**Table 4.**

Comparison of CyclinD1 and BCL-2 levels before and after treatment in both groups.

| Group                    | Treatment time   | CyclinD1(ng/mL)        | BCL-2(U/mL)             |
|--------------------------|------------------|------------------------|-------------------------|
| Observation group (n=36) | Before treatment | 11.09±2.76             | 52.96±6.85              |
|                          | After treatment  | 4.80±1.36 <sup>#</sup> | 30.47±4.02 <sup>#</sup> |
| Control group (n=42)     | Before treatment | 10.97±2.52             | 54.37±5.82              |
|                          | After treatment  | 6.85±1.83 <sup>*</sup> | 39.50±5.33 <sup>*</sup> |

Note: Compared with before treatment, <sup>\*</sup> $P<0.05$ ; compared with the control group after treatment, <sup>#</sup> $P<0.05$

## 4. Discussion

Due to the special and complex oral structure, the vascular and lymphatic networks are abundant, and malignant tumors are easy to metastasize. Among them, tongue squamous cell carcinoma has high invasiveness and recurrence, and it is difficult to cure, and the mortality rate is extremely high, which seriously endangers the life of patients[9,10]. At this stage, squamous cell carcinoma is mainly treated by radical surgery. PF chemotherapy is often used in the perioperative period, clinically, this program has a good effect on tongue squamous cell carcinoma[11]. However, postoperative PF chemotherapy alone can inhibit the immune function of patients, activate oxidative stress, multidrug resistance and increase toxic side effects. Lentinan is a widely used immunopotentiator with immunomodulatory and antitumor effects. Thymopentin has a two-way regulation of immune function, and also has the effect of inhibiting tumor differentiation, proliferation, and preventing tumor spread and metastasis.

The immune function of patients after radical tumor resection is generally disordered. Therefore, it is important to adopt appropriate chemotherapy after surgery to improve the immune function of patients[12]. IgA, IgG and IgM are important serum immunoglobulins for regulating humoral immunity of the body. IgA is an important factor in mucosal local immunity, which is antiviral and pathogenic; IgG can eliminate free toxins and regulate phagocytic cells; IgM is the first appeared antibody in humoral immunity, which are closely related to the severity of the disease[13,14]. This study showed that the levels of IgA, IgG and IgM in the observation group were significantly increased than those before treatment, and the observation group was significantly higher than the control group. This result is similar to the results reported by Shen Huajian[15]. It is suggested that lentinan combined with thymopentin-assisted chemotherapy can significantly enhance the immune function

of patients. The reasons may be: (1) lentinan can promote the proliferation and differentiation of T lymphocytes, promote the production of cytotoxic T lymphocytes, and improve the immune function of patients[16,17]; (2) Thymosin is secreted from the thymus and has a two-way immunomodulatory effect, which can effectively enhance the body's immune function[18,19].

During the radical operation of the patient, blood circulation disorder is caused by the removal of the diseased lymph nodes and tissue incision[20]. In addition, the stress damage generated during the process causes the body to generate massive free radicals, causing lipid peroxidation, resulting in the formation of lipid peroxides, such as MDA; and inhibiting the production of antioxidants such as SOD. In addition, after radical surgery, factors such as nutritional intake disorders, immunosuppression and the use of anti-tumor drugs will also cause a large number of free radicals, causing the patient's body to be in an oxidative stress state. If this could not be improved, it will also have a certain impact on the patient's postoperative recovery. This study showed that patients with postoperative PF chemotherapy alone, the SOD level was significantly lower than before treatment, and MDA was significantly higher than before treatment, indicating that chemotherapy can further strengthen the oxidative stress response of patients after surgery, reduce its antioxidant function; Lentinan and thymopentin-assisted PF chemotherapy, the SOD level was significantly higher than before treatment, and MDA was the opposite, indicating that this program can enhance the antioxidant function of postoperative and contribute to postoperative recovery. The reason may be related to the immunomodulatory effects of lentinan and thymopentin.

Studies have shown that[21] MMP-2 and MMP-9 are key proteases for degrading extracellular matrix components, and their high expression is closely related to the degree of disease of various tumors. The mechanism of action is to cause tumor cell growth and invasion through destroying the tissue structure and the basement membrane barrier, while also regulate vascular endothelial growth factor, promote the formation of tumor new blood vessels. This study showed that the levels of matrix metalloproteinases in the two groups obviously reduced after treatment, and the levels of MMP-2 and MMP-9 in the observation group were significantly lower than those in the control group. It is suggested that lentinan combined with thymopentin-assisted chemotherapy can effectively improve the level of matrix metalloproteinase in patients and inhibit the invasion and metastasis of tumor cells to some extent. The reason may be related to the immunomodulatory effects of lentinan and thymopentin, which promotes the proliferation and differentiation of immunoglobulins and T lymphocytes and enhances the body's ability to clear tumor cells. Meanwhile, thymopentin itself can inhibit proliferation and metastasis of tumor cell.

For patients with oral squamous cell carcinoma undergoing PF-

induced chemotherapy, CyclinD1 expression is highly sensitive prediction of chemotherapy[22]. High expression of BCL-2 can inhibit the apoptosis of tumor cells, which is considered to be one of the targets of treatment of tongue squamous cell carcinoma and has a certain correlation with CyclinD1[23]. This study investigated the inhibitory effect of lentinan combined with thymopentin-assisted PF chemotherapy on tumor cell growth, invasion and metastasis. The results showed that the levels of CyclinD1 and BCL-2 were significantly decreased in the two groups after treatment, and the levels of CyclinD1 and BCL-2 in the observation group were significantly lower than those in the control group. It further confirmed the superiority of lentinan combined with thymopentin-assisted chemotherapy for tumor cell growth and disease condition control.

In summary, lentinan and thymopentin-assisted PF chemotherapy can enhance immune function, improve oxidative stress and inhibit tumor cell proliferation and metastasis in patients with tongue squamous cell carcinoma after radical surgery. It has clinical promotion significance.

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