Efecto de Eucalyptus Limonensis en el suero TGF- b1, IL-8 y subconjuntos de linfocitos T en pacientes con carcinoma nasofaríngeo

Effect of Eucalyptus Limonensis on Serum TGF- β1, IL-8 and T Lymphocyte Subsets in Patients with Nasopharyngeal Carcinoma

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Resumen
Se estudió el efecto de la IMRT sobre los subgrupos de tgf-31 y linfocitos T en suero en pacientes con NPC. El grupo control y el grupo de observación fueron tratados con Eucalyptus limonensis bidimensional e IMRT respectivamente. La tasa de supervivencia a 5 años, recurrencia, reacciones adversas, tgf-31 sérico, subconjuntos de linfocitos L-8 y T se compararon antes y después del tratamiento. Los resultados mostraron que la tasa de supervivencia libre de recurrencia regional y la tasa de supervivencia libre de tumor de los pacientes en el grupo de observación fueron significativamente más altas que las del grupo de control, la tasa de recurrencia local de los pacientes en el grupo de observación fue significativamente menor que en el grupo de observación. grupo control, y la tasa de supervivencia libre de metástasis a distancia de los pacientes en el grupo de observación fue ligeramente mayor que la del grupo control; la incidencia total de reacciones adversas en el proceso de Eucalyptus limonipin en el grupo control fue del 41%, y en el grupo de observación fue del 32%; los niveles séricos de tgf-31 e IL-8 de los pacientes en los dos grupos después del tratamiento fueron más altos que los del grupo control. El nivel de CD8 después del tratamiento fue significativamente más bajo que antes del tratamiento (P <0.05). Conclusión: la IMRT tiene un efecto significativo en la APN, que puede lograrse mejorando los niveles de los subgrupos de linfocitos T, tgf-31, IL-8 y suero.

Palabras clave: Palabras clave carcinoma nasofaríngeo; Eucalyptus limonensis; Factor de transformación del suero-β 1; Interleucina-8; Subconjuntos de linfocitos T

Abstract
The effect of IMRT on serum tgf-31 and T-lymphocyte subsets in patients with NPC was studied. The control group and the observation group were treated with two-dimensional Eucalyptus limonensis and IMRT respectively. The 5-year survival rate, recurrence, adverse reactions, serum tgf-31, L-8 and T-lymphocyte subsets were compared before and after treatment. The results showed that the regional recurrence free survival rate and tumor free survival rate of the patients in the observation group were significantly higher than those in the control group, the local recurrence rate of the patients in the observation group was significantly lower than that in the control group, and the distant metastasis free survival rate of the patients in the observation group was slightly higher than that in the control group; the total incidence of adverse reactions in the process of Eucalyptus limonipin in the control group was 41%, and that in the observation group was 32%; the serum tgf-31 and IL-8 levels of the patients in the two groups after treatment were higher than those in the control group. The level of CD8 after treatment was significantly lower than that before treatment (P < 0.05). Conclusion: IMRT has a significant effect on NPC, which may be achieved by improving the levels of serum tgf-31, IL-8 and T lymphocyte subsets.

Key words: Keywords nasopharyngeal carcinoma; Eucalyptus limonensis; Serum transforming factor-β 1; Interleukin-8; T lymphocyte subsets

1. Introduction

Nasopharyngeal carcinoma is a common malignant tumor in clinic. Because the structure of nasopharynx is very complex, and it is adjacent to the important organs, blood vessels and nerves, Eucalyptus limonensis is one of the main methods of clinical treatment of NPC[1]. The Eucalyptus type of NPC mainly includes two-dimensional eucalyptus, three-dimensional eucalyptus and Eucalyptus. Recently, the relevant literature reported that compared with the conventional two-dimensional eucalyptus, the technical advantages of three-dimensional eucalyptus and strengthening Eucalyptus can transform clinical benefits, such as improving the prognosis of
patients, reducing the incidence of toxic reactions in normal tissues and prolonging the survival period of patients[2-3]. So, what is the main mechanism of IMRT (intensity modulated radiation therapy) in the treatment of NPC to obtain clinical benefits is a common concern in clinical practice. In recent years, it has been reported that serum transforming factor (TGF) is a kind of cytokine secreted by tumor cells and widely exists in patients’ serum, which mainly plays the role of "promoting factor" to stimulate tumor cells to differentiate and reproduce continuously; IL-8 belongs to C-X-C chemokines, which originates from monocytes, macrophages and vascular endothelial cells. There is no report about the effect of IMRT on the levels of TGF - β 1, IL-8 and T lymphocyte subsets in the serum of NPC patients[4]. The purpose of this study is to investigate the effect of IMRT on the serum levels of these indexes in patients with NPC, and to elucidate the mechanism of IMRT in the treatment of NPC.

2. Data and methods

2.1 Clinical data
115 NPC patients admitted to our hospital from August 2017 to August 2018 were selected as the research objects, all of which were confirmed by clinical symptoms and signs, chest X-ray, abdominal B-ultrasound, bone ect, CT or MRI and pathological sections. They were divided into control group (57 cases) and observation group (58 cases). Control group: 41 male, 16 female; age 35-70 years old, average (56.29 ± 5.71) years old; pathological type: whoi type 0, who type II 29, who type III 28; UICC stage in 2002: stage III 46, IV A 11. In the observation group, there were 43 males and 15 females, aged 32-70 years with an average age of (55.16 ± 5.88) years, 0 whoi, 31 who II and 27 who III. In 2002, UICC was divided into 45 patients in stage III and 13 in stage VA. There was no significant difference in general data between the two groups. The follow-up time of both groups was 5 years.

2.2 Inclusion criteria
Inclusion criteria: 1. patients with NPC confirmed by clinical symptoms and signs, chest X-ray, abdominal B-ultrasound, bone ect, CT or MRI and pathological sections; 2. patients without contraindications to conventional cineole and IMRT; 3. patients can tolerate chemotherapy; 4. patients without other malignant tumors; 5. patients without mental disorders and mental disorders. Exclusion criteria: 1. those who do not meet the above inclusion criteria; 2. those who give up treatment halfway.

2.3 Treatment
The observation group was treated with IMRT: 6 MV x-ray was used to design a 7-9 field Eucalyptus limonensis plan, the dosage DT was 70Gy, 5 times a week, a total of more than 6 weeks, a total of 32 times Eucalyptus limonensis. The control group was treated with two-dimensional Eucalyptus limonensis: the dose was 70Gy, 5 times a week, a total of 35 times. At the same time, chemotherapy was given: 80mg / m² cisplatin (DDP) was given intravenously for 2 days; 500mg / m² 5-FU, D1-5 was given intravenously every 3 weeks.

Under general anesthesia, all patients were operated by the same physician under nasal endoscopy. The inflammatory lesions were removed, the normal mucosa was preserved, and the operation cavity was filled with calcium alginate dressing. Antibacterial treatment: 3-4 days before operation and 5-7 days after operation, cefuroxime sodium was infused intravenously, with an average of 4 g per day; then cefuroxime capsule was orally taken for about 1 week, 0.1 g per day. Nasal cleaning: clear the dressing of calcium alginate on the third day after operation, and then clean the nasal cavity with normal saline once a day, morning and evening, for 2 consecutive months; after each cleaning, spray the nose with budesonide hydrochloride twice a day, twice a day, once a week later, once a day, for 3 consecutive months. The patients in the control group were treated with the above-mentioned antibacterial treatment and nasal cleaning, and did not take the Eucalyptus limonin enteric capsule. On the basis of the above antibacterial treatment and nasal cleaning, the patients in the treatment group took Eucalyptus limonensis enteric coated capsules before operation, 300mg each time, three times a day, for 3-4 days continuously; the patients in the treatment group resumed taking 300mg each time, three times a day, for 4 weeks continuously, and then changed to 300mg each time, twice a day, for 2-4 weeks continuously.

2.4 Test method
2.4.1 IL-8 level determination
Radioimmunoassay was used for the determination. The kit was provided by CIS company of France. The specific experiment was carried out in strict accordance with the instructions on the kit.

2.4.2 Determination of TGF - β 1 level
Enzyme linked immunosorbent assay (ELISA) was used for the determination. The kit was produced by Shanghai Xinyu Biotechnology Co., Ltd. in strict accordance with the instructions on the kit.
2.4.3 Determination of T lymphocyte subsets

The mononuclear cells were separated by the lymphocyte layering solution with the specific gravity of 1.077:1 (produced by Shanghai Guyan science and Technology Co., Ltd.), washed with Hanks solution for 2-3 times, cultured in 1640 medium, adjusted the cell concentration level to $2.0 \times 10^6$ / ml, took out the partial cell smear, dried it and placed in the incubator with the temperature of 37°C overnight, and then Store in refrigerator. The air dried smear was fixed for 5min, dried and washed with PBS for 2-3 times, then anti-CD4/8 monoclonal antibody was added, anti mouse IgG and apaab complex were added, then alkaline phosphatase substrate was added to fix red for staining, and glycerin gelatin was used to seal the smear. Under high power microscope, 200 nucleated cells (colored red) were counted as positive cells.

2.4.4 Adverse reactions

Acute response and late injury were evaluated according to RTOG / EORTC.

2.5 Observation indicators

The 5-year survival rate and recurrence, the incidence of adverse reactions, and the changes of serum TGF-β1, IL-8 and T lymphocyte subsets before and after treatment were compared between the two groups.

2.6 Statistical methods

The data in this study were all treated with SPSS18.0 statistical software. The 5-year survival rate, recurrence and adverse reaction rate were expressed as percentage. Serum TGF-β1, IL-8 and T lymphocyte subsets before and after treatment. The water level is expressed in the form of $\bar{x} \pm s$, and the inspection level is $\alpha = 0.05$.

3. Results

3.1 Comparison of 5-year survival rate and recurrence of 2 groups of patients

The patients without regional recurrence and tumor-free survival were significantly higher than the control group (P<0.05). The local recurrence rate of the observation group was significantly lower than that of the control group (P<0.05). The observation group had no distant metastasis survival rate. Greater than the control group (P>0.05), see Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Regional recurrence free survival</th>
<th>Tumor free survival</th>
<th>Survival without distant metastasis</th>
<th>Local recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>57</td>
<td>43(75.44)</td>
<td>38(66.67)</td>
<td>42(73.68)</td>
<td>17(29.82)</td>
</tr>
<tr>
<td>Observation group</td>
<td>58</td>
<td>54(93.10)</td>
<td>49(84.48)</td>
<td>44(75.86)</td>
<td>3(5.17)</td>
</tr>
<tr>
<td>$X^2$ value</td>
<td></td>
<td>5.402</td>
<td>3.927</td>
<td>1.029</td>
<td>7.771</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.027</td>
<td>0.041</td>
<td>0.088</td>
<td>0.015</td>
</tr>
</tbody>
</table>

3.2 Comparison of the incidence of adverse reactions in two groups

The total incidence of adverse reactions in the control group was 40.35% (23 / 57) and the observation group was 34.48% (20 / 58). There was no significant difference in the incidence of adverse reactions between the two groups (P > 0.05, $X^2 = 1.203$), as shown in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Oral cavity</th>
<th>Digestive system</th>
<th>Blood system</th>
<th>Skin</th>
<th>Total occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>57</td>
<td>5(8.77)</td>
<td>7(12.28)</td>
<td>6(10.53)</td>
<td>5(8.77)</td>
<td>23(40.35)</td>
</tr>
<tr>
<td>Observation group</td>
<td>58</td>
<td>4(6.90)</td>
<td>6(10.34)</td>
<td>5(8.62)</td>
<td>5(8.62)</td>
<td>20(34.48)</td>
</tr>
</tbody>
</table>

3.3 Comparison of serum TGF - β 1 and IL-8 levels before and after treatment in two groups

The levels of TGF - β 1 and IL-8 in the two groups after treatment were significantly lower than those before treatment (P < 0.05), and the decrease of the above indexes in the observation group was significantly greater than that in the control group (P < 0.05), as shown in Table 3.
The ostium of the nasal sinuses is enlarged, the symptoms of nasal obstruction are significantly improved, the bacterial growth environment in the nasal sinuses is changed, and the growth of bacteria is inhibited, but there is no significant improvement in the cilia transport function, wound cleaning and healing of the nasal mucosa, and the normal ventilation and drainage function of the nasal cavity were restored significantly.

Conventional two-dimensional Eucalyptus limonensis has a high incidence of side effects, such as bone marrow inhibition, oral mucosal inflammation, parotid gland injury, occlusion difficulties and other reactions. It is difficult to further increase the target radiation dose. In recent years, the newly introduced IMRT is a relatively advanced Eucalyptus limonensis technology, which can not only ensure the target dose, but also protect the normal tissue, and reduce the incidence of Eucalyptus limonensis reaction.

The epithelium of paranasal sinuses is the first line of defense against the external damage of the nasal cavity and paranasal sinuses. It can pass through the physical barrier mechanism, mucociliary clearance system and damage repair mechanism, mainly mucociliary clearance system to remove pathogenic factors and avoid the pathogenic factors stimulating the immune cells under the skin, so as to protect the columnar epithelial cells of the nasal mucosa and reduce the hyperplasia of submucous glands. When cilia or mucous membrane dysfunction, mucus discharge is blocked, mucus is over secreted, and mucus is rich in lipopolysaccharide or proinflammatory factors, thus forming a vicious cycle, leading to airway inflammatory response. When the lesion was removed completely, the structure and mucosal function of the nasal cavity and sinuses were not damaged, the normal ventilation and drainage function of the nasal cavity were maintained, and the structure and function of the operative cavity were restored significantly. However, kemppainen and others believed that frequent surgical treatment was not conducive to the improvement of mucosal epithelization and symptoms. Some studies have shown that after the removal of the inflammatory lesions in the nasal sinuses, the nasal cavity is in an open state, the ostium of the nasal sinuses is enlarged, the symptoms of nasal obstruction are significantly improved, the bacterial growth environment in the nasal sinuses is changed, and the growth of bacteria is inhibited, but there is no significant improvement in the cilia transport function, wound cleaning and healing of the nasal mucosa. Therefore, it is the beginning of epithelial regeneration after operation, and the quality of postoperative treatment has a great influence on the effect of operation.

The results showed that the local inflammatory recovery and the recovery of mucociliary clearance system were the important factors affecting the mucosal epithelization after operation. Inflammatory reaction on the one hand led to the increase of gland secretion, on the other hand, it affected the function of mucociliary cells in...
mucociliary clearance, and further affected the clearance and defense function of mucociliary clearance system\[12-14\]. Therefore, it is an important method to eliminate inflammatory reaction and restore mucociliary clearance system. Eucalyptus limonene and pinene enteric coated capsule is a new generation of slime promoting drug. It is composed of Eucalyptus olein, limonene and α-piocene. It is widely used in the treatment of respiratory diseases. Among them, eucalyptus oil essence has analgesic, anti-inflammatory, antibacterial and antiasthmatic effects; limonene has antiinhibitory, expectorant and antibacterial effects, α-piocene has antiinhibitory, expectorant and antifungal effects[15]. By adjusting the proportion of the three components, it can make them play a role in coordination in the body, so as to alkalize the mucus, regulate the pH of the mucus to return to the normal value, reduce the viscosity of the mucus, and play the role of diluting the mucus; or by playing the β-sympathetic effect, stimulate the cilia to swing, enhance the activity of the cilia, and play the role of promoting the mucus removal; or by adjusting the composition proportion of the mucus blanket, ensure the mucus blanket In order to break the vicious circle of inflammatory reaction, promote the recovery of mucociliary clearance system function, and adjust the mechanism of mucociliary clearance defense[16-17]. It can improve the symptoms of bleeding, swelling of mucous membrane, excretion of mucus and inflammatory reaction, promote the epithelization of the operating cavity, reduce the growth of vesicles, granulation and polyps, and significantly improve the postoperative recovery and reduce the recurrence. Hang Wei et al. Studied the clinical effect of Eucalyptus limonensis enteric coated capsule in the treatment of rhinosinusitis. The results showed that eucalyptus limonensis enteric coated capsule can significantly improve patients' subjective feelings and objective indicators, significantly improve patients' nasal congestion, enhance nasal defense function and reduce infection[18].

In recent years, it has been found that many tumor cells can inhibit the growth of tumor cells by autocrine or paracrine TGF - β 1, and promote the growth of tumor cells. The results showed that the serum TGF - β 1 level of NPC patients was significantly higher than that of normal people (P < 0.01). 1. TGF - β 1 can inhibit the immune system and make tumor cells escape from the “immune surveillance” of the body, which is conducive to the formation of tumor. 2. the body's responsiveness to TGF - β 1 decreased. Under normal circumstances, TGF - β 1 can inhibit the expression of c-myc gene after transcription and transcription, make the cell growth stagnate at the G1 stage level, and inhibit the formation of tumor. Once the body's response to TGF - β 1 is reduced, the c-myc gene can be over expressed, and the dephosphorylation of RB protein and p53 protein is also relieved, resulting in excessive cell growth and malignant transformation. 3. TGF - β 1 can cause the over expression of VEGF, promote tumor angiogenesis, increase the synthesis and degradation of extracellular matrix (E (m), provide a good local environment for the rapid growth and metastasis of tumor cells, and lead to the progress of cancer.

IL-8 is a cytokine produced mainly by monocyte macrophages. The results showed that the level of serum IL-8 in patients with NPC was significantly higher than that in normal people (P < 0.01). The mechanism of the increase could be the stimulation of monocytes and epithelial cells in patients' own growth, which is basically connected with the literature. Near.

The results showed that the CD4 / CD8 ratio of c4t cells decreased and the number of d8 cells increased in NPC patients, which indicated that there was a disorder of cellular immune function in NPC patients. Moreover, the level of TGF - β 1 was negatively correlated with (D4 cells, (D4 / D8 ratio), and positively correlated with (D8 cells number. Under normal conditions, tgf-ftgf - β 1 and T cells inhibit and coordinate with each other, and they are in a dynamic balance. Because of the increase of TGF - β 1 level, the cellular immune function is more disordered. Tumor cells can escape the monitoring and elimination of cellular immunity, which is conducive to the occurrence and development of tumor.

IMRT has been recognized and applied in clinic. In general, the two-dimensional Eucalyptus limonensis program is designed according to the practical experience of doctors. The selection of the direction, energy and dose weight of the radiation field is also determined according to the experience of doctors. Then the dose distribution of Eucalyptus was calculated and thecurative effect was evaluated to determine the final scheme. It can be seen from this that this Eucalyptus project needs to be repeatedly tested, so as to finally select an "acceptable Eucalyptus project", which increases the difficulty of work and the risk of adverse reactions. The treatment scheme design function of IMRT's Eucalyptus and limonine plan system is reverse. The eucalyptus and limonine plan adopted can be determined according to the expected results of the treatment. Moreover, this scheme can also screen hundreds of Eucalyptus and limonine plan schemes by computer, and finally select the eucalyptus and limonine plan with the best effect. One of the most important reasons for failure is that after regular radiation therapy, there will still be local residual or recurrence. The anatomical structure of nasopharynx is very complex, so it is difficult to effectively avoid the complications caused by irradiation of normal tissues when increasing the dose of target irradiation. The IMRT technology developed in the 1990s is mainly based on the reconstruction function of CT simulator and 3D treatment planning system to construct the three-dimensional image of the target volume and the surrounding oar, which can more accurately irradiate the tumor target volume, so as to fully protect the oar; the reverse treatment planning system can make the
distribution of high dose curve and the shape of the tumor target volume. It can effectively reduce the radiation dose and surface area of the surrounding oar.

In recent years, clinical research has found that TGF-β1 is a tumor cell promoting factor, which is mainly manifested in: (1) TGF-β1 has an inhibitory effect on the immune system, making tumor cells escape the "immune supervision" of the body, so as to promote the growth and reproduction of tumor cells; (2) TGF-β1 can promote the over expression of VEGF, accelerate tumor angiogenesis and the secretion of extracellular matrix (ECM). Obviously increased, thus for the growth and reproduction of tumor cells to create a very ideal living environment, accelerate the proliferation and reproduction of malignant tumor cells. Interleukin-8 (IL-8) is a kind of cytokine produced by monocyte macrophage, which can act on the process of neutrophil chemotaxis, degranulation and T cell chemotaxis. The level of the above-mentioned inflammatory factors in the serum of patients with NPC is significantly higher than that of normal people. The possible mechanism of the increase of the level of the inflammatory factors is the stimulation of monocytes and epithelial cells during the growth of patients themselves. The number of CD4+ T cells and CD4/CD8 water in the serum of patients with NPC was significantly lower than that of normal people, indicating that there was a disorder of cellular immune function. The study also showed that TGF-β1 was negatively correlated with CD4 cell number and CD4/CD8, and positively correlated with CD8 cell number. Under normal circumstances, TGF-β1 and T cells are in a dynamic balance, which is in mutual inhibition and coordination. Due to the increase of TGF-β1 level, the cellular immune function is more disordered. Tumor cells can escape the monitoring and elimination of cellular immunity, which is conducive to the occurrence and development of tumor.

5. Conclusion

The results of this study showed that the levels of TGF-β1 and IL-8 in the two groups after treatment were significantly lower than those before treatment (P < 0.05), and the levels of CD3, CD4 and CD4/CD8 in the observation group were significantly higher than those in the control group (P < 0.05), and the levels of CD8 after treatment were significantly lower than those before treatment (P < 0.05). The results showed that the levels of TGF-β1, IL-8 and T-lymphocyte subsets could be effectively changed by IMRT and two-dimensional Eucalyptus.

In conclusion, IMRT has a significant effect on NPC, which may be achieved by improving the levels of serum tgf-31, IL-8 and T-lymphocyte subsets.

References


