

The effect of three-dimensional conformal radiotherapy on locally recurrent nasopharyngeal carcinoma and on the expression of succinate dehydrogenase B

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Abstract. – **OBJECTIVE:** Investigate the effect of three-dimensional conformal radiotherapy (3DCRT) on locally recurrent nasopharyngeal carcinoma (NPC) on the expression of succinate dehydrogenase B (SDHB).

PATIENTS AND METHODS: Eighty-six patients diagnosed with locally recurrent NPC in our hospital were selected and divided into the control group (43 cases) and observation group (43 cases). Conventional two-dimensional radiotherapy was applied in the control group, and 3DCRT was adopted in the observation group. The curative effect of both groups was compared.

RESULTS: The effective rate and the degree of alleviation of the observation group were higher than those of the control group, and the differences were statistically significant ($p < 0.05$). There were no differences in the occurrence rate of complications from radiotherapy between the two groups ($p > 0.05$). The survival rate and median survival time of the observation group were significantly higher than those of the control group ($p < 0.05$). The positive expression rate of SDHB in the observation group after radiotherapy was significantly higher than that of the control group ($p < 0.05$), and the median survival time of patients with positive expression of SDHB was significantly higher than patients with negative expression ($p < 0.05$).

CONCLUSIONS: 3DCRT applied for treatment of locally recurrent NPC was safe and effective. It also improved the positive expression rate of SDHB, which was associated with increased survival time.

Key Words:

Three-dimensional conformal radiotherapy, Nasopharyngeal carcinoma, Succinate dehydrogenase, Median survival time.

Introduction

Nasopharyngeal carcinoma (NPC) is a form of a malignant tumor with high prevalence in the head and neck. It is sensitive to radiotherapy, but 50-65% of NPC are in the middle-advanced period when treated, and the three-year recurrence and metastasis rate is about 60-75%, with poor prognosis¹. Three-dimensional conformal radiotherapy (3DCRT) effectively improved the dose to the target area, destroyed components of tumor cells to the greatest extent, reduced damage of normal tissue, and reduced occurrence of adverse reactions². Recent studies³ showed that glucose metabolism of NPC cells could play an important role in proliferation, differentiation, and invasion of tumors. Succinate dehydrogenase B (SDHB) is located in the inner mitochondrial membrane and is an important component of the mitochondrial respiratory chain. Deficiency of SDHB results in a disorder of energy metabolism of the tricarboxylic acid cycle, which regulates tumor occurrence⁴. Several studies⁵⁻⁶ have shown that the rate of mutation of the SDHB gene was high in pheochromocytoma and paraganglioma. Based on this observation, the present study evaluated the effect of 3DCRT on locally recurrent NPC and the expression of SDHB, to identify new targets for the improvement of cancer treatment.

Patients and Methods

Patients

From January 2012 to January 2015, 86 patients diagnosed with locally recurrent NPC in our hos-

pital were selected. The inclusion criteria included: 1. NPC was pathologically diagnosed by nasopharyngeal CT or MRI, and fiber nasopharyngeal biopsy; 2. Patients were diagnosed with locally recurrent NPC and over 1 year passed since patients were initially treated with radiotherapy; 3. There were no distant metastases. The exclusion criteria included: 1. Complications with other primary malignant tumors, such as metastatic tumors of the nasal cavity; 2. Complications from radiotherapy did not resolve; 3. Complications with basic diseases, such as of the heart, lung, brain, liver, kidney and other organ dysfunctions and bone marrow transplantation; 5. Incomplete medical history.

This study was carried out under the approval of the Ethics Committee of our hospital, and all patients signed the informed consent for contrast-enhanced ultrasonography examination. The eighty-six patients were divided into the control group (43 cases) and observation group (43 cases) by parity digit at the end of admission number. The control group consisted of 23 men and 20 women; aged from 40-75 years old with an average age of 51.6 ± 17.5 years; the recurrence time was 13-20 months, with an average of 15.5 ± 7.3 months. There were 20 cases of differentiated squamous cell carcinoma, 10 cases of non-keratinizing carcinoma, seven cases of undifferentiated carcinoma, five cases of adenocarcinoma, and one case of other carcinomas. The observation group consisted of 22 men and 21 women; aged from 40-80 years old, with an average age of 53.4 ± 18.6 years; the recurrence time was 14-22 months, with an average time of 15.5 ± 7.3 months. There were 18 cases of differentiated squamous cell carcinoma, 13 cases of non-keratinizing carcinoma, six cases of undifferentiated carcinoma, five cases of adenocarcinoma, and one case of other carcinomas. There were no significant differences in the comparisons of general parameters between the two groups ($p > 0.05$).

Methods

The two groups of patients were treated by the same radiotherapy and nursing team. Two-dimensional radiotherapy was adopted in the control group and 3DCRT was applied in the observation group. The specific methods were as follows: 1. Position and fixation: the supine position, a headrest, and a thermal plastic mask were adopted for fixation. Basic information was recorded on the mask. The mask, surface, and pillows were marked with a relative location marker. Setup errors were minimized within 2-3 mm; 2. CT scan-

ning: the scan center was determined in the simulator. Under the three-dimensional laser lamp, projection on the skin was tagged with a metal wire to ensure recognition of scanned images. From the top of the head to under the clavicle (3 cm), reinforced and continuous helical scanning with a thickness of 3 mm was adopted to scan images, transmit them to the workstation, and reconstruct them with Inspace software. Target volumes of organs of interest were maximized and sketched to reduce the position error; 3. Program design and implementation of 3DCRT: according to the results of sketching, the treatment design was made. Prescription dose and critical organ dose were determined, target volume was covered by 90% dose, and dose difference was controlled within 3%. Doses of important tissues and organs were controlled within acceptable ranges. Coplanar or non-coplanar irradiation methods were taken with 5-7 conformal fields, under the premise of meeting the requirements of the prescribed dose. Segment number was minimized and exposure time was shortened. A multi-leaf collimator (1 mm thickness) was produced and verification of simulation was made in the linear accelerator. After ensuring treatment parameters were correct, radiotherapy was given five times per week at 2.0 Gy/dose. The total treatment time was 6-7 weeks and the total dose was 60-70 Gy.

Observational Indexes and Evaluation Methods

The effects of radiotherapy, adverse reactions, follow-up median survival time, and survival rate were compared. The evaluation of effects of radiotherapy were with reference to WHO standards as follows; complete remission: lesions and clinical symptoms disappeared completely and were maintained for over 4 weeks; partial remission: the reduction of tumor size was $> 50\%$ and maintained for over 4 weeks; no remission: the reduction of tumor size was $< 50\%$, tumor metastasis occurred and was increased in size. Adverse reactions mainly included mucosal inflammation, injury of cranial nerves, and hearing loss.

The positive rates of expression of SDHB in tissue before radiotherapy were compared. Tissue specimens were obtained by endoscopy. Immunohistochemistry was applied for detection and the kit was from Zhongshan Jinqiao (Biological Co., Ltd., Beijing, China). The SDHB monoclonal antibody was from Sigma-Aldrich (St. Louis, MO, USA). PBS was used instead of primary antibody as the negative control.

Table I. The comparison of radiotherapy effects and complications [cases (%)].

Group	Cases	Complete remission	Partial remission	No remission	The effective rate	Mucosal inflammation	Injury of cranial nerves	Hearing loss	Incidence of complications
The control group	43	4 (9.3)	19 (44.2)	20 (46.5)	23 (53.5)	2	2	2	6 (14.0)
The observation group	43	12 (27.9)	20 (46.5)	11 (25.6)	32 (74.4)	3	1	1	5 (11.6)
χ^2		6.639	4.086				0.104		
p		0.036	0.043				0.747		

Manufacturer instructions were strictly followed. The positive expression of SDHB was mostly located in the cytoplasm, which showed as light brown to dark brown granules. Five high power fields (200 $\times$) were randomly selected for analysis. Criteria of positive results: (1) Cells without staining, 0 points; (2) Cells were stained pale yellow, 1 point; (3) Cells were stained yellow, 2 points; (4) Cells were stained brown, 3 points. Judgment was made based on the number of positive cells: (1) The number of positive cells was < 5%, 0 points; (2) The number of positive cells was 5-25%, 1 point; (3) The number of positive cells was 26-50%, 2 points; (4) The number of positive cells was 51-75%, 3 points; (5) The number of positive cells was > 75%, 4 points. The two kinds of points were added; 0 points meant negative (-), 1-4 points meant weakly positive (+), 5-8 points meant moderately positive (+ +) and 9-12 points meant strongly positive (+ + +).

Statistical Analysis

SPSS19.0 software (SPSS Inc. Chicago, IL, USA) was used for data analysis. Measurement data are presented as $\bar{x} \pm s$ and t -test was applied for statistical analyses. Count data are presented as ratio and χ^2 -test was used for statistical analyses. Rank sum test was applied for ranked data, Kaplan-Meier model, and log-rank χ^2 -test was applied for analysis of survival. $p < 0.05$ was taken as statistically significant.

Results

The Comparison of Effects of Radiotherapy and Complications

The effective rate and the degree of alleviation in the observation group were higher than in the

control group, and the differences were statistically significant ($p < 0.05$). There were no differences in the occurrence rate of complications from radiotherapy between the two groups ($p > 0.05$) (Table I).

The comparison of Median Survival Time and Survival Rate

The follow-up time of the two groups was 10-45 months and the average time was 35.2 ± 10.3 months. The survival rate was 51.2% (22/43) in the observation group and 30.2% (13/43) in the control group. The difference in median survival time between the two groups was statistically significant ($\chi^2 = 3.903$, $p = 0.048$). The survival rate and median survival time of the observation group were significantly higher than those of the control group (30.5 months and 24.8 months, respectively, log-rank $\chi^2 = 10.632$, $p < 0.001$).

The Comparison of Positive Rate of Expression Of Succinate Dehydrogenase B In Tissue Before And After Radiotherapy

There were no significant differences in the comparison of the positive rate of expression of SDHB in tissue before radiotherapy ($p > 0.05$). After radiotherapy, the positive expression rate of SDHB in the observation group was significantly higher than in the control group ($p < 0.05$) (Table II).

The Relationship Between Positive Expression of Succinate Dehydrogenase B and Survival Time

After radiotherapy, the median survival time of patients with positive expression of SDHB was significantly higher than patients with negative expression (38.0 months and 25.0 months, respectively. Log rank $\times 1 = 174.760$, $p < 0.001$) (Figure 1).

Table II. The comparison of the positive rate of expression of succinate dehydrogenase B in tissue before and after radiotherapy [cases (%)].

Group	Cases	Before radiotherapy		After radiotherapy	
		0-4 points	5-12 points	0-4 points	5-12 points
The control group	43	31 (72.1)	12 (27.9)	28 (65.1)	15 (34.9)
The observation group	43	30 (69.8)	13 (30.2)	18 (41.9)	25 (58.1)
χ^2		0.056		4.674	
p		0.812		0.031	

Discussion

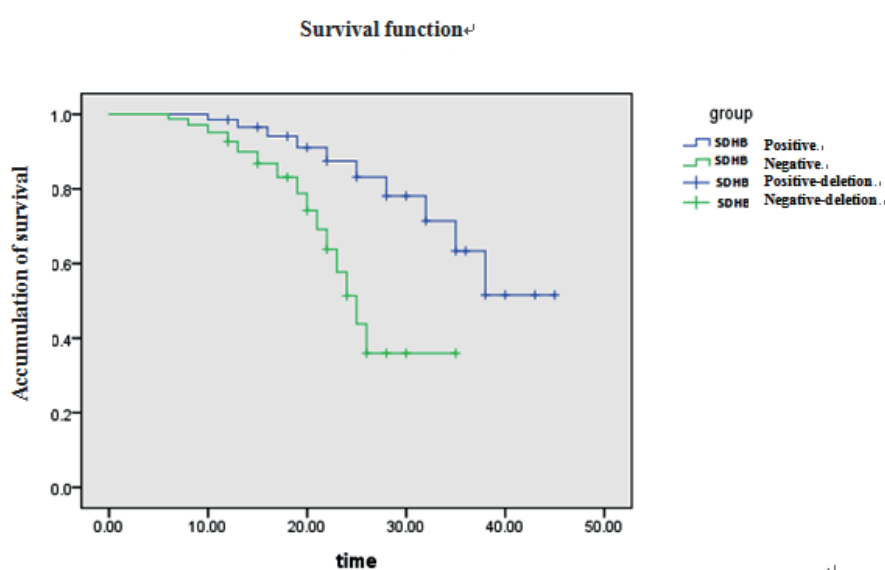
The Treatment of Locally Recurrent Nasopharyngeal Carcinoma

Because of limitations of surgery and chemotherapy, radiotherapy is the main method for treatment of NPC with non-remote metastasis⁷. After treatment, the tumor recurrence rate is high and has become a key issue in the treatment of NPC after controlling radiotherapy. For cases with larger tumors, common doses of conventional radiotherapy cannot kill tumor tissues. Additionally, conventional radiotherapy has some drawbacks, which may cause tumor recurrence. For example, to reduce the radiation dose to the brainstem and spinal cord, a low dose was used, which was generally used in the spinal cord near the higher recurrence zone of the tumor. This was one of the reasons that caused tumor recurrence⁸.

Because of the limitation of vision, repeated and high doses of irradiation on the cheek gland, optic nerve and temporomandibular joint resulted in severe complications from radiotherapy⁹.

Imaging technology has developed such that there are now techniques that involve an irradiation field that exclude healthy tissues, such as small fields, multi-fields and projective techniques with multi-direction¹⁰. 3DCRT has been increasingly used for the treatment of various malignant tumors¹¹. The technology can make the distribution shape of the irradiation field and the shape of the target area similar, meet the required coverage dose of the target area, and greatly improve the accuracy of radiotherapy. Therefore, it boasts the advantages of accurate positioning, precise planning and precise radiotherapy¹².

The technology not only enables high-dose radiotherapy on sites with easily recurrent tumor

**Figure 1.** The relationship of the positive expression of succinate dehydrogenase B and survival time.

development in proximity to the brainstem and spinal cord, but also reduced repeated high-dose irradiation on the parotid, optic nerve, temporomandibular joint and other organs. The injury was thereby reduced, which was important for improving the quality of life of patients with NPC¹³. We concluded that the curative rate of radiotherapy in the observation group was 74.4%, the complete remission rate was 27.9%, the survival rate was 51.2%, the median survival time was 30.5 months, and the occurrence rate of complications from radiotherapy was 11.6%. 3DCRT had higher safety and effectiveness.

The Effect of Three-Dimensional Conformal Radiation Therapy on the Expression of Succinate Dehydrogenase B

SDHB is part of the cytochrome oxidase complex, which is bound to the inner mitochondrial membrane and composed of four subunits (A, B, C, and D). Succinate dehydrogenase links oxidative phosphorylation to electron transport and is a marker that reflects mitochondrial function¹⁴. Mutations in SDHB reduced its activity, which resulted in a metabolic disorder of the tricarboxylic acid cycle. This also resulted in inhibition of prolyl hydroxylase, activation of HIF-1 α , increased expression of platelet-derived growth factor receptor, TGF- β 1, and epidermal growth factor receptor, which resulted in the formation of pheochromocytoma and paraganglioma^{15,16}.

3DCRT can target SDHB and reduce expression of SDHB, thus effectively prolonging the survival time of patients¹⁷. We determined that the positive expression rate in the observation group after radiotherapy was significantly higher than that of the control group, and the median survival time of patients with positive expression of SDHB was significantly higher than in patients with negative expression.

Conclusions

The application of 3DCRT on locally recurrent NPC was safe and effective, and it improved the positive expression rate of SDHB, which was associated with increased survival time.

Conflicts of interest

The authors declare no conflicts of interest.

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