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ORIGINAL ARTICLE

Relationship between cyclooxygenase-2 (COX-2) content and prognosis in nasopharyngeal carcinoma before and after radiochemotherapy

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Summary

Purpose: *To identify new effective prognostic indicators for patients with nasopharyngeal carcinoma (NPC).*

Methods: *The immunohistochemical staining method was used to detect cyclooxygenase-2 (COX-2) protein in tumor tissues of 100 patients before and after chemoradiation. All the patients had stage III poorly differentiated NPC.*

Results: *The positive expression of COX-2 was decreased before and after chemotherapy. The expression levels of COX-2 in patients before treatment was associated with T stage ($p<0.05$). The changes in the positive expression of COX-2 following treatment was also associated with T stage ($p<0.05$). The clinical response rate (CR+PR) exhibited significant differences ($p<0.05$) in patients with negative, weakly positive, partially positive, and strongly positive COX-2 expression before treatment. The clinical response rate (CR+PR) exhibited significant differences ($p<0.05$) compared with the patients who were negative or weakly positive. The COX-2 positive expression level of patients with NPC before treatment was closely associated with the survival time and survival rate of the patients ($p<0.05$). The changes of COX-2 positive expression were associated with the survival time and survival rate ($p<0.05$) in patients with NPC following treatment. T stage, COX-2 expression before treatment and changes in COX-2 expression after treatment were independent factors affecting NPC prognosis ($p<0.05$).*

Conclusion: *Changes in COX-2 expression levels before and after treatment may be a useful indicator for assessing the prognosis of NPC after chemoradiotherapy.*

Key words: *circulating tumor cells, Cox-2, nasopharyngeal carcinoma, prognosis*

Introduction

It is commonly agreed that NPC is a multigene hereditary disease involving the interaction between multiple genes or between genes and the environment, which may be caused by multi-factorial interactions and long-term effects [1]. Modern medical research has indicated that the occurrence and development of NPC are closely associated with genetic factors, viral infection, biochemical environment, geographical distribution, dietary habits and individual differences [2].

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Due to the hidden location of the nasopharynx, patients with NPC often have no symptoms at the early stage of disease. The proportion of early diagnosis and early treatment is low, while the 5-year survival rate of patients with advanced NPC is estimated between 30 and 50% [3]. The main reason for the poor prognosis of patients is local recurrence and distant metastasis. Local recurrence and distant metastasis are still the main factors affecting the prognosis of this disease. The occurrence and development of NPC are a complex and multi-step process. The evolution of this disease is the result of the combined action of various factors inside and outside the body. Recent studies have shown that the Epstein-Barr virus (EBV) infection, genetic factors, and environmental factors are all associated with the occurrence of NPC [4]. Previous studies have shown that cyclooxygenase- 2 (COX-2) is overexpressed in several tumors. This enzyme participates in tumor cell proliferation, angiogenesis, invasion and metastasis and is closely related to tumor occurrence, development, and prognosis [5].

In the present study, patients with stage III NPC were selected and unified and treated with standardized radiotherapy and chemotherapy. Immunohistochemical methods were used to qualitatively detect changes in COX-2 positive rate in tumor tissues after radiotherapy and chemotherapy in patients with NPC . The aim of this study was to investigate whether the protein expression of COX-2 is a prognostic factor for NPC patients.

Methods

Clinical data

A total of 100 patients were included in this study. They all had stage III (T1N2M0, T2a-2bN2M0, T3N0-2M0) poorly differentiated squamous cell NPC and were treated at the hospital from 2008 to 2012. Other stages were excluded at the time of diagnosis. All patients had pathologically clear fibronasal microscopy biopsy. The initial examination included medical history and physical examination (including complete head and neck examination and indirect nasopharyngoscopy if clinically indicated), nasopharyngeal and skull base to clavicle-enhanced magnetic resonance imaging (MRI) and enhanced computed tomography (CT), chest MRI and/or CT, abdominal ultrasound, blood routine and biochemical examinations. NPC staging was determined based on the American Joint Committee on Cancer (AJCC) NPC TNM staging system, sixth edition.

Inclusion criteria

1. All patients were diagnosed with NPC by biopsy, and the initial treatment of TNM stage III (T1N2M0, T2a-2bN2M0, T3N0-2M0) was determined by imaging, such as head and neck MRI, chest radiography and abdominal ultrasound.
2. The blood, urine and stool routine examination of all patients were basically normal before treatment and no apparent ulcers were noted in the oral cavity. The patient general condition could tolerate radiotherapy and chemotherapy.
3. KPS score ≥80 points.
4. Before treatment, the patients were informed of the possible efficacy and risks of treatment, and possible toxic and side effects. The patients agreed to receive chemoradiotherapy and signed the radiotherapy and chemotherapy consent form under the guidance of the physician.

Exclusion criteria

The following exclusion criteria were used:
1. Patients with staged cancer other than stage III.
2. All non-nasopharyngeal cancer patients.
3. Presence of other malignancies.
4. People with severe cardiovascular and cerebrovascular diseases.
5. Pregnant or lactating women.
6. Subjects who refused to take a suspicious tissue biopsy under nasal endoscopy.

Main reagents and instruments

An immunohistochemical method was employed for determining the positive rate of COX-2 expression in tumor tissues before and after chemoradiation. The following reagents were used: mouse anti-human COX-2 monoclonal antibody, diaminobenzidine (DAB) color development kit, phosphate buffered saline (PBS), citrate buffer, alcohol, IHC antigen retrieval and antibody dilution were purchased from Shenzhen Newbond Biotechnology Co., Ltd, Shenzhen, China.

The nasal endoscopic imaging system purchased from STORZ, Germany, was used as the main instrument for immunohistochemical determination of COX-2 expression in tumor tissues before and after chemoradiation. The paraffin embedder, virtual section scanner, CUT4060 rotary paraffin sectioner and bio-optical high-power microscope were all from Eltra, Germany. The MDF 382 -70°C ultra-low temperature refrigerator and 0°C refrigerator were from Sanyo, Japan. Ordinary refrigerators and microwave ovens were from Haier Group, China. The ultra-pure water treatment machine was from USA5415R. The Eppendorf pipette was purchased from Eppendorf, Germany.

Method

Before radiotherapy and chemotherapy, bronchoscopy was used to remove lesions or suspicious tissues in each NPC tissue, and paraffin blocks were made within 48 h following formalin fixation. Paraffin blocks were made into specimen sections and each one of them was selected as five pathological sections. At the 1st 3rd, 6th and 12th month and the 5th year after the end of chemoradiotherapy, the NPC tissues were obtained, stained, and the changes in the positive rate of COX-2 were observed. In case the primary NPC was no longer visible to the naked eye after treatment, the last microscopical observation of COX-2 expression that was recorded in

solid tumor specimens was used for the analysis. The specific staining steps were as follows:

(1) A rotary microtome was used to make a 5 μm-thick slices, followed by (2) xylene dewaxing, (3) gradient alcohol dehydration, (4) antigen repair solution (PBS) and rinsing for 5 min repeated three times. (5) Microwave high-temperature and high-pressure antigen retrieval was performed for 2 min and subsequently the samples were allowed to stand at room temperature for 20 min. (6) Incubation with 3% hydrogen peroxide was used to eliminate endogenous peroxidase activity, 3 times, 5 min each time. (7) The primary antibody was added dropwise and incubated overnight at 4°C with uniform distribution. (8) The samples were rinsed with PBS for 5 min, 3 times and (9) the secondary antibody was added for 30 min. (10) The samples were rinsed for 5 min, 3 times, and (11) freshly prepared DAB solution was added dropwise and allowed to react for 5 min. (12) Finally, the samples were rinsed in tap water, counterstained with hematoxylin, dehydrated with alcohol, baked dry and sealed with neutral gum.

Five high-power areas of normal mucosal tissues and 5 high-power areas of NPC tissues rich in cancer cells were selected under light microscope according to the Axiotis score [6]. The cell cytoplasmic expression was evaluated under high magnification (×400) by the ratio of positive cells. The comprehensive staining intensity was measured in a semi-quantitative way. The staining intensity scoring criteria were the following: 0 points uncolored; 1 point yellow; brownish yellow 2 points; yellow-brown 3 points. The scoring standard for the proportion of positive cells (the proportion of positive expression in NPC tissue) was as follows: number of positive cells <10% 1 point; 10% to 50% 2 points; 50% to 75% 3 points;> 75% 4 points. Finally, the points obtained by the two measurement methods were multiplied and the scores were estimated to 0, 1, 2, 3, 4, 6, 8, 9, and 12. If the result was 0, the expression was negative (-). ; 1 to 2 points were recorded as weakly positive (+), 3 to 6 points were recorded as positive (++); 8 to 12 points were recorded as strongly positive (+++). In this test, two pathologists counted the sections separately. When the scores were inconsistent, the data from a third pathologist were used.

Contrast design

As a self-control, the positive rate of COX-2 expression in tumor tissues before radiotherapy and chemotherapy was compared with the positive rate of COX-2 expression in tumor tissues after radiotherapy and chemotherapy.

Assessment criteria of the change in expression levels after COX-2 treatment

1. The positive expression of COX-2 was decreased as follows: from strong positive to partial positive (+++ → ++), strong positive to weak positive (+++ → +), and strong positive to negative (+++ →-). Some positive samples became weakly positives (++ → +) and negatives (++ →-), whereas some weak positive samples became negatives (+ →-). These changes were collectively referred to as positive COX-2 expression after treatment. The decline was described as follows:
2. Persistent positive: persistent strong positive, persistent partial positive and persistent weak positive. These changes were collectively referred to as COX-2 persistent positive after treatment;
3. Continuous negative: COX-2 expression was negative before and after treatment, which was defined as continuous negative.

Statistics

The experimental data were uniformly analyzed using the SPSS Statistics 20.0 software. The measurement data were expressed as mean±standard deviation. Spearman correlation analysis was also performed; the results were analyzed by the x2 test and one-way analysis of variance (ANOVA). $P<0.05$ was considered as statistically significant.

Results

The expression levels of COX-2 in nasopharyngeal carcinoma tissues following immunohistochemical staining.

Before radiotherapy and chemotherapy, 100 cases of NPC tissues were collected, of which negative COX-2 expression was noted in 7 cases (Figure 1)

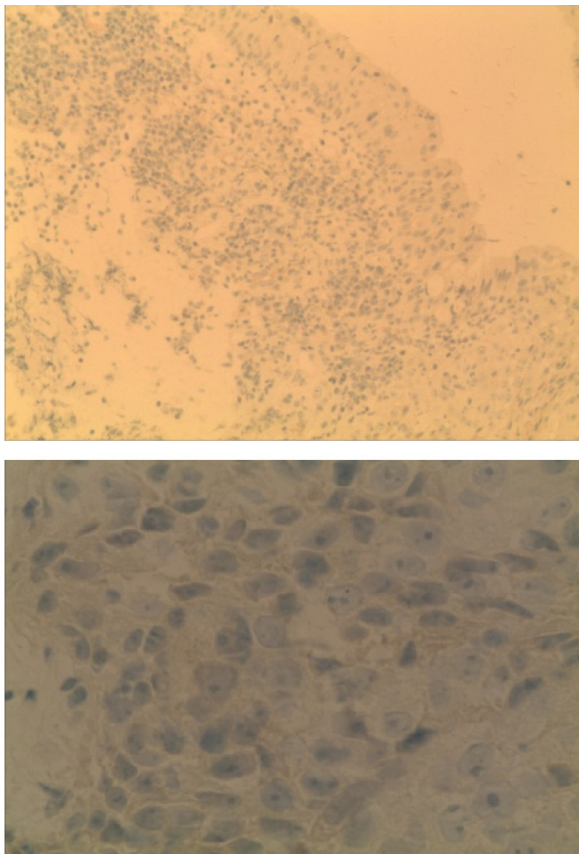

Figure 1. Negative expression of COX-2 in nasopharyngeal carcinoma tissue. **A:** ×400, **B:** ×100.

and positive expression in 93 cases (Figure 2). The positive expression rate was 93.00% (93/100) (Figure 3). COX-2 protein was mainly located in the cytoplasm of NPC cells and around the necrotic foci. Brown-yellow particles were diffusely distributed throughout the cytoplasm or linearly along the periphery of the nuclear membrane following COX-2 positive staining.

Association in the expression changes before and after COX-2 treatment with gender and T stage

The positive expression of COX-2 in patient with NPC before and after treatment was determined. The positive rate of COX-2 before treatment was 93%, of which 91.04% was noted in male patients and 96.97% in female patients. No significant differences were

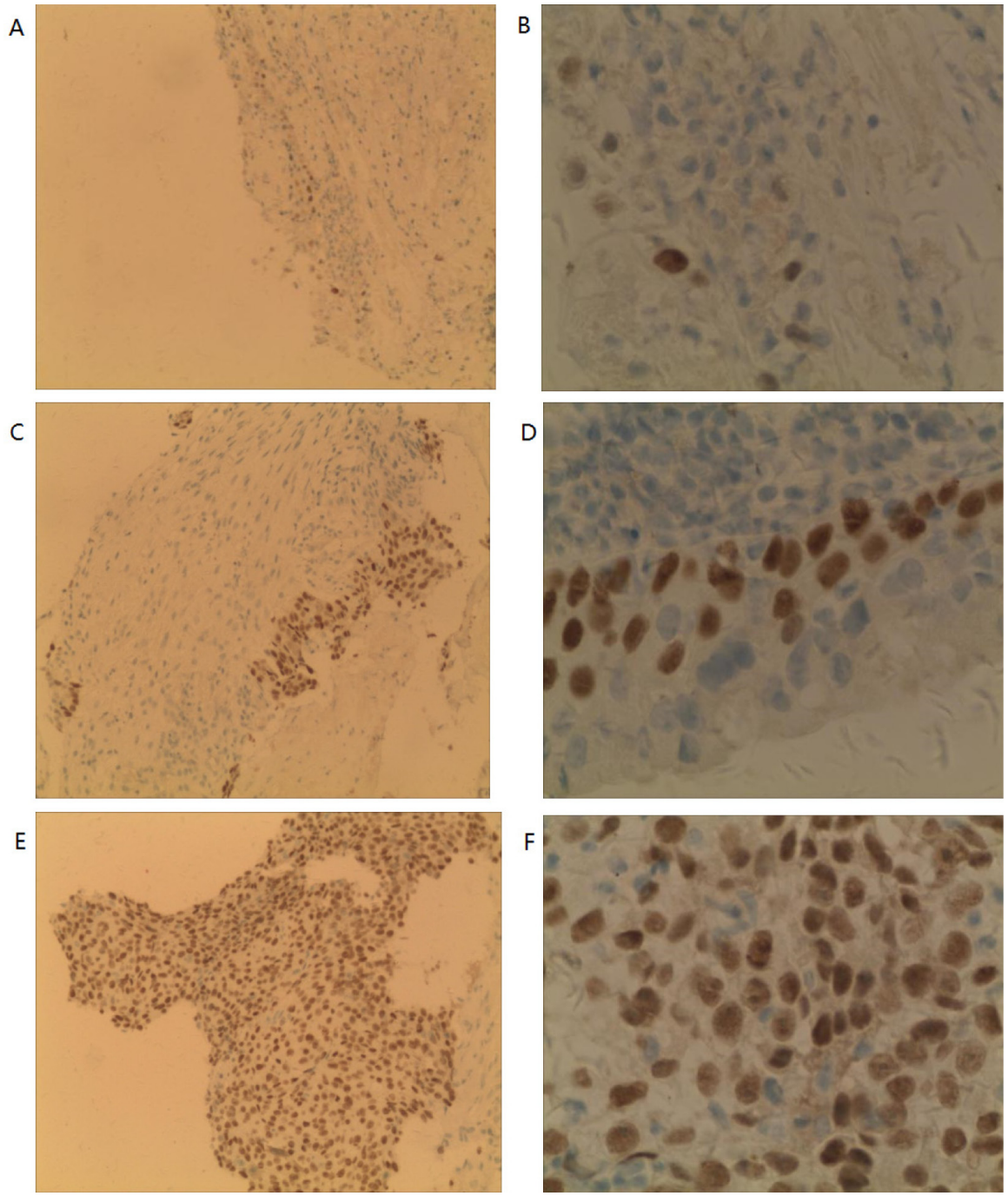

Figure 2. Positive expression of COX-2 in nasopharyngeal carcinoma tissues. **A:** COX-2 weakly positive expression in nasopharyngeal carcinoma tissues. Expression + (×100). **B:** COX-2 weakly positive expression in nasopharyngeal carcinoma tissues. Expression + (×400). **C:** COX-2 positive expression in nasopharyngeal carcinoma tissues. Expression ++ (×100). **D:** COX-2 positive expression in nasopharyngeal carcinoma tissues. Expression ++ (×400). **E:** COX-2 strongly positive expression in nasopharyngeal carcinoma tissues. Expression +++ (×100). **F:** COX-2 strongly positive expression in nasopharyngeal carcinoma tissues. Expression +++ (×400).

noted (p>0.05). The expression of pre-COX-2 positive rate was not associated with the patient gender.

The COX-2 positive expression rates in patients with T1n2m0, T2 n2m0, and T3n2m0 before radiotherapy and chemotherapy were 81.82%, 91.30%, and 97.67%, respectively. The COX-2 positive expression rates in the three T stage patients after radiotherapy and chemotherapy were 45.45%, 65.22% and 86.05%, respectively, whereas the positive expression rates were decreased by 36.37%, 26.08%, and 11.62%, respectively. The changes in the COX-2 positive rate before and after chemoradiotherapy were associated with T stage (Table 1).

After treatment, the positive COX-2 expression in the tumor tissue of patients with NPC was decreased and the distribution of persistent positive and persistent negative expression in male and female patients was not different (p>0.05).

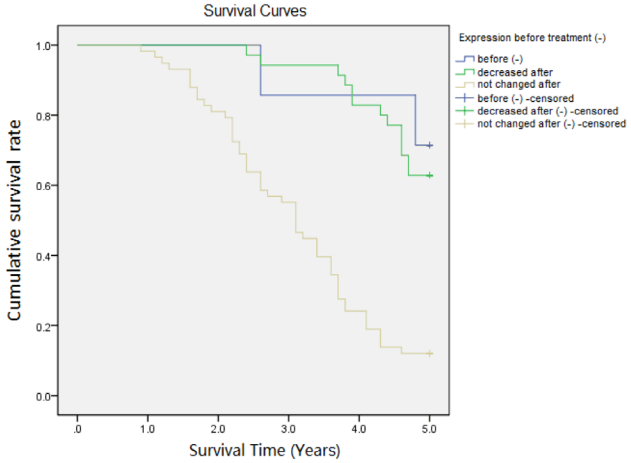

Figure 3. Overall survival of patients with decreased COX-2 expression, persistently positive patients and persistently negative patients after chemoradiotherapy were associated with changes in COX-2 positive expression (<0.01). Overall survival of patients with decreased COX-2 expression, persistently positive patients and persistently negative patients after chemoradiotherapy were associated with changes in COX-2 positive expression (<0.01).

The in the positive expression of COX-2 after treatment was also related to T stage. The higher the level of the T stage, the smaller the change in the positive rate of COX-2 after treatment. The T stage is a clear factor affecting the prognosis. It can be speculated that the change noted in the positive expression of COX-2 was related to the disease prognosis. Statistical analysis demonstrated that the changes in the expression of COX-2 exhibited significant differences in the distribution of patients with stages T1-3 of the disease in tissues of patients with NPC after treatment. The patients with the same degree of positive expression had mainly T3 stage tumors. The patients with T1 stage tumors were most likely to indicate changes in positive expression of COX-2 after radiotherapy and chemotherapy. The changes in COX-2 expression after treatment in patients with T2 tumors was intermediate compared with that in patients with stage T1 and T3 tumors. The expression changes between these different types of patients are shown in Table 2.

Association of COX-2 expression level changes before and after COX-2 treatment with toxic and side effects

The toxicity and side effects of the patients treated during follow-up are recorded in Table 3.

Positive to negative suggests that the immunostaining was changed from positive (+, ++, +++) to negative (-); continuous positive refers to staining before and after treatment. All refer to the following staining patterns:+, ++, or +++.

The proportion of patients with decreased COX-2 positive expression after radiotherapy and chemotherapy that experienced oral, skin and blood system side effects was 80.25%, 66.67%, and 90.48%, respectively. The positive levels of COX-2 expression remained unaltered before and after treatment. The proportion of patients with toxicity and side effects in the oral cavity, skin and blood system was 86.11%, 55.56%, and 91.67%, respectively (which was not statistically significant (p>0.05).

Table 1. Association between COX-2 expression and clinical characteristics before treatment

		Clinical expression					
Case characteristics	*n*	*Cox-2 expression of nasopharyngeal carcinoma tissue before radiochemotherapy*				*Fisher exact probability*	*Spearman correlation analysis*
Gender		0	+	++	+++		
Male	67	6	7	19	35	p=0.9203	r=0.216
Female	33	1	3	5	24		p =0.462
T staging							
T1	11	2	2	5	2	p<0.001	r=0.548
T2	46	4	5	16	27		p =0.0001
T3	43	1	3	9	30		

Association between changes before and after COX-2 treatment and recent response rate

Regular follow-up patients were included and statistical analysis was performed on the patient COX-2 expression status and recent remissions. The detailed analysis of the clinical data is reported in Tables 4 and 5.

The clinical response rates (CR+PR) of patients with negative, weakly positive, partially positive, and strongly positive COX-2 expression before the treatment were 100%, 90.00%, 67.50%, and 44.07%, respectively. Patients with different levels of COX-2 expression exhibited significant differences in clinical response rates, with a calculated p<0.001.

After treatment, the positive rate of COX-2 expression was decreased (including positive to negative, strong to partial or weak, and partial to weak to positive). The clinical response rate (CR+PR))

Table 2. Association between the positive degree of COX-2 expression after treatment and clinical cases

Clinicopathological features	*n*	*Cox expression changes after treatment*			*Fisher exact probability*	*Spearman correlation analysis*
		Decrease in positivity	*Continuous ang*	*Persistent overcast*		
Gender						
Male	67	22	39	6	p=0.8204	r=0.110
Female	33	13	19	1		p=0.428
T staging						
T1	11	8	1	2		r=0.572
T2	46	15	27	4	p<0.0001	p=0.0001
T3	43	12	30	1		

Table 3. COX-2 positive changes and toxicity after treatment

Expression change	*n*	*Side effects*			*Fisher exact probability*	*Spearman correlation analysis*
		Oral cavity	*Skin*	*Blood system*		
Decrease in positivity	21	17	14	19	p=0.1479	r=0.042
Positive degree unchanged	72	62	40	66		p=0.289

Table 4. COX-2 expression and recent remission status before treatment

Cox-2 expression before treatment	*n*	*Clinical response*				*Fisher exact probability*	*Spearman correlation analysis*
		CR	*PR*	*SD*	*PD*		
0	7	6	1	0	0	p<0.001	r=0.552
+	10	8	1	1	0		p=0.0001
++	24	15	5	2	2		
+++	59	14	12	26	7		

Table 5. Relationship between positive changes in COX-2 expression after treatment and recent response rates

Cox-2 expression after treatment:	*n*	*Gender*		*Clinical response*				*Fisher exact probability*	*Spearman correlation analysis*
		Male	*Female*	*CR*	*PR*	*SD*	*PD*		
Decrease in positivity	35	22	13	23	9	2	1	p<0.001	r=0.507
Positive degree unchanged	58	39	19	14	9	27	8		p=0.0001
Persistent negative	7	6	1	6	1	0	0		

was 80.95%, of which 23 patients achieved complete response (61.90%). The clinical response rate (CR+PR) was 51.39% in patients with positive COX-2 after treatment, of which 14 patients achieved complete response (33.33%). The data of the two groups were statistically analyzed and were significantly different (p<0.001).

Associations between changes before and after COX-2 treatment and five-year survival rate

Of the 7 patients with negative COX-2 expression before treatment, one died of accidental injury during the second year of follow-up. The censored data were processed for lack of survival and non-NPC incidence during follow-up.

The overall survival curves of patients with decreased COX-2 expression, persistently positive patients and persistently negative patients after chemoradiotherapy were combined and plotted by the SPSS software, as shown in Figure 3.

Survival analysis was performed using the Kaplan-Meier method, with an overall p<0.001, indicating a significant difference. Therefore, the survival time and survival rate of the patients with advanced NPC were associated with changes in COX-2 positive expression. Among them, negative expression of COX-2 before treatment in NPC patients was compared with decrease in COX-2 positive expression after treatment. These data demonstrated that these two groups exhibited the highest 5-year survival.

The pre-treatment (-) and post-treatment COX-2 positive expression subjects were combined into a group named group M. The subjects with continuous expression were defined as group N and the survival curve of MN is shown in Figure 4. The Kaplan-Meier method and log-rank test showed significant difference (p<0.001), suggesting that the 5-year survival of patients with positive COX-2 expression before and after treatment (-) was better than that with persistent positive COX-2 expression.

Survival analysis was performed on the relationship between different COX-2 positive expression rates and 5-year survival in patients before treatment, as shown in Figure 5. The Kaplan-Meier method plus log-rank test showed an overall p=0.001. The survival time and survival rate of cancer patients were associated with the expression of COX-2 in tumor tissues before treatment.

The pre-treatment (-) and pre-treatment (+) subjects were combined into the group A; the pre-treatment (++) and pre-treatment (+++) were combined into the group B and the survival curves of the two groups (A+B) are shown in Figure 6. The calculated p<0.001 indicated a significant differ-

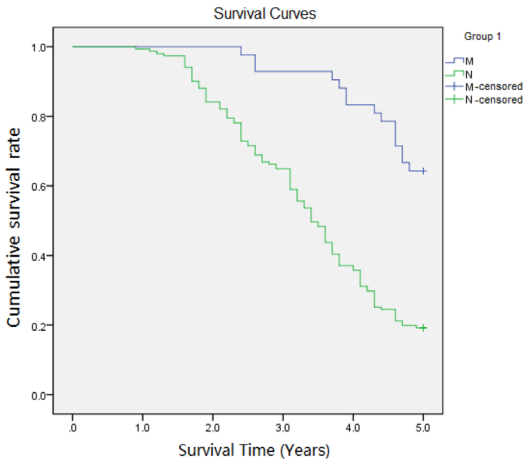

Figure 4. Survival curves of patients in Group M and group N. Group M was defined as the combination of pre-treatment (-) and post-treatment COX-2 positive expression subjects. The subjects with continuous expression were defined as Group N (p<0.001).

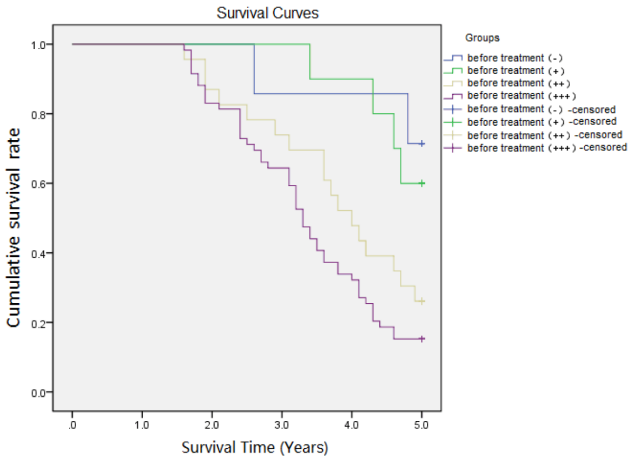

Figure 5. Survival curves with different levels of COX-2 expression in tumor tissues of patients with nasopharyngeal carcinoma before treatment (p<0.001).

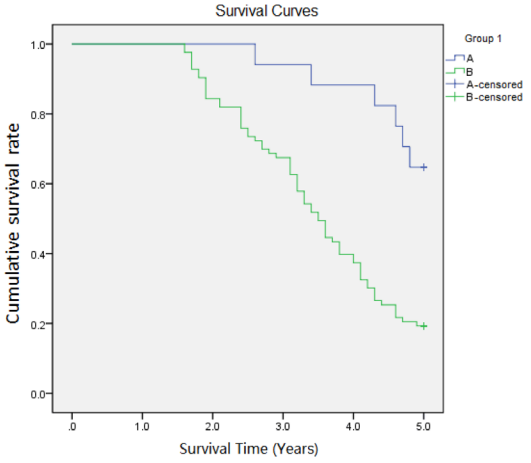

Figure 6. Survival curves of patients in Group A and B. Group A: before treatment (-) merged with before treatment (+); Group B: before treatment (++) merged with before treatment (+++) (p<0.01).

Table 6. Multivariate analysis of prognosis after chemoradiation in 100 NPC patients

Project	*B*	*SE*	*Wald*	*Df*	*p*
Age	0.282	0.963	0.089	1	0.772
Gender	0.053	0.068	0.925	1	0.941
T staging	1.581	0.624	6.607	1	0.036
Cox expression before radiotherapy and chemotherapy	1.631	0.735	4.189	1	0.041
Changes in cox expression after treatment	3.739	1.397	7.975	1	0.002

ence between the two groups. Therefore, the two levels of COX-2 expression before treatment (-) and after treatment (+) were good prognostic indicators. A predictor indicates that the 5-year survival of patients with such expression levels is more optimistic than that of patients with two COX-2 expression levels before treatment (++) and/or (+++).

Multivariate analysis of COX risk regression model

The parameters age, sex, T stage, COX-2 expression before treatment and CsOX-2 expression change after treatment of the 100 NPC patients were included into a Cox risk regression model. At multivariate analysis of survival COX-2 expression and changes in COX-2 expression after treatment were independent factors affecting the prognosis of NPC ($p<0.05$) (Table 6).

Discussion

The anatomical structure of NPC is deep and irregular and the majority of the tissue types are low-differentiated squamous cell carcinomas with lively biological characteristics. The tumor tissue lacks the stroma surrounding the cancer nest, so it is easy to invade surrounding organs and tissues. Approximately 17% of patients with NPC have intracranial metastasis [4] and the rate of cervical lymph node metastasis can reach from 67.14 to 81.26% [5]. The influencing factors of prognosis related to NPC can suggest more accurate treatments. Therefore, the individualized treatment for NPC patients can be targeted, which can greatly improve the clinical effect of patient prognosis.

This article selected patients with NPC who exhibited COX-2 expression changes with regard to the positive rate of tissue COX-2 expression. Moreover, patients with stage III NPC were included. A series of changes and prognostic factors of patient COX-2 expression levels before and after treatment were developed. In view of the fact that radiation therapy has been mostly used in the early treatment of NPC, the study of prognostic molecules related to the intermediate and advanced stages of this disease is highly required. Through this study, clinicians could select timely treatment options and can greatly improve the overall survival rate of patients with this malignancy

COX-2 expression and clinical characteristics

The present study demonstrated that before radiotherapy and chemotherapy, 93 out of 100 cases of NPC exhibited positive COX-2 protein expression and the remaining 7 cases exhibited negative expression. The positive expression rate was 93%. The COX-2 protein was mainly localized in the cytoplasm of NPC cells. Around the necrotic areas, brown-yellow particles were diffusely distributed throughout the cytoplasm or linearly along the periphery of the nuclear membrane following COX-2 positive staining. COX-2 was highly expressed in NPC and its expression was significantly higher than that of other noted malignant tumors. This result may be attributed to the anatomical location of NPC. COX-2 is an inflammatory response-related molecule and its role in tumors has been gradually clarified in recent years. The positive rates of COX-2 expression in the patients of the present study were high before treatment. Considering that the nasopharynx is an exposed anatomical structure, it constantly exchanges gas with the air. Dust particles and polluted gases in the air can continuously stimulate the open nasal cavity, thereby activating the overexpression of COX-2, thus leading to an overlap of the inflammatory and tumor factors. The causes of a high positive rate of COX-2 in NPC have been described previously [7]. The expression of COX-2 in tumor tissues of patients with NPC and the changes in the positive rate of COX-2 protein expression after treatment are not related with the patient gender. However, multiple factors depend on the gender. Epidemiological investigations have shown that the parameter gender affects the prognosis of NPC [8]. Moreover, the morbidity and mortality are related to the gender. The present study included 67 male and female patients. A total of 33 subjects were included in the present study, resulting in a relatively small sample size compared with that noted in large-scale epidemiological surveys, which include different number of

male and female patients. Therefore, the lack of large sample size and the difference in gender of the included group may cause bias in the experimental results. Therefore, a comprehensive study of the expression of COX-2 and prognosis-related factors, such as gender, age and clinical stage is a direction that can be used to explore the factors affecting the effective prognosis of NPC. The expression of COX-2 in tumor tissues of patients with NPC is an important biomarker than can be investigated in future studies with larger sample sizes. The positive expression rate of COX-2 was increased and this was associated with the increase in the T stage. The current study analyzed different COX-2 positive expression rates in tumor tissues of NPC patients nasopharyngeal carcinoma of different T stages before treatment and the results demonstrated that the positive expression rate of COX-2 was increased with the increase in the T stage. Gallo et al [9] and other similar studies demonstrated that the overexpressed region of COX-2 protein is correlated with the increased blood vessel density (Spearman product-moment correlation coeficcients (r)=0.450, p=0.007) and lymph node metastasis. The patient tumor microvessel density was considerably higher than that of patients with head and neck tumors without lymphatic metastasis. It was speculated that the content of COX-2 is positively correlated with lymph node metastasis. This experiment validated the conclusions drawn by Gallo et al and further supplemented information regarding the association of the expression levels of COX-2 with the T stage of the tumors examined.

COX-2 expression and side effects

The changes in the COX-2 positive expression rate noted in patients with NPC before and after radiotherapy and chemotherapy was not associated with the side effects of treatment. Tumor patients often exhibit different degrees of toxic and side effects after frequent chemotherapy and radiotherapy. The side effects of radiotherapy and chemotherapy can be alleviated in these patients. The side effects of treatment were mainly manifested in the functional damage of the digestive, central nervous and blood systems. At present, the clinical side effects of patients are generally estimated according to the selected chemotherapeutic drugs and doses. The present study examined the medical records of 100 NPC patients with oral mucosal damage, skin and blood system toxicity and radiotherapy treatment in association with the changes in COX-2 positive rate before and after chemotherapy and radiotherapy. A clear relationship was noted with the toxic and side effects of treatment.

COX-2 expression and recent remission

The decrease in COX-2 expression in patients with NPC after treatment was associated with recent remission. The present study demonstrated that the levels of COX-2 before treatment in patients with NPC were related to the short-term efficacy of these patients; the patients who had decreased levels of COX-2 after treatment were evaluated for the short-term efficacy of the chemoradiotherapy. A previous study by Chen et al [10] demonstrated that COX-2 before treatment was associated with the short-term remission of patients with NPC. In view of the association between the patient COX-2 expression and recent remissions after treatment, a limited number of studies have provided evidence suggesting the use of this enzyme as an effective prognostic factor for NPC.

COX-2 expression and 5-year survival rate

During the 5-year follow-up performed in the present study, the data indicated that the expression of COX-2 before treatment was associated with the 5-year survival. Chen et al [11] examined the prognostic value of COX-2 in 27 patients with NPC by multivariate analysis and demonstrated that the content of COX-2 and the 5-year survival rate of the patients were closely associated, which was consistent with the findings of the present study. The magnitude and speed of the decline in the expression of COX-2 protein in patients with NPC before and after treatment were closely associated with the 5-year survival rate. The decrease in the expression levels of COX-2 was associated with the 5-year survival rate of the patients. The faster the decline of the positive degree of COX-2, the higher the 5-year survival rate of the patients, suggesting that the change in COX-2 expression after treatment could be used as a prognostic predictor for patients with NPC.

Multivariate regression analysis revealed that T-stage, COX-2 expression levels before treatment and COX-2 expression changes after treatment were independent prognostic factors for patients with NPC.

COX-2 is not only found in tumors, but also in the blood and body fluids of normal subjects and non-cancer patients. The diagnosis of malignant tumors requires high specificity and sensitivity of tumor markers, which is not always possible. Therefore, it is not recommended to conduct general screening of asymptomatic people. At present, it is not possible to obtain a diagnosis of the disease based on COX-2 expression alone, which could be beyond the normal reference range. Therefore, this biomarker can only be used for the auxiliary diag-

nosis of malignant tumors. The practical significance and clinical application of COX-2 in the early diagnosis of NPC requires additional studies. The present study confirmed the association between COX-2 protein expression levels in patients with stage III NPC before and after treatment and their short-term remission rate, 5-year survival rate and associated toxicities.

In summary, the data indicated that the change of COX-2 expression levels before and after treatment of NPC patients may be a potentially useful indicator for assessing the prognosis of NPC after chemoradiotherapy.

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Conflict of interests

The authors declare no conflict of interests.

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