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Research Article

Relationships between ADC Value of Nasopharyngeal Carcinoma Lesion and Pathological Indices

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Abstract

Objective: To investigate the correlations between Apparent Diffusion Coefficient (ADC) values of patients with nasopharyngeal carcinoma (NPC) and the cell density, microvessel density or nuclear proliferation labeling index, and further study mechanisms of primary lesion's ADC variation of. **Patients and Methods:** Patients with locally advanced NPC treated with intensity modulated radiation therapy were enrolled from April 2010 to November 2011. MRI Conventional and DWI scans were performed at two time points: before therapy and during radiotherapy (just two weeks thereafter). The ADC values of the primary lesion were measured. Histological specimens were detected with hematoxylin and eosin staining and immunohistochemical staining. The cell density, microvessel density and nuclear proliferation index values of the primary lesions were measured. Finally, the relationships between the ADC value before therapy, the ADC difference between the two time point and those pathology indices were analyzed by correlation analysis. **Results:** There is negative correlation between the ADC value difference and the cell

density (r=-0.426，*P*=0.001), and a positive correlation between the ADC value difference and the microvessel density (r=0.429，*P*<0.001). However, there is not significant correlation between the ADC value difference and nuclear proliferation index (*P*=0.291). No significant correlation exists between the ADC values before therapy and the three pathological labeling indices (*P*>0.05). **Conclusion:** ADC variation of NPC may have correlations with the cell density and the microvessel density, which provides p athological mechanism for ADC value of prediction for prognosis of NPC received radiotherapy.

Keywords: Nasopharyngeal carcinoma; Diffusion weighted imaging; Apparent diffusion coefficient value; Cell density; Microvessel density; Nuclear proliferation index.

Introduction

With the development of diagnosis and treatment technology, nasopharyngeal carcinoma (NPC) therapy has significantly improved, anyhow，the local recurrence and distant metastasis rate is still high [1]. Prediction of the malignancy degree and treatment sensitivity early is very significant for the individualized treatment plan and its timely adjustment, and genes and signal transmission channel related to radiosensitivity were reported [2]. However, these studies are not suitable for clinical practice, and there are few viable and effective methods to predict nasopharyngeal carcinoma radiation sensitivity.

Magnetic Resonance Diffusion Weighted Imaging (MR-DWI) detects the motion state of to analyze the proliferation changes in the extracellular space and intracellular and extracellular water molecules in pathological state [3]. MRI as functional imaging plays an important role in the diagnosis and treatment of nasopharyngeal carcinoma [4, 5]. It is learned that DWI can tell the abnormality in the cellular and molecular level earlier than that in organization morphological level [6], and may become a new prediction method of radiation sensitivity of NPC, which is helpful for therapy direction and adjust.

It is reported that ADC value of hepatic carcinoma, bone tumors, prostate cancer, breast cancer [7-10] are closely related to pathological index. But there is not report about the relationship between nasopharyngeal carcinoma ADC value and pathological index. We conducted this study to investigate the pathological mechanism of NPC primary lesion’s ADC value change, and analyze the correlations between ADC values of DWI in patients with locally advanced NPC and pathological indice, including cell density, microvessel density and nuclear proliferation index.

Subjects and methods

Subjects

NPC patients in Fujian Provincial Hospital were enrolled from April 2010 to November 2011.

Inclusion criteria

Patients diagnosed nasopharyngeal carcinoma by pathologic biopsy in Fujian Provincial Hospital; patients with locally advanced (Tumor T3 & T4 stage) nasopharyngeal carcinoma (NPC); patients who were performed conventional and DWI MRI before treatment and 2 weeks after intensity-modulated radiotherapy; patients who agreed to accept the study.

Exclusion criteria

Patients who failed to finish intensity-modulated radiotherapy or failed to perform either MRI exam.

This study was approved by the Ethics Committee of the Fujian Provincial Hospital and written informed consent was obtained from all subjects prior to the interview and sample collection.

Instruments and reagents

Signa 1.5 T EXCITE III HD superconducting magnetic resonance imaging system from the United States G E company; OLYMPUS BX51 microscope and E-620 camera from Japan.

Reagents Maxim Company in Fuzhou: CD34 immunohistochemical monoclonal antibody (rat against human MAB-0034, batch number 111214034 F), Ki-67 immunohistochemical monoclonal antibody (rat against human MAB-0129, batch number 111227129 M), EliVison TM Super two-step KIT (KIT-9933, batch number 1202039933).

MRI scanning methods

GE Signa 1.5 T EXCITE III HD superconducting magnetic resonance imaging system and head and neck joint coil were used. The patients were performed regular and DWI scanning, positioning scanning range from sella turcica to the inferior margin of the seventh cervical vertebra. The scanning followed the following parameters: the spin echo-echo planar imaging (SE-EPI) sequence, b value 0 and 800 s/mm2, a single shot, 5 mm

layer thick, 1 mm layer spacing.

The ADC values measurement

The data collected from MRI scan were transferred to GE Adw workstation. The outline of the maximum tumor range was draw on the DWI image of the largest tumor section, and ADC values before therapy and two weeks from the beginning of radiotherapy were measured respectively.

Treatment

Patients all received Intensity modulated radiotherapy [1].

Individualized chemotherapy was carried out, including induction chemotherapy, concurrent chemoradiotherapy or both.

HE and immunohistochemical staining

All the biopsy specimens were soaked by formalin, embedded by paraffin, cut into slice within 2 um, heated at 60 °C for a whole night, and then dealt with HE and immunohistochemical staining.

Pathological index calculation

Cell density was observed in HE staining section. For each pathological section, five microscopic fields were chosen randomly in 400X OLYMPUS BX51 microscope, and were taken photos by OLYMPUS E-620 camera .The photos were transferred into computer and analyzed by IMAGE-Z analysis. The result that the tumor cell nuclear field area divided the whole field area was expressed as a percentage. The average of the five microscopic fields was recorded as the whole specimen cell density [11, 12]. Microvessel-density calculation was referred to methods reported by Weidner [13] and observed in CD34 immunohistochemical staining section. For each pathological section, five microscopic fields were chosen randomly in 200X OLYMPUS BX51 microscope, and were taken photos by OLYMPUS E-620 camera. Different from the tumor cell or connective tissue cell, an endothelial cell or cell clump was counted as a micro blood vessel. As long as the structure was not connected, the branch was counted as one as well. Transverse lumen of a microvessel contains less than 8 red blood cells. Microvessel number was counted for each photo, and the average of the five was recorded as MVD value. Nuclear proliferation index was observed in Ki-67 immunohistochemical staining section. For each pathological section, five microscopic fields were chosen randomly in 400X OLYMPUS BX51 microscope, and were taken photos by OLYMPUS E-620 camera. The average of the five was the final result. Ki-67 expressed in the cell nucleus, and the index was achieved as follows: no shading 0 point, light yellow 1 point, tan 2 points, fulvous 3 points. Score of the positive tumor cell proportion were achieved as the follows: positive cells less than 5% 0 point, 6-25% 1 point, 26%-50% 2 points, 51% - 75% three points, more than 75% four points. The multiplication of the two score was the final score [14].

Statistics

SPSS for Windows 17.0 statistical software package was used. Inspection significant level $\alpha = 0.05$, $P < 0.05$ is deemed to be statistically significant difference. Double variable Pearson correlation analysis method was adapted to analysis the ADC value and pathologic indice. The ADC value included the one before therapy and the difference between the two time points. Stepwise multiple linear regression analysis was also taken to analyzed the relationship between ADC values with the pathological index, pathology classification, chemotherapy or not, age, gender, T stage and so on.

Results

Totally 89 cases were enrolled initially, 3 cases changed to conventional radiotherapy and 7 cases failed to perform MRI at the right time. So there were 79 cases included for final analysis, ranged from18 to 79 years old, and the median age is 48. Seventy-three patients were diagnosed as non-keratinizing undifferentiated type squamous cell carcinoma, 4 non-keratinizing differentiation type of squamous cell carcinoma, and 2 keratinizing squamous cell carcinoma. Thirty three were T3 cases and 46 were T4 cases. Thirty five patients treated with pure induction chemotherapy, 29 patients were given concurrent chemoradiotherapy, 31 patients with induction chemotherapy plus synchronous chemotherapy, and 4 patients without chemotherapy.

Table 1. ADC values and ADC difference, pathological index data list

	ADC before therapy (x10-4mm2/s)	2 weeks' ADC (x10-4mm2/s)	ADC difference (x10-4mm2/s)	Cell density (%）	CD34	Ki-67 (point）
mean	8.15	13.32	5.16	54	66.46	6.89
StDve	1.28	2.44	2.33	19	23.94	3.18
Maximum	5.24	19.4	10.95	88	116	12
Minimum	12.1	9.1	1.68	4	18	0

Table 2. Bivariate ananlysis statistical results of ADC values difference and pathological index

	Cell density	CD34	Ki-67
r*	-0.426	0.429	-0.120
P	0.000	0.000	0.291

*: r, Correlation coefficient

ADC values and case index results are shown in Table1. Bivariate analysis was shown in Table 2 and Fig 1 (correction between ADC value difference and pathological index) and Table 3 and Fig 2 (correction between ADC value and pathological index. There is negative correlation between the ADC value difference and the cell density (r=-0.426, *P*<0.001), and positive correlation between the ADC value difference and microvessel density (r=0.429, *P*<0.01). However, there is not significant correlation between the ADC value difference and nuclear proliferation index (*P*>0.05) (Table 2). There are not significant correlations between the ADC values before therapy and the cell density, microvessel density and nuclear proliferation index values (*P* values were 0.896, 0.517, and 0.201, respectively) (Table 3).

Table 3. Bivariate analysis statistical results of ADC value (before radiotherapy) and pathological index

	Cell density	CD34	Ki-67
r*	-0.115	0.015	0.145
P	0.896	0.517	0.201

*: r, Correlation coefficient

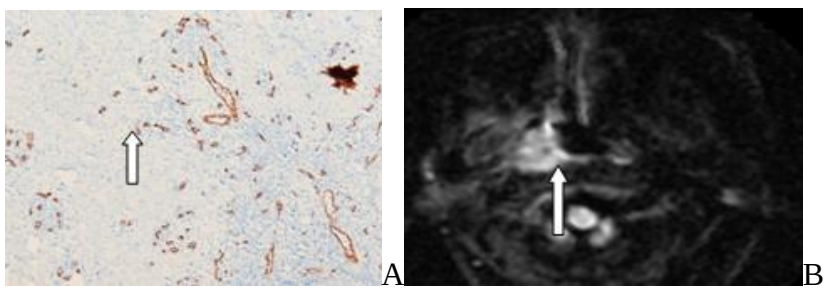

Figure 1. Male, 63 years old, non-keratinizing undifferentiated NPC. (A) for HE dyeing slice in the 400 X microscope. The white arrow shows the typical empty bubble tumor cells. The cell density is 0.53. (B) for MRI-DWI figure, the white arrow shows the high signal for NPC lesion, ADC value is 0.952 x10-3mm2/s.

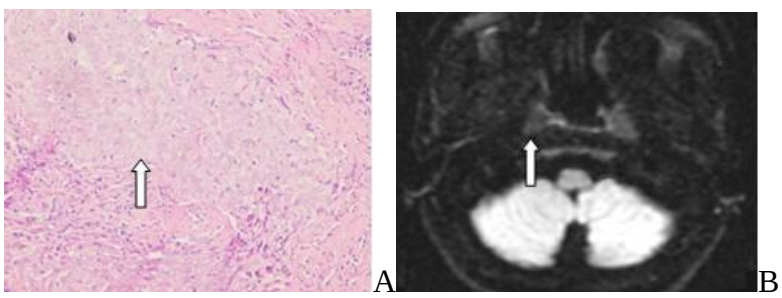

Figure 2. Female, 38 years old, non-keratinizing undifferentiated NPC. (A) for the CD34 immunohistochemical dying slice in 200 x microscope. The white arrow shows the typical microvessel.The MVD is 65.(B) for MRI – DWI. The white arrow shows the NPC lesion. The ADC value is 0.893x10-3mm2/s.

Linear correlations exist between ADC values before therapy and ages and T stage (Table 4). Linear correlations also exist between ADC value differences and ages, the cell density, microvessel density. However, there is not linear correlation between ADC value before therapy and gender, chemotherapy or not, pathological types, pathologic indices. There is not linear correlation between ADC value differences and gender, T stage, chemotherapy or not, pathologic types, Ki-67 (Table 5 and Figure 3) Therefore, the ADC value differences may reflect the cell density, microvessel density indirectly. The larger the difference is, the smaller the cell density is and the larger the microvessel density is.

Discussion

This research shows ADC value difference related to the cell density. As tumor cell density decreased, the ADC difference increased. The cell density decreased, and then the oxygen deficiency level decreased. Lack of oxygen is currently accepted as one of the important factors of sensitivity and curative effect of radiotherapy [15, 16]. The micro environment will change when lacking of oxygen, and tumor cells will adapt to a series of changes, which leads to the decline of radiotherapy sensitivity. The lower oxygen deficiency is, the higher the radiotherapy sensitivity is, the better the short-term effect is, and the ADC difference is larger. This study showed ADC value

Table 4. Multivariate linear regression analysis results between ADC values (before radiotherapy) and gender, age, T stage, respectively.

variable	β	SE	T	*P*	95% CI*
Age	0.027	0.01	2.721	0.008	0.007, 0.047
T stage	0.692	0.271	2.558	0.013	0.153, 1.231

*CI: confidential intervals

Table 5. Multivariate linear regression analysis results between ADC difference and gender, age, T stage, chemotherapy or not, pathologic types, pathology index, respectively.

variable	β	SE	T	*P*	95% CI*
CD34	0.033	0.01	3.449	0.001	0.014, 0.052
Cell density	-4.037	1.264	-3.539	0.001	-6.870, -1.922
Age	-0.036	0.016	-2.245	0.028	-0.069, -0.004

*CI: confidential intervals

before treatment had no relationship with cell density. This result is different from the close relationship between liver cell carcinoma, bone tumor, prostate cancer, breast cancer cell and ADC value [7-10]. It is analyzed that there were so many lymphocytes among NPC cells that ADC value may be influenced.

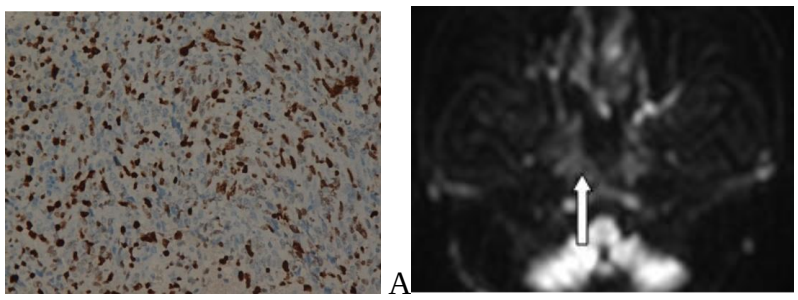

Figure 3. Male, 39 years old, non-keratinizing undifferentiated NPC. (A) for the Ki – 67 immunohistochemical dying slice in 400 x microscope. The nuclear proliferation index is 12. (B) for MRI - DWI .The white arrow for NPC lesion, and the ADC value is 1.12x10-3mm2/s.

This research showed that the ADC difference and microvessel density were positively correlated. The larger the short-term ADC value difference was, the higher the microvessel density was. Microvessel density is one of the most important indicators for forecasting tumor invasion, metastasis and recurrence, which was confirmed in variety of tumor [17, 18]. The radiation sensitivity of microvessel played an important role in the sense of radiotherapy, and destruction degree determined the total radiotherapy. It is studied that microvessel density had close relationship with radiosensitivity; and the higher the MVD was the higher radiation sensitivity was [19]. This study supported our research results. The higher the MVD was, the more sensitive radiotherapy was, and the larger the ADC value difference was.

Our studies have not yet found that ADC values and and Ki - 67 nuclear proliferation index had obvious correlation. Ki – 67 nuclear proliferation index represents the tumor biological behavior, whose performance was determined by a variety of factors (age, constitution, family history, etc.). And ADC value represents the imaging findings, which is also affected by many kinds of factors (breathing, age, tumor size, focal fibrosis/liquefied necrotic, etc.). The results of current study were consistent with other researches [20, 21], which also considered that there were no significant correlation between ADC values and Ki-67 nuclear proliferation index. Of course, it should be further confirmed by larger sample size studies.

In this study, linear correlations exist between ADC values before therapy and ages and T stage. Linear correlations also exist between ADC value differences and ages, the cell density, microvessel density. However, there is not linear correlation between ADC value before therapy and gender, chemotherapy or not, pathological types, pathologic indices. There is not linear correlation between ADC value differences and gender, T stage, chemotherapy or not, pathologic types, Ki-67.

It is considered that physiological state is different as the age is different. Extracellular diffusion, intracellular diffusion and water molecules' diffusion across the membrane are so different, which influences the diffusion, resulting in the influence of ADC.

Following imitations should be declared in this study. Nasopharyngeal carcinoma is unable to be cured by surgery due to its special anatomical structure. It is impossible to take pathology by complete excision, so only biopsy sample can be achieved. Due to the objective conditions, pathology inspection results from biopsy sample cannot conclude all information of the nasopharynx cancer lesion.

In conclusion, the short-term ADC relevant to tumor cell density and change in early radiotherapy of NPC is microvessel density. This may be the pathology foundation for ADC to predict NPC radiotherapeutic curative effect. This study provides the theoretical basis for MR – DWI, this non-invasive, simple and dynamic observing molecular imaging technique, to be applied to clinical prediction of nasopharyngeal carcinoma radiotherapeutic curative effect.

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