IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 14, Issue 5 Ver. VII (May. 2015), PP 104-109
www.iosrjournals.org

CT Quantitative Analysis Reflects the Degree of Ischemic Necrosis and the Level of Glucose Metabolism of Non-Small Cell Lung Cancer

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Abstract
Objective: *To evaluate the capability of CT quantitative analysis reflecting the degree of ischemic necrosis and glucose metabolism level of non small cell lung cancer.*
Methods: *52 NSCLC cases, measuring the ischemia necrosis CT quantitative value (INCTQ) for NSCLC tumor on early enhancement CT images before surgery. Tumor specimen expressions of glucose transporter (Glut1) carbonic anhydrase (CAⅨ) were detected by immunohistochemistry.*
Results: *The mean INCTQ values of expression of Glut1-negative, grade 1 and grade 2 were 0.35 ± 0.23, 0.52 ± 0.55 and 1.55 ± 1.20, respectively. It showed that higher INCTQ value associated with higher expression of Glut 1, F=10.6,p=0.000; The one-way analysis showed that group of co expression of Glut 1 and CA IX had significantly higher INCTQ value than single expression group and the mean INCTQ value of single expression group was higher than non expression group significantly also, F=4.449, p=0.017.*
Conclusion: *The early enhancement CT can be used to appraise the ischemia necrosis degree, and reflect the level of glucose metabolism of NSCLC which help to determine treatment plan for advanced NSCLC*
Keywords: *Non-small cell lung cancer; X-ray computed tomography; ischemia necrosis; Glucose transportation protein 1; Carbonic anhydrase Ⅸ*

I. Introduction

Early enhancement CT which can reflect the degree of tumor angiogenesis has been already proven by the various researches, but the level of tumor glucose metabolism can be reflected in early-enhancement CT has not been reported. The metabolism of malignant tumor cells is characterized by enhanced uptake and utilization of glucose, a phenomenon known as Warburg effect [1]. A large number of studies have reported that the cause of this phenomenon is due to rapid tumor growth, surpassing the rate of tumor angiogenesis, and hypoxia-induced tumor necrosis. HIF-1 is a major regulator of cell adaptation to hypoxic stress and plays a crucial role in tumor angiogenesis [2], As a result of decreased dependency on aerobic respiration, there is metabolic change to glycosis, including increased expression of glucose transporters (Glut1). Significantly, Glut1 act as primary marker for hypoxia [3]. Carbonic anhydrase IX (CAIX) is an HIF-1 target gene, whose product carbonic anhydrase has been explored as an endogenous hypoxia marker [4], Airley et al[5] detected various tissue types, tumors including non-small cell lung cancer by immunohistochemical staining of the tissue microarray, Glut 1 and CAIX expressed mainly in the perinecrotic areas in many tumor types [5, 6]. Importantly Glut1, CAIX over-expressed and associated with poor prognosis in an extensive range of tumors [7, 8]. The main purpose of this study was to explore whether the early enhanced CT can be used to evaluate the degree of tumor necrosis and the glucose metabolism level of non small cell lung cancer.

1.1 Materials and methods

Patients and specimens

Preoperative CT scan of 52 patients with non small cell lung cancer were collected at Affiliated Renmin Hospital of Jiangsu University in between December 2010 to May 2013. The patients age extent was 39 to 87 years, mean age is 61.6 years, 36 males and 16 females. Among them, 18 cases were diagnosed as squamous cell carcinoma, 31 cases as adenocarcinoma, and 3 cases as adenosquamous cell carcinoma. Paraffin embedded tissue specimens of NSCLC were obtained from the pathology department of the hospital. The study was approved by the ethics committee of our hospital.

Chest CT scan

We used the Siemens Sensation 64-slice CT system (Siemens AG,Muenchem,Germany), PHILIPS Brilliance iCT 256-slice CT system.The contrast agent was iohexol with iodine 300 mg/mL. We performed a plain chest scans. The CT scans were performed 35 seconds after injection with iohexol at the rate of 3ml /s and 1.5 ml/kg. The scan parameters were as follows: 120 kV, 120 mAs, a scan thickness of 0.5 or 0.625 and a reconstruction thickness of 3 mm.

Measurement of ischemic necrosis CT quantitative (INCTQ) value

Computed tomographic values were measured at the the center plane of the tumor axis section with the thickness of 3 mm and to evaluate the degree of necrosis, we examined both sagittal and coronal sections of contrasted images at the level of 50/250 (window centre/window width).The CT value of the ischemic necrosis (non enhanced area) was named as H_N, and ischemic necrosis size was named as S_N. The CT value of the non-ischemic necrosis (enhanced area) was named as H_C, and non-necrosis size was named as S_C (see Fig.1), the value of INCTQ is calculated as follows: $(H_C \div H_N) \times (S_N \div S_C)$.

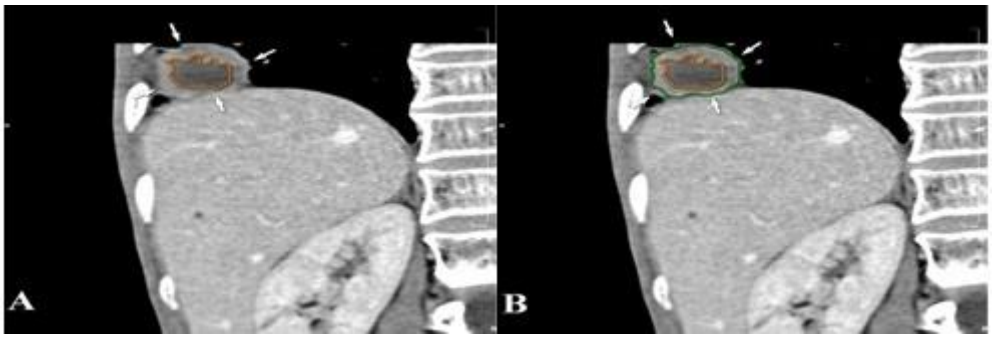

Fig.1. Measurement method of INCTQ on constrast-enchanced CT image of lung tumor; Three millimeter-thick CT image in the mass centre plane (white arrows point to the tumor): we observed the shape of the mass at 50/250 of windows centre and width, distinguishing between the enhanced region and non enhanced region. A, we drew the shape of non enhanced area with red line and recorded the CT value as (H_N) and necrosis size as (S_N).The enhanced area is the region between the red and green line; the CT value were recorded as (H_C) and non necrosis size as (S_C).

1.2 Histochemical examination

Rabbit anti-human Glut1 polyclonal antibody was purchased from USA bioworld companies. Rabbit anti-human CAIX polyclonal antibody was purchased from PROTEINTECH (Chicago, I11), Streptavidin , horse-radish Peroxidase and secondary antibodies were purchased from BOSTER (Wuhan, China). Paraffin embedding machine,Pathology slicing machine were purchased from Electronic Instrument Factory (Changzhou, China), Electric heat thermostat were purchased from Pharmacia Corporation (U.S.A), Stained section viewed with an optical microscope(Olympus CX41 Japanese type) and fluorescence microscopy (Olympus Corporation PX53 Japanese type), Pathological image analysis system was purchased from Kingstar Winning software (Shanghai, china)

All specimens were routinely fixed in 10% buffered neutral formalin and embedded in paraffin. Each section (3.0 μm) was stained with the standard hematoxylin and eosin method for screening review by experienced pathologists. The paraffin was removed from the slides by xylene, and the tissue was rehydrated in various concentrations of ethanol. Antigen retrieval was performed by placing slides in a high-pressure cooker in a citrate buffer, at 4℃ overnight; Endogenous peroxidase activity was blocked by incubating the section in 3% H_2O_2 for 10 min, followed by rinsing in PBS solution three times. Immunohistochemical staining was performed with the two-step EnVision. The sections were incubated with primary antibody at 4℃for 12 hours, followed by dextran polymer conjugated with horseradish peroxidase enzyme and secondary antibody at 37 ℃ for 30min. Slides were stained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) chromogen and counter-stained with hematoxylin. Negative controls were conducted by adding PBS solution. USA bioworld .BOSTER and PROTEINTECH provided section that were used as positive controls for staining)

Evaluation method of Glut1 and CA IX expression grade: The immunoreactivity of Glut1 and CAIX was scored according to the method of Ozbudak et al [9]. Glut1 and CA IX expression of tumor cells were classified from grade 0 to grade 2.The absence of the brown staining of the tumor cell membrane were assigned grade 0, $\leq$ 20% positive cells; grade 1, > 20% positive cells; grade 2. All the specimens were examined and scored by two independent pathologists .The entire specimens were observed under low-power magnification (10x10)

1.3 Statistical Analysis

Statistical Analysis was performed using SPSS 17.0 software (SPSS Inc Chicago,I11).The associations of Glut 1 , CA IX and INCTQ value were analyzed by using one way analysis of variance, if the variance was heterogeneity by test, the mathematical correction were performed, and then test with one way analysis, $p < 0.05$ was considered significant.

II. Results

2.1 NSCLC tissue expression of Glut 1

In 52 NSCLC cases, the expression of Glut1 were focal and membranous brown staining with a varying degree of cytoplasmic reactivity in tumor cells; positive tumor cells were localized mainly to the surrounding necrotic and microvascular areas. The percentage of Glut 1 expression was 71.2% (37/52), the number of expression grades 0, 1 and 2, were 15, 13 and 24 respectively.

2.2 NSCLC tissue expression of CA IX

In 52 NSCLC cases, the expression of CAIX were focal and membranous brown staining with a varying degree of cytoplasmic reactivity in tumor cells, positive tumor cells were mainly distributed around the necrotic areas. The percentage of CA IX positive expression was 48.1% (25/52); the numbers of expression grade 0, 1 and 2 were 27, 12 and 13 respectively.

2.3 Relationship of INCTQ and Glut 1 expression

The average INCTQ value in 52 NSCLC cases was 0.95 ± 1.03; the numbers of Glut 1 expression grade 0, 1, 2 were 15, 13 and 24 respectively. The corresponding average INCTQ values were 0.35 ± 0.23, 0.52 ± 0.55 and 1.55 ± 1.20,where high INCTQ value were significantly correlated with high Glut 1 expression, F = 10.633, P = 0.000. See Fig.2

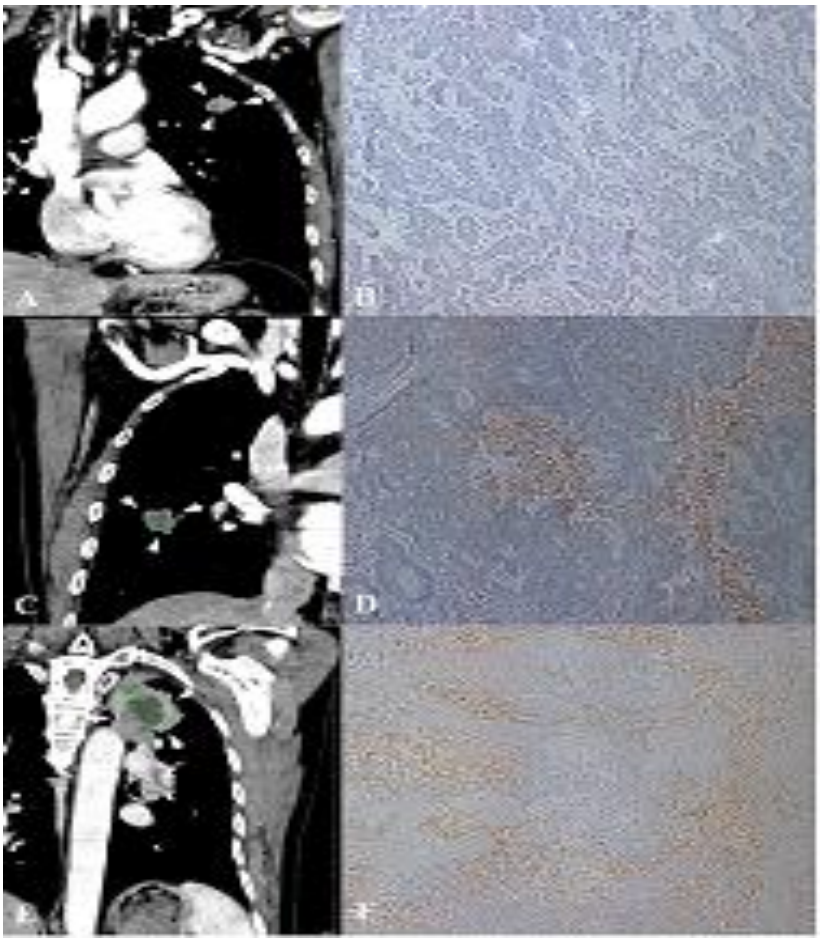

Fig.2. Correlation between INCTQ and Glut1 expression of non-small cell lung tumor(white arrows direct tumors) A, displays left upper lobe tumor with small, fairly well circumscribed foci of necrosis with slightly low density area (shown in green coil),The INCTQ value was 0.18 B, negative staining for Glut1 immunohistochemical staining(no brown staining) in the membrane and cytoplasm of the cancer cells(original

magnification10 ×10). C, displays right lower lobe tumor with large necrosis and low density area (shown in Green coil), INCTQ value was 0.53 D, Positive staining for Glut1 immunohistochemical staining (brown staining) in the membrane and cytoplasm of the cancer cells (original magnification10 × 10). Glut1 expression grade1 (20% +) E, display upper left para-mediastinum lung tumor with central large necrosis and very low density area (shown in green coil), INCTQ value was 2.7 F, positive staining for Glut1 immunohistochemical staining(brown staining) in the membrane and the cytoplasm of the cancer cells(original magnifictaion10 × 10).Glut1 expression grade 2 (90% +).

2.4 Relationship of INCTQ values and CA IX expression

In 52 NSCLC cases, the numbers of CA IX expression grades 0, 1 and 2 were 27, 12 and 13 respectively, the average of corresponding INCTQ values were 0.69 ± 0.77,1.10 ± 1.27 and 1.34 ± 1.18. There were no significant expression correlation between INCTQ value and CA IX expression, $F = 1.980$, $p = 0.149$. Fig.3

2.5 Relationship of INCTQ values and co expression of Glut 1 and CA IX

11 of 52 tumor samples stained negative for Glut1and CA IX expression, the INCTQ average value of this group were 0.32 ± 0.25; Twenty of 52 tumor samples stained positive for Glut 1 or CA IX expression, the INCTQ average value of this group were 0.85 ± 0.83; twenty-one of 52 tumor samples stained positive for co expression of Glut 1 and CA IX, INCTQ average value of this group were 1.37 ± 1.27. The INCTQ average value of these three group were significantly different, $F = 4.449$, $p = 0.017$. As shown in See Fig.3

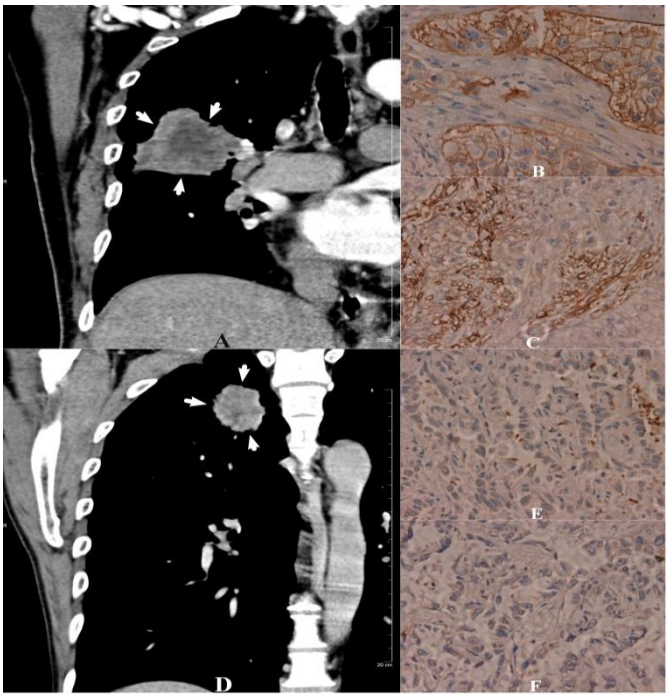

Fig.3. Correlation between INCTQ value with Glut1 and CAIX expression of non small cell lung cancer;(white arrows direct tumors) A, displays large irregular tumor with central necrotic low density area in right upper lobe , INCTQ value was 2.15, the number of Glut1 expression grade 2 (B:10 x 40), while CA IX expression grade 2 (C:10 x 40); D displays right upper lobe tumor with no significant low density area, INCTQ value was 0.21, Glut1 expression negative (E:10 x40) and CA IX expression was also negative (F: 10x 40)

III. Discussions

Necrosis is one of the characteristic features of malignant tumors; rapid growth of the malignant tumor is complemented by development of tumor vascularization. Hence most of the tumors encounter hypoxia early in their development and are more likely to undergo necrosis [10]. According to Airley et al Cancer cells require a steady source of metabolic energy in order satisfy the demand of uncontrolled growth and proliferation. Glucose transport and metabolism are indispensible for the post treatment survival of tumor cells, leading to poor prognosis [11] Glucose transporter 1 was expressed in varieties of epithelial malignancies. Over-expression of GLUT1 regulates mechanisms that support tumor growth at the expense of host tissues [12].Therefore; we examined GLUT1 expression, because higher levels of GLUT1 in cancer cells indicate a poor prognosis. Carbonic anhydrase (CA) is a type of zinc-containing metalloenzyme. In mammals at least 16 types of CA

isozymes have been described with different tissue distribution and catalytic properties.CA IX and CA XII are over-expressed in many tumors signifying that this is a common feature of cancer cells that may be required for tumor progression and metastasis [13]. In our study, we aimed to explore the expression of CAIX in NSCLC and its correlation with pathologic characteristics of NSCLC.

Early enhanced CT scanning is the modality to visualize malignant tumors blood supply that were usually greater in malignant tumors and cannot be distinguish in plain CT scan. Hypoxia necrosis areas in tumor where there is inadequate blood supply and no enhancement also shows low density in early enhanced CT images. The chest CT scans were obtained 35 seconds after injection of constrast agent for the measurement of the tumor necrosis degree .Our previous research found that necrosis detected in early enhanced CT images of breast cancer related to key hypoxia biomarkers expression status [14], this study was the further extension for the evaluation of hypoxia necrosis degree in NSCLC. We performed ischemic necrosis CT quantitative (INCTQ) analysis that included the size and density of hypoxia necrosis in NSCLC, the formula is an objective and comprehensive. Firstly, we need to take into account the density of hypoxia necrosis, the lower density correlates with the more severe necrosis; secondly, we measured the size of hypoxia necrosis areas, the larger the size of necrotic areas indicates the more severe hypoxia necrosis. Ganeshan [15]et al detected the texture of NSCLC lesion with a software named TexRAD, CT texture analysis can help quantify tumor heterogeneity by assessing the distribution of gray levels, heterogeneity is a recognized feature of malignancy, reflecting areas of high cell density, necrosis, hemorrhage, and myxoid change[16], Ganeshan [15]et al study demonstrated that high tumor heterogeneity correlated with excessive Glut 1 in NSCLC tissue, and increased level of glucose metabolism .The results of our study showed that INCTQ values for NSCLC had a strong correlation with the expression of Glut 1, p=0.000, and INCTQ values for NSCLC had no obvious correlation with CA IX expression. But INCTQ values were strongly correlated with co expression of Glut 1 and CA IX, F=4.449，P=0.017.

Hypoxia is an adverse biologic feature associated with an aggressive phenotype and resistance to both chemotherapy and radiation therapy [17, 18, 19]. Association between INCTQ value and tumor necrosis degree suggests potential clinical application for CT analysis in treatment of patients with NSCLC. This study contributed to the validation of INCTQ value analysis as a potential prognostic biomarker for patients with NSCLC. Furthermore, future trials could assess the feasibility of using INCTQ value analysis in the patients of NSCLC undergoing radiation therapy who would benefit from the use of hypoxia-modifying agents or a higher radiation dose to compensate for hypoxia-associated radio resistance.

Conflict of Interest

The authors declare that they have no conflict of interest.

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