CLINICAL VALUE OF COMBINING ^{18}F-FDG PET/CT SCANNING WITH SERUM LEVELS OF CYFRA21-1 AND HIF-1AFOR EARLY DETECTION OF LUNG CANCER

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ABSTRACT

Objective*: To explore the value of combining 2-Fluoro-2-Deoxy-D-Glucopyranose (18F-FDG) (Positron emission tomography/computer tomography, PET/CT) scanning with serum levels of (Cytokeratin 19 fragment antigen), Cyfra21-1 and (hypoxia inducible factor-1α, HIF-1α) αfor early detection of lung cancer.*

Method*: A total of 80 treatment-free patients with lung cancer confirmed through imaging, chemical pathology diagnosis and cytology in our hospital from January 2015 to December 2016 were placed in the early lung cancer group, while 25 others with benign lung diseases who reported in our hospital during the same period were placed in the control group. Serum levels of Cyfra21-1 and HIF-1α in both groups were quantified, and then 18F-FDG PET/CT scanning was done, so as to evaluate the clinical significance of both methods in early diagnosis of lung cancer.In a third approach, tissue levels of deoxyglucose and serum levels of Cyfra21-1 and HIF-1α were determined simultaneouslyusing18F-FDG PET/CT scanning.*

Results*: The scan revealed that the standard uptake value (SUV) of 80 patients with early lung cancer (10.51±4.23) was significantly higher than that of the control group (P <0.01). Levels of Cyfra21-1 and HIF-1α were also significantly higher than in the control group (P <0.01), and also significantly higher in patients with squamous cell carcinoma than in those with adenocarcinoma or small cell carcinoma (P <0.05). However, there were no significant differences in Cyfra21-1 and HIF-1α levels amongadenocarcinoma, small cell carcinoma and unclassified cancerpatients (P>0.05). The sensitivity, specificity, and accuracy of Cyfra21-1 independently determined were 60.53%, 90.34%, and 80.52%, respectively, while those of HIF-1α were 49.56%, 84.91%, and 65.23% respectively. The sensitivity, specificity, and accuracy of 18F-FDG PET/CTindependently determined were 88.75%, 90.57%, and 90.05% respectively, while those of 18F-FDG PET/CT determined jointly with Cyfra21-1 and HIF-1α were 97.04%, 98.62%, and 98.75%, respectively. There was statistical difference between the values of the independent and combined determinations (P <0.05) .*

Conclusion*: The technique of combining 18F-FDG PET/CT scanning with determination of serum Cyfra21-1 and HIF-1α levels is a considerably improved, accurate and sensitive approach for diagnosis of early stages of lung cancer.*

Keywords*: 18F-FDG PET/CT, Cyfra21-1, HIF-1α, lung cancer.*

DOI: 10.19193/0393-6384_2018_1_30

Received *November 30, 2017;* ***Accepted*** *January 20, 2018*

Introduction

Mortality from lung cancer, one of the malignant tumors with greatest threat to human health and life, tops those of other malignant tumors. With rapid increase in incidence and mortality, lung cancer has been one of the major causes of death. Most patients with lung cancer are asymptomatic in the early and middle stages. This is particularly worrisome because most lung cancers reach advancedstageby the timepatientsshow up in the hospital. At that stage, any chance of successful surgical intervention would have been lost[1]. Therefore, it is important to explore and improve on techniques that make the diagnosis of early lung cancer possible.

Positron emission tomography/computer tomography (PET/CT) reveals cell metabolism at the molecular level in living organisms, and plays a vital role in specific disease diagnosis, monitoring of therapeutic efficacy and strategies for new drug development and selection.

These molecular imaging techniques use radioisotope-tagged molecular probes to bind and identify specific target organs. The uptake of 18F-FDG reveals sites of sugar metabolism, and since tumor cells exhibit a high propensity for glucose uptake, this is an important way to diagnose tumor and monitor treatment responses[2, 3].

One of the major directions of modern tumor research is the identification and detection of tumor markers in the serum, and considerable attention have been focused on the identification of lung cancer markers. Among those markers is Cyfra21-1 which hasbeen shown to be poorly expressed in benign lung cancer. However, in patients with lung cancer, proteasesare activated, which degrade cytokeratinwith attendant increase in Cyfra21-1 levels. Thus serum Cyfra21-1 is regarded as a tumor marker for lung cancer detection[4, 5].

Researchers claim that hypoxicenvironment exists in many tumors and stimulates a series of biochemical reactions whose rates vary depending on the tumor type. Hypoxia inducible factor- 1α (HIF-1α) has crucial influence on these reactions, as well as on the activation of several key target genes related to tumor growth such as vascular endothelial growth factor[6, 7].

In this study, the serum levels of Cyfra21-1and HIF-1α of lung cancer patients and those of different lung cancers were compared.The specificity and sensitivity of independent determinations of Cyfra21-1and HIF-1α., and combined18F-FDG PET/CT and tumor markerdeterminations for evaluating patients with early lung cancer were assessed.

Materials and methods

Subjects

A total of 80 patients diagnosed with lung cancer in our hospital (Liaocheng Peoples' Hospital, China) from January2015 to December 2016 and scheduled for treatment were selected for this study. Their statuses were confirmed by imaging, pathology, and cytology. There were 35 females and 45 males, with age ranging from 20 to 71 years. Their mean age was 57.42± 5.92. Among them were patients with symptoms of lung cancer such as dry cough, asthma, and chest pain. Apart from these, there were 25 patients with benign lung disorders who reported at the hospital during the same time and so were placed in the control group, comprising 15 males and 10 females, whose ages ranged from 30 to 69 years (mean age was 49.22 ± 3.86). There was no statistically significant difference in mean age between patients in both groups.

Inclusive and exclusive criteria

Patients who after undergoing 18F-FDG PET/CT examination had satisfactory images indicative of good load of lung cancer with detectable levels of Cyfra21-1 and HIF-1α, and who fell within age range 18 to 80 years were selected. They were also expected to have normal body temperature and acceptable full blood count.

Other inclusion criteria were absence of other malignancies, and a requirement that the patient was not on any other any other treatment before hospital admission. Patients who not eligible were those with metastatic lung cancer or receiving any type of cancer therapy.In addition, those who were allergic toimaging agents, as well as those who considered 18F-FDG PET/CT examination or Cyfra21-1 and HIF-1α test unacceptable, orhad severe liver and kidney diseases, or even mental illness were also not eligible.

Methods

^{18}F-FDG PET/CT scanning was done using Pet/CT Discovery model with 64 detectors (GE Healthcare, USA). In order to ensure that the fasting blood glucose levelremained below 5.5mmol/L, the patients fasted before the procedure. Patients remained in bed for 60min after intravenous injection of ^{18}F-FDG at a dose of 4.40 ~ 8.10 MBq/kg. Their urine samples were analyzed for radio-labeledmetabolites.The patients underwent body scan with 7~9 window levels, ranging from mid-femur to scalp, and images werefused and reconstructed through Ordered Subsets Expectation Maximization (OSEM).

The images werevisually evaluated by two doctors who are experts in nuclear medicine, for evidence pathology, and regions of interest (ROIs) were delineated. They also measured maximum standardized uptake value (SUVmax) of lesion areas.Workstation was used for automatic calculation of SUV. Values of SUV ≥2.5 indicated malignancy; SUVs greater than 1.0 and less than 2.5 indicated benign lesions, while SUVs < 1.0 indicated absence of lesion. Fasting venous blood samples (5mL each) were taken in the morning

and left standing for 30min. Thereafter they were centrifuged at 4000 rpm for 30 minand the sera samples preservedat -80°C. The levels of serum Cyfra21-1 and HIF-1α were assayed with ELISA kits (ShenzhengDakewe Biotechnology Co. Ltd, China), according to manufacturer's instructions.

The results of 18F-FDG PET/CT scanning, and ELISA assays for determination of serum Cyfra21-1 and HIF-1α were compared, and their relationships, sensitivities, specificities and accuracies evaluated.

Estimation of sensitivity and specificity

Sensitivity = cases of true positive / cases of true positive + cases of false negative.

Specificity =cases of true negative / cases of true negative + cases of false positive.

In combined determinations, one positive out of three determinations was considered positive, but if all determinations are negative, this indicated negative.

Statistical analysis

Data were processed with statistical analysis software SPSS17.0. All quantitative data were expressed as mean ± SD ($\bar{x}$±*SD*) and analyzed by Student's t- test. The enumeration data were expressed as %and analyzed byχ2 test. P values < 0.05 were considered statistically significant.

Results

^{18}F-FDG PET/CT scan and serum Cyfra21-1 and HIF-1α determination

The scan showed that SUV value of 80 patients with early lung cancer was 10.51±4.23, which was significantly higher than that of the control group which was 5.36±2.03. The concentration of Cyfra21-1 was 26.14±10.86 ng/ml, which again was significantly higher than that of the control group (5.56±8.22 ng/ml) .The level of HIF-1α in the lung cancer group was 201.86±35.48 pg/ml, which was significantly higher than that of the control group (123.56±20.69 pg/ml) (P <0.01; Table 1).

Comparison of the levels of Cyfra21-1 and HIF-1α in serum of patients with different types of lung cancer

The 80 patients with early lung cancer were carefully divided into four types namely adenocarcinoma, squamous cell carcinoma, small cell carcinoma, and unclassified carcinoma, to evaluate their serum levels of Cyfra21-1 and HIF-1α. The values were compared as shown in Table 2. The Cyfra21-1 level of patients with squamous cell carcinoma was significantly higher than that of patients with adenocarcinoma and small cell carcinoma (P <0.05) ,but was not significantly different from that of the unclassified carcinoma group (P>0.05).

	^{18}F-FDG PET/CT (SUV)	Cyfra21-1 (ng/ml)	HIF-1α (pg/ml)
Early lung cancer group (n=80)	10.51±4.23	26.14±10.86	201.86±35.48
Control group (n=25)	5.36±2.03	5.56±8.22	123.56±20.69
T value	5.866	8.716	10.47
Pvalue	<0.01	<0.001	<0.001

Table 1: Results of 18F-FDG PET/CT scanning and serum Cyfra21-1 and HIF-1α analyses [$\bar{x}$±SD]

Types	Cases	Cyfra21-1 (ng/ml)	HIF-1α (pg/ml)
Squamous cell carcinoma	21	28.63±13.27^{ab}	234.32±39.85^{ab}
Adenocarcinoma	36	21.36±2.95	196.38±43.15
Small cell carcinoma	20	18.95±2.44	203.85±27.16
Unclassified carcinoma	3	26.63±4.32	206.67±21.58

Table 2: Comparison of levels of Cyfra21-1 and HIF-1α in serum of different lung cancer patients[$\bar{x}$±SD].
^{a}P <0.05 compared with adenocarcinoma patients; ^{b}P <0.05 compared with small cell carcinoma patients.

Values of Cyfra21-1 from patients with adenocarcinoma, small cell carcinoma and unclassified carcinoma were not significantly different from each other. The mean level of serum HIF-1α of patients with squamous cell carcinoma was significantly higher than that of patients with adenocarcinoma and small cell carcinoma (P <0.05), but was not significantly different when compared with corresponding value for unclassified carcinoma (P>0.05). Serum HIF-1α of patients with adenocarcinoma, small cell carcinoma and unclassified carcinoma were not significantly different from each other (P>0.05).

Sensitivity and specificity of Cyfra21-1 and HIF-1α as parameters forassessing patients with early lung cancer

The sensitivity, specificity and accuracy of Cyfra21-1independently determined, were 60.53%, 90.34% and 80.52% respectively, while those of HIF-1α also determined separately were 49.56%, 84.91% and 65.23%, respectively. This implies that the sensitivity, specificity and accuracyof Cyfra21-1

and HIF-1α when determined separately, were not high, and so are of little clinical value in early lung cancer detection. On the other hand, the sensitivity, specificity and accuracy of the combined determination of the two serum tumor markers were 83.59%, 93.21%and 90.84%, respectively. These are better than the percentages obtained from the independent determinations, which were not remarkable (Table 3).

Parameter	Sensitivity	specificity	accuracy
Cyfra21-1	60.53	90.34	80.52
HIF-1α	49.56	84.91	65.23
Combined determination	83.59	93.21	90.84

Table 3: Assessment of serum Cyfra21-1 and HIF-1α detection as agents for early lung cancer detection in patients (%).

Analysis of sensitivity and specificity of combining^{18}F-FDG PET/CT scanning with serum Cyfra21-1 and HIF-1α determination for assessing patients with early lung cancer

The sensitivity, specificity and accuracy of ^{18}F-FDG PET/CT separately determined, were 88.75%, 90.57% and 90.05%, respectively, but those of combined ^{18}F-FDG PET/CT scanning and serum Cyfra21-1 and HIF-1α determination were 97.04%, 98.62% and 98.75%, respectively (Table 4).

Parameters	Sensitivity	Specificity	Accuracy
^{18}F-FDG PET/CT	88.75	90.57	90.05
^{18}F-FDG PET/CT and Serum Cyfra21-1 and HIF-1α combined determination	97.04	98.62	98.75
χ2value	4.4.6968	5.769	4.737
P value	<0.05	<0.05	<0.05

Table 4: Assessment ofrelevance of 18F-FDG PET/CTcombined with Cyfra21-1 and HIF-1α for detection of early stage lung cancer.

It was only in one patient that the combined determination failed as a diagnostic parameter for lung cancer. The combinedapproach is evidently more meaningful and clinically relevant for early detection of lung cancer.

Discussion

In recent years, lung cancer has been one of the diseases with very high incidence and mortality, and has continued to threaten human life and health. In terms of incidence, lung cancer ranks first among men and second in women, but its mortality rate in both sexes is topmost in most countries[8]. Lung cancer has no obvious clinical features when it starts. It may start with low fever, cough or chest pain. Consequently, most patients may already have developed advanced lung cancer, or metastasis may have set in when they are diagnosed. This makes most patients have very low five-year survival rate (about 15%)[9]. Therefore, improving on the diagnostic methods and technical skills are very important requirements for raising the survival rate of lung cancer patients.

^{18}F-FDG PET/CT is a relatively new approach for diagnosis of cancer. ^{18}F-FDG PETrefers to a substance which mimics the principal energy nutrient of cells. When tumor cells have grown to some extent, their ability to absorb FDG increases. Hence ^{18}F-FDG PET can be used as an injectable imaging agent for assessing patients' pathological conditions[10]. However this technology has some limitations in spatial resolution which may easily lead to false positive results for broncho-alveolar carcinomaand adenocarcinoma. The present study shows that the ^{18}F-FDG PET/CT determination is a better technique for early lung cancer detection.

Increasing advances in molecular biology have led to better appreciation of the concept of tumor markers (TMds), which are generally peptides produced by tumor cells or their hosts during tumor growth and reproduction. Tumor markers for various tumors are specifically bound to tumor cells and may not drain into body fluids. So with TMs, not only can tumors be detected, but their growth can also be evaluated. Consequently, although the process maycause few injuries and cost some money, it is readily acceptable. TM assessment is of crucial clinical value in lung cancer diagnosis and evaluationof therapeutic outcomes and prognosis[11]. Cyfra21-1 is a solublefragment generated in caspase-lysedepithelial cell, which is present in normal tissues. When an epithelial cell undergoes malignanttransformation, protease is activated to enhance the degradation of cytokeratin, resulting in release of large amounts of Cyfra21-1 into the blood. Cyfra21-1 is lowin benign diseases, and even in patients with kidney failure or benign liver disorder. Usually, it is not higher than 10 ng/mL.Thusit is regarded as an important marker for detecting lung cancer[12-14].

Studieshave established that hypoxia is a feature of solid tumors, and the main factor that propels malignant growth, metastasis and chemo-resis-

tance[15]. As the key transcription factor that induces cells to generateadaptive response in hypoxic conditions, HIF-1α regulates the expression of several cancer-associated genes including vascular endothelial growth factor (VEGF). The expression of HIF-1α increases in hypoxic micro-environment, which causes indirect VEGF up-regulation, and promotes angiogenesis, vascular permeability, and lymphangiogenesis, to facilitate supply oxygen and nutrients to tumor cells. Hypoxia inducible factor can regulate key elements of apoptosis, or enhance sugar transport and glycolysis intumor cells[16]. Some studies have reportedthat the levels of HIF-1α in normal human tissues and benign tumorsare lower than in lung cancer tissues[17]. Results from the present study showed that the level of HIF-1α in patients with lung cancer was significantly higher than that in the control group, and that HIF-1α level in patients with squamous cell carcinoma was significantly higher than those in patients with adenocarcinoma and small cell carcinoma.The results also showed that combining ^{18}F-FDG PET/CT scanning with Cyfra21-1 and HIF-1α determination markedly increased the accuracy and sensitivity in detection of early lung cancer.

Conclusion

The results obtained in this study demonstrate that Cyfra21-1 and HIF-1α levels in patients with early lung cancer were significantly higher than corresponding values for patients with benign lung diseases. This implies that Cyfra21-1 and HIF-1α can be used asTMs for diagnosis and detection of early lung cancer.The results also revealed that combining ^{18}F-FDG PET/CT scanning with determination of Cyfra21-1 and HIF-1α improved the accuracy and sensitivity of detection of early lung cancer.

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