RESEARCH ARTICLE

Research on the Relationship Between Serum Levels of Inflammatory Cytokines and Non-small Cell Lung Cancer

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Abstract

Aims: This study was conducted to evaluate the levels of TNF-α, IL-6, IL-8 and VEGF in serum of patients with non- small cell lung cancer, for assessing their possible diagnostic and prognostic roles. Methods: We enrolled 48 patients newly diagnosed with non-small cell lung cancer and 40 healthy controls. TNF- α, IL-6 and IL-8 levels were measured in the serum of all the subjects with specific radioimmunoassay kits, while EGF was analyzed by sandwich enzyme immunoassay techniques. Results: A statistically significant difference was observed between lung cancer patients and the control group regarding the values of TNF-α, IL-6, IL-8 and VEGF in serum. Moreover, TNF-α, IL-8 and VEGF levels were higher in patients with advanced stages compared to early stages. In addition, higher serum levels of TNF-α, IL-6, IL-8 and VEGF were found in smokers than in non-smokers, both in patients and controls. Conclusion: Serum levels of TNF-α, IL-6, IL-8 and VEGF were all elevated in lung cancer patients, suggesting that inflammatory cytokines could be jointly used as a screening tool. Though TNF-α, IL-8 and VEGF levels were related to advanced disease, long-term survival studies of NSCLC patients should be performed to confirm whether they can act as biomarkers of advanced disease. In addition, smoking would be an important contributor to the processes of inflammation and lung cancer.

Keywords: TNF-α - IL-6 - IL-8 - VEGF - serum values - non-small cell lung cancer

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Introduction

Lung cancer, which characterized by uncontrolled cell growth in tissues of the lung, is the leading cause of cancer death and the third most common form of malignancy. Moreover, this disease brings a devastating effect to 1.30 million people worldwide in 2004 (Ferlay et al., 2007; Dalaveris et al., 2009; Bodelon et al., 2013) and reveals a general upward trend especially in developing countries, such as China and India. Clinically, it always leads to shortness of breath, coughing and weight loss. Among of them, non-small cell lung cancer (NSCLC) occupies the lion's share of lung cancer, reaching up to more than 80%. Because of its prevalence and poor prognosis, how to select appropriate biomarkers for evaluating severity, monitoring progression and estimating the efficacy of a specific therapy becomes an unavoidable issue.

The hypothesis that tumorigenesis may be induced by chronic inflammation was first proposed by Virchow in as early as 1863 (Il'yasova et al., 2005). Then, numerous researches focused on their relationship elucidate that inflammatory cytokines, which were secreted by both immunocompetent cells and tumor cells, played a pivotal role in the pathogenetic processes (Tas et al., 2005). Among of these, tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) are the typical studied ones.

TNF-α, an important immune regulatory factor, has toxic effect on tumor cells and inhibits their growth (Saito et al., 2013). It is supposed that adequate TNF-α could help to maintain the metabolic as a protective response to external stress, whereas excess TNF-α would lead to inflammatory, multiorgans injury and septic shock. IL-6 is a phosphorylated glycoprotein containing 185 amino acids, which influence tumor cell proliferation. Many recent researches have proved that IL-6 has ability to influence the tumor size (Wojcik et al., 2010). Although the cause of the IL-8 is unclear, it can be detected in all nude mice models, related with respiratory acidosis, tumor burden as well as has negative correlation with survival (Millar et al., 2008; Cheng et al., 2013). In terms of VEGF, it plays an important role in tumor biology, especially in tumor progress and growth of metastasis (Takigawa et al., 1998).

The aim of the present study was to investigate the diagnostic and prognostic roles of serum inflammatory cytokines prior to and after chemotherapy, as well as give further support to the relationship between these inflammatory biomarkers and NSCLC.

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Materials and Methods

Characteristics of the patients and controls

In this research, 48 consecutive patients with histological evidence of primary lung cancer admitted to the Beijing chest hospital in China between 2008 and 2010 were enrolled (Table 1). There were 40 males and 8 females in patients, whose mean age was 52.4 years (ranged 36 to 68). In all, 25 patients were smokers until now, while 16 and 7 patients were ex- smokers and never smokers respectively. Normal controls (n=40) were recruited from individuals participating health examination at the same hospital and all were in excellent health at the time of the study. The written informed consent was obtained from each patient upon approval of the study by the Ethic Committees of the hospital.

For all of these patients, a diagnosis of lung cancer was confirmed by histologic examinations of biopsy and/ or cytology specimens obtained during fiberoptic bronchoscopy or with chest CT guided scans. Before the disease had been diagnosis, none of them had received any form of anti-cancer therapy, invasive diagnostic procedure or primary lung surgery.

Pathologic stages were determined according to the criteria of World Health Organization (Beasley et al., 2005). It is evident that sufferers in stage I, II, III, IV account for 33.3%, 20.8%, 12.5% and 33.3% of the total patients respectively, in which 39.6% appear metastasis. In terms of hystotype, the population can be divided into squamous cell carcinoma (47.9%) and adenocarcinoma (52.1%). Eventually 26 patients receive treatment in our hospital.

Table 1. Distribution of Selected Characteristics Among Patients and Controls

	NSCLC Patients (%)	Controls (%)	*P*
Number	48	40	
Sex			
Male	40(83.3%)	0	0.33
Female	8(17.7%)	10(25.0%)	
Age	52.4±9.8	49.6±10.2	0.19
Race			
Chinese Han	42(87.5%)	34(85.0%)	0.73
Non- Chinese Han	6(12.5%)	6(15.0%)	
Smoking habit			
Current smokers	25(52.1%)	15(37.5%)	<0.01
Ex- Smokers	16(33.3%)	2(5.0%)	
Non-Smokers	7(14.6%)	23(57.5%)	
Stage NSCLC			
Stage I	16(33.3%)	-	-
Stage II	10(20.8%)	-	
Stage III	6(12.5%)	-	
Stage IV	16(33.3%)	-	
Metastasis			
M0	29(60.4%)	-	-
M1	19(39.6%)	-	
Hystotype			
Squamous cell carcinoma	23(47.9%)	-	-
adenocarcinoma	25(52.1%)	-	
Taking chemotherapy treatment			
Yes	26(54.2%)	-	-
No	22(45.8%)	-	

Table 2. Serum Levels of TNF-α, IL-6, IL-8 and VEGF in Patients with Lung Cancer and Healthy Controls (pg/ml)

	Controls (n=48)	Patients (n=40)	*P*
TNF-α	23.1±3.7	42.2±6.9	<0.01
IL-6	4.6±2.1	28.9±7.5	<0.01
IL-8	15.8±3.5	38.2±8.2	<0.01
VEGF	174.8±17.2	352.4±48.1	<0.01

Blood sample collection

4 ml venous blood were collected from both patients and controls and subsequently centrifuged at 3000×g for 10 min at 4 °C. The supernatant was transferred into microtubes and frozen at -80°C until use. In order to avoid any influences of the circadian rhythm to the measured biomarkers, all blood sample collections were conducted early in the morning.

Measurements of TNF-α, IL-6, IL-8, VEGF

TNF-α, IL-6 and IL-8 levels were measured with a specific radioimmunoassay kit (Beijing North Institution of Biological Technology, Beijing, China) according to the manufacture's protocol. While VEGF (R&D systems Inc., Minneapolis, MN, USA) employed the quantitative sandwich enzyme immunoassays techniques according to the manufacture's protocol. The intra-assay and inter-assay variability were less than 10%.

Treatment

All of the patients were treated with combination therapy consisting of cisplatin plus vinorelbine chemotherapy. Response to chemotherapy was determined after two cycles of chemotherapy according to standard WHO criteria.

Statistical Analysis

Data were expressed as means±S.E.M. Comparisons of levels of these biomarkers either between controls and patients or between patients before and after chemotherapy were performed using t- test, while Student-Newman-Keuls (SNK) method was used for multiple comparisons among each stage of patients and controls. ANOVA test was used to compare these serum levels among different responses. Statistical significance was defined as p values<0.05. All statistical analyses were performed with the SPSS statistical software program package (SPSS version 15.0 for windows, SPSS Inc., Chicago, Illinois, USA).

Results

Comparison of serum levels of TNF-α, IL-6, IL-8 and VEGF between healthy controls and patients with lung cancer

It can be seen from Table 2 that all levels of these inflammatory cytokines including TNF-α, IL-6, IL-8 and VEGF in patients were significantly higher than these in control group (P<0.01).

Comparison of serum levels of TNF-α, IL-6, IL-8 and VEGF among each stages of patients and control groups

As significant difference can be obtained among

Table 3. Serum Levels of TNF-α, IL-6, IL-8 and VEGF in Patients with Lung Cancer Before and after Chemotherapy

	TNF-α		IL-6		IL-8		VEGF	
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment
CR+PR	35.2±5.4	25.3±2.3	21.8±6.1	7.2±3.9	24.2±4.1	18.3±3.2	218.7±41.0	189.2±26.8
SD	43.1±6.8	58.1±8.2	29.1±8.3	34.2±10.3	40.5±10.8	49.4±14.2	339.1±56.2	390.1±49.6
PD	47.8±8.1	62.1±12.3	36.2±11.0	56.4±12.2	48.2±9.1	78.9±18.5	484.8±65.4	966.3±108.6

CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease

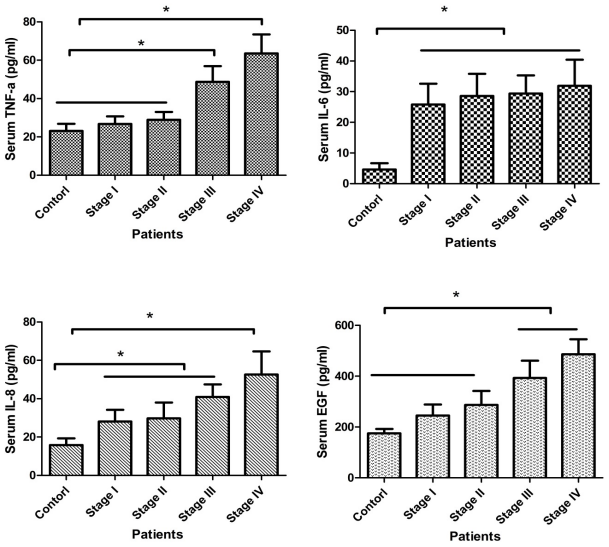

Figure 1. Comparison of Serum TNF-α, IL-6, IL-8, VEGF Among Different Stages of Lung Cancer and Healthy Control (*significant difference)

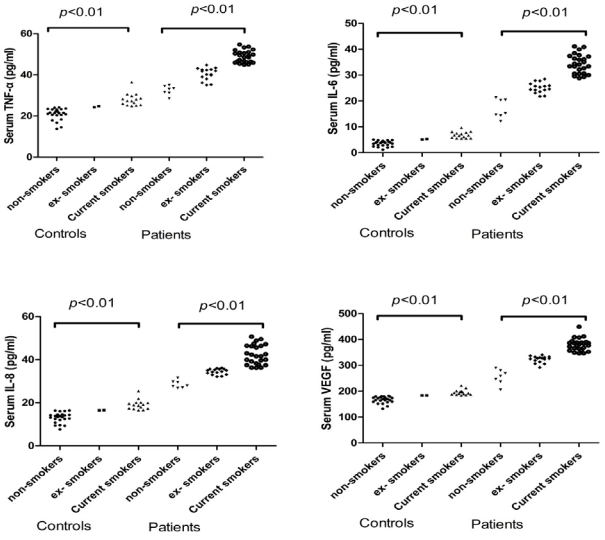

Figure 2. Comparison of Serum Levels of TNF-α, IL-6, IL-8 and VEGF Between Smokers and Non- smokers

different stages of patients and healthy controls, it is necessary to conduct the multiple comparisons among them. From Figure 1, the serum TNF-α level of patients in stage III is significant higher than these in controls, stage I and II, while that in stage IV is highest. For serum IL-6 concentration, there is no significant difference among each stage of patients while they are significant higher than controls. The topping level of serum IL-8 also exists in stage IV and there is no difference can be found among stage I, II and III. As far as VEGF levels, stage III and IV are obviously higher than controls, stage I and II.

Comparison of serum levels of TNF-α, IL-6, IL-8 and VEGF between smokers and non- smokers

From Figure 2, it is interesting that higher serum levels of TNF-α, IL-6, IL-8 and VEGF were observed in smokers than these in ex- smokers and non- smokers both in patients and controls.

Comparison of serum levels of TNF-α, IL-6, IL-8 and VEGF in patients with lung cancer before and after chemotherapy

After two cycles of chemotherapy, 26 persons obtained overall responses: 1 complete response (CR), 6 partial response (PR), 15 stable disease and 4 progressive disease. Effective rate and clinical benefit response of chemotherapy were 26.9% and 84.6%, respectively.

Table 3 shows that TNF-α (P<0.01), IL-6 (P<0.01), IL-8 (P<0.01) and VEGF (P<0.01) values were increased by chemotherapy as compared with pretreatment in PD patients, while no significant changes in CR+PR and SD patients. Moreover, serum levels of TNF-α (P<0.01), IL-6 (P<0.01), IL-8 (P<0.01) and VEGF (P<0.01) in patients with lung cancer were significant difference among CR+PR, SD and PD patients after chemotherapy. Specifically, TNF-α values were higher in PD and SD patients than in patients with CR+PR (P<0.01) after chemotherapy, while the significant difference between PD and SD patients were not detected (P=0.195). Compared with CR+PR and SD patients, IL-6 (P were both <0.01), IL-8 (P were both <0.01) and VEGF (P were both <0.01) values were much higher in PD patients.

Discussion

According to induction hypothesis theory, excessive cell proliferation and activation of a cascade of cellular actions can be led by chronic inflammation, thereby bringing about the induction of irreversible DNA damage. Persistent irritation and inflammation consequently promote these initiated cells, leading to tumor growth, progression of metastatic disease, and immunosuppression (Coussens and Werb, 2002). It is suggested that inflammatory cytokines released through inflammatory response would promote tumor growth, while tumor growth further stimulate the inflammatory response (Scott et al., 2002). On the other hand, some argues that the immune response of the host is studied as a consequence of tumor growth itself. Grivennikov et al. illustrated that several stages of carcinogenesis can be induced by inflammation, through mechanisms containing genomic instability, epigenetic modifications, localized immunosuppression, and angiogenesis (Grivennikov et

al., 2010). Thus, it suggests the inflammatory cytokines may have the potential to act as biomarkers of lung cancer (Hashimoto et al., 2005; Siemes et al., 2006).

In the present study, serum concentrations of TNF-α, IL-6, IL-8 and VEGF seem to be higher in the NSCLC patients compared to healthy controls. These findings are in corresponding with some other reports. Dalaveris et al. (2009) and Tas et al. (2006) have published that both in serum and exhaled breath condensate of patients, overproduction of VEGF and TNF-α can be seen, while some recent studies suggested that serum concentrations of IL-6 and Il-8 were also increased (Lagiou and Trichopoulos, 2011; Pine et al., 2011). Furthermore, it can be seen that the serum levels of TNF-α, IL-8 and VEGF in the serums of subjects increased along with the advanced stages of lung cancer, suggesting a possible local production of inflammatory cytokines, which is also in accordance with some previous studies (Boldrini et al., 2000). In addition, serum levels of TNF-α, IL-6, IL-8 and VEGF increased in PD patients by chemotherapy compared with pretreatment. Moreover, all these four inflammatory cytokines were much higher in PD patients than these in CR+PR and SD patients. Because TNF-α, IL-6, IL-8 and VEGF are potent angiogenic meditators with important effects on tumor growth, as well as associated with increased tumor vessel density and cancer prognosis (Volm et al., 1999; Matsuyama et al., 2000), they may be suggested to be surrogate biomarkers of angiogenesis and prognosis in lung cancer.

Finally, the higher levels of all these four inflammation biomarkers were expressed in smokers compared with non-smokers. This indicates that tobacco smoking play a pivotal role in inflammatory processes, thereby contributing to lung cancer etiology. Moreover, in line with some related research, although there is a gradual reduction of the lung cancer risk after cessation of smoking, it is difficult for these ex-smokers to reach the low lung cancer risk levels of the never-smokers (Peto et al., 2000). Besides, the fact that higher levels of inflammation biomarkers in NSCLC patients regardless of smokers or no-smokers indicate their increase is not only due to tobacco use but also affected by lung cancer.

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The author(s) declare that they have no competing interests.

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