HOSTED BY
ELSEVIER
Contents lists available at ScienceDirect
Journal of Acute Disease
journal homepage: www.jadweb.org

Original article http://dx.doi.org/10.1016/j.joad.2016.05.003

The correlation between serum contents of TGF-β1 and IL-6 and acute radiation pneumonitis in patients with lung cancer

Guang-Yuan Liu*, Qiang Li, Xiao-Jun Yang, Zhong-Hua Xiong

Lung Tumor Unit, Sichuan Cancer Hospital and Institute, Chengdu, Sichuan Province, 610041, China

ARTICLE INFO

Article history:
Received 14 Apr 2016
Received in revised form 3 May 2016
Accepted 15 May 2016
Available online 13 Jun 2016

Keywords:
Non-small cell lung cancer
Radiation pneumonitis
Transforming growth factor β1
Interleukin-6

ABSTRACT

Objective: To explore the correlation between contents of serum transforming growth factor β1 (TGF-β1) and interleukin-6 (IL-6) and acute radiation pneumonitis (RP) in patients with lung cancer.

Methods: A total of 138 patients with non-small cell lung cancer receiving three-dimensional conformal radiation therapy in our hospital from May 2012 to October 2015 were selected as the research objects. According to the presence or absence of RP in the process of radiotherapy, those patients were divided into the RP group and non RP group to detect the contents of TGF-β1 and IL-6 in serum.

Results: Before radiotherapy, there was no significant difference in the serum contents of TGF-β1 and IL-6 in the RP group and non RP group. At weeks 2, 4, 6, 8 and 10 after radiotherapy, the serum contents of TGF-β1 and IL-6 in patients of the RP group were all higher than those in the non RP group. Differences of the contents of TGF-β1 and IL-6 in each time point after radiotherapy between two groups had statistical significance ($P < 0.05$). In RP group, the higher the RP grade was, the more the serum contents of TGF-β1 and IL-6 became. Differences of the contents of TGF-β1 and IL-6 in patients with different RP grade were statistically significant in each time point ($P < 0.05$).

Conclusions: In the process of radiotherapy, the increase of the serum contents of TGF-β1 and IL-6 in patients with lung cancer is closely related to the occurrence of acute RP.

1. Introduction

Lung cancer is one of malignant tumors with the highest incidence rate in our country, in which non-small cell lung cancer is the most common type. The disease has a lower rate in early diagnosis, and most patients already developed into the locally advanced stage at diagnosis[1,2]. Concurrent chemoradiotherapy is the standard therapeutic regimen for the treatment of non-small cell lung cancer in locally advanced stage. The ray in radiation can kill cancer cells as well as injury the normal cells, and then cause the occurrence of radiation pneumonitis (RP)[3]. RP is a relatively common complication occurring in patients with lung cancer in the radiation process, and its main clinical symptoms are cough, anhelation, dyspnea, *etc*. Severe patients will suffer from respiratory failure. Acute RP refers to radiation pneumonitis that happens within 90 days from the beginning of radiotherapy, while advanced RP refers to radiation pneumonitis that happens after 90 days from the beginning of radiotherapy[4].

Local pathological features of RP including inflammatory cell infiltration and interstitial fibrosis will cause lung functions injury in patients with lung cancer and influence conditions of prognosis[5,6]. At present, RP has already become the main factor limiting radiotherapy dose, which will further impact the killing effect of radiotherapy on lung carcinoma cell and the survival time of lung cancer patients. In clinical practice, the diagnosis of RP mainly depends on X-ray, CT scan and other imaging methods. transforming growth factor β1 (TGF-β1) and interleukin-6 (IL-6) are the import *in-vivo* cell factors, which respectively involve in the regulation of organizing fibrosis process and inflammatory response process, and are related to pathological process of acute RP. In this study, we analyzed the correlation between serum contents of TGF-β1 and IL-6 and acute RP in patients with lung cancer.

*Corresponding author: Guang-Yuan Liu, Lung Tumor Unit, Sichuan Cancer Hospital and Institute, Chengdu, Sichuan Province, 610041, China.
Tel: +86 13880010292
E-mail: liuguangyuan80@163.com

Foundation Project: Supported by Scientific Research Project of Health Department, Sichuan Province (Grant No. 120051).
Peer review under responsibility of Hainan Medical College. The journal implements double-blind peer review practiced by specially invited international editorial board members.

2. Materials and methods

2.1. Research objects

A total of 138 patients with non-small cell lung cancer receiving three-dimensional conformal radiation therapy in our hospital from May 2012 to October 2015 were selected as the research objects. Inclusion criteria included: (1) patients were diagnosed with advanced non-small cell lung cancer through pathological examination or cytologic examination; (2) patients conformed to the indications of radiotherapy and could tolerate radiotherapy; (3) patients' expecting life span was over 6 months at diagnosis; (4) patients have no chronic obstructive pulmonary disease, bronchial asthma and other diseases; (5) patients had complete medical records; (6) patients' serum specimens were all kept every week before and after radiotherapy; (7) the study was approved by Hospital Ethics Committees and patients signed the informed consent. The grouped patients consisted of 83 males and 53 females with the mean age of (56.5 ± 8.3) years and body mass index of (21.5 ± 3.9) kg/m^2.

2.2. The diagnosis of RP and grouping

Radioactive damage evaluation criterions made by American Radiation Therapy Oncology Group (RTOG) were used to diagnose RP. The standards were described as follow: (1) no obvious change was considered as Grade 0; (2) slight dry cough or dyspnea after tired work was considered as Grade I; (3) continuous cough or dyspnea after light activity and required antitussive drugs were considered as Grade II; (4) antitussive drug cannot effectively relive dry cough, patient showed dyspnea in resting state, or imageology showed changes were considered as Grade III; (5) patients with severe dyspnea that required mechanical ventilation or uninterrupted oxygen were in Grade IV. Patients in Grades II–IV were diagnosed with RP and included in the RP group. Patients in Grade 0 and Grade I were included in the non RP group.

2.3. Detection methods of clinical index

Serum specimens before and two cycles after radiotherapy were collected. TGF-β1 and IL-6 kits (Shanghai Westang Bio-Tech Co., Ltd) were used to detect the contents of TGF-β1 and IL-6 in serum.

2.4. Statistical methods

SPSS 20.0 was used to input data and process statistical analysis. Measurement data were tested by homogeneity of variance. After this, data conformed to normal distribution were conducted with *t*-test or ANOVA, while data conformed to skewed distribution were conducted with nonparametric rank-sum test. Differences were statistically significant ($P < 0.05$).

3. Results

3.1. Serum content of TGF-β1 in patients of the RP group and non RP group

Before radiotherapy, there was no significant difference in the serum content of TGF-β1 [(4.29 ± 0.61) *vs.* (4.41 ± 0.55) μg/L] in patients of the RP group and non RP group. At weeks 2, 4, 6, 8 and 10 after radiotherapy, the serum contents of TGF-β1 [(6.51 ± 0.88) *vs.* (4.82 ± 0.58) μg/L, (7.96 ± 1.03) *vs.* (5.04 ± 0.72) μg/L, (8.73 ± 1.22) *vs.* (4.95 ± 0.66) μg/L, (9.31 ± 0.94) *vs.* (5.26 ± 0.75) μg/L, (10.76 ± 1.65) *vs.* (5.37 ± 0.81) μg/L] in patients of the RP group were all higher than those in the non RP group. Contents of TGF-β1 in each time point after treatment between two groups had statistical significance ($P < 0.05$) (Table 1).

Table 1
Serum contents of TGF-β1 (μg/L) in patients of the RP group and non RP group before and after radiation treatment.

Time	RP group	Non RP group	*P*
Before radiotherapy	4.29 ± 0.61	4.41 ± 0.55	> 0.05
Week 2 after radiotherapy	6.51 ± 0.88	4.82 ± 0.58	< 0.05
Week 4 after radiotherapy	7.96 ± 1.03	5.04 ± 0.72	< 0.05
Week 6 after radiotherapy	8.73 ± 1.22	4.95 ± 0.66	< 0.05
Week 8 after radiotherapy	9.31 ± 0.94	5.26 ± 0.75	< 0.05
Week 10 after radiotherapy	10.76 ± 1.65	5.37 ± 0.81	< 0.05

3.2. Content of serum IL-6 in patients of the RP group and non RP group

Before radiotherapy, the contents of serum IL-6 in patients of the RP group and non RP group had no significant difference ($P > 0.05$). At weeks 2, 4, 6, 8 and 10 after radiotherapy, the contents of serum IL-6 [(59.2 ± 8.8) *vs.* (44.8 ± 7.2) ng/L, (77.1 ± 10.3) *vs.* (50.1 ± 6.4) ng/L, (94.4 ± 11.8) *vs.* (47.6 ± 7.1) ng/L, (120.6 ± 16.7) *vs.* (53.1 ± 8.9) ng/L, (133.7 ± 19.3) *vs.* (52.1 ± 7.2) ng/L] in patients of the RP group were all higher than those in the non RP group. Differences of the contents of serum IL-6 in each time point after radiotherapy between the two groups were statistically significant ($P < 0.05$) (Table 2).

Table 2
Contents of serum IL-6 (ng/L) in patients of the RP group and non RP group.

Time	RP group	Non RP group	*P*
Before radiotherapy	45.2 ± 7.1	43.1 ± 6.7	> 0.05
Week 2 after radiotherapy	59.2 ± 8.8	44.8 ± 7.2	< 0.05
Week 4 after radiotherapy	77.1 ± 10.3	50.1 ± 6.4	< 0.05
Week 6 after radiotherapy	94.4 ± 11.8	47.6 ± 7.1	< 0.05
Week 8 after radiotherapy	120.6 ± 16.7	53.1 ± 8.9	< 0.05
Week 10 after radiotherapy	133.7 ± 19.3	52.1 ± 7.2	< 0.05

3.3. Contents of serum TGF-β1 in patients with different RP grades

The contents of serum TGF-β1 [(4.41 ± 0.55) *vs.* (4.18 ± 0.64) *vs.* (4.30 ± 0.62) μg/L] in patients of RP Grade II, RP Grade III and RP Grade IV showed no significant differences ($P > 0.05$). At weeks 2, 4, 6, 8 and 10 after radiotherapy, the contents of serum TGF-β1 in patients of RP Grade II, RP Grade III and RP Grade IV were different. The higher RP grades led to the more contents of serum TGF-β1 [(5.22 ± 0.72) *vs.* (6.31 ± 0.89) *vs.* (7.67 ± 0.95) μg/L, (6.87 ± 0.83) *vs.* (7.76 ± 0.94) *vs.* (9.41 ± 1.36) μg/L, (7.31 ± 1.04) *vs.* (8.45 ± 1.30) *vs.* (9.85 ± 1.45) μg/L, (8.03 ± 1.12) *vs.* (9.22 ± 0.79) *vs.* (11.32 ± 1.32) μg/L, (8.74 ± 0.93) *vs.*

(11.03 ± 1.61) *vs.* (13.68 ± 1.56) μg/L]. Differences of the contents of TGF-β1 in each time point after treatment in patients with different RP grades had statistical significance ($P < 0.05$) (Table 3).

Table 3
Contents of serum TGF-β1 (μg/L) in patients of different RP grades.

Time	RP Grade II	RP Grade III	RP Grade IV	*P*
Before radiotherapy	4.41 ± 0.55	4.18 ± 0.64	4.30 ± 0.62	> 0.05
Week 2 after radiotherapy	5.22 ± 0.72	6.31 ± 0.89	7.67 ± 0.95	< 0.05
Week 4 after radiotherapy	6.87 ± 0.83	7.76 ± 0.94	9.41 ± 1.36	< 0.05
Week 6 after radiotherapy	7.31 ± 1.04	8.45 ± 1.30	9.85 ± 1.45	< 0.05
Week 8 after radiotherapy	8.03 ± 1.12	9.22 ± 0.79	11.32 ± 1.32	< 0.05
Week 10 after radiotherapy	8.74 ± 0.93	11.03 ± 1.61	13.68 ± 1.56	< 0.05

3.4. Contents of serum IL-6 in patients with different RP grades

The contents of serum IL-6 [(46.4 ± 6.1) *vs.* (44.1 ± 7.7) *vs.* (44.8 ± 7.3) ng/L] in patients of RP Grade II, RP Grade III and RP Grade IV had no significant difference ($P > 0.05$). At weeks 2, 4, 6, 8 and 10 after radiotherapy, the contents of serum IL-6 in patients of RP Grade II, RP Grade III and RP Grade IV became different. Besides, higher RP grades made more contents of serum IL-6 [(51.2 ± 7.6) *vs.* (58.5 ± 9.1) *vs.* (64.2 ± 10.2) ng/L, (70.4 ± 8.9) *vs.* (78.3 ± 10.8) *vs.* (84.7 ± 11.6) ng/L, (79.7 ± 10.3) *vs.* (98.7 ± 11.3) *vs.* (110.3 ± 14.3) ng/L, (100.3 ± 15.4) *vs.* (118.4 ± 19.3) *vs.* (139.4 ± 28.5) ng/L, (116.7 ± 23.1) *vs.* (135.1 ± 20.5) *vs.* (149.5 ± 24.1) ng/L]. Differences of the contents of IL-6 in each time point after treatment in patients with different RP grades had statistical significance ($P < 0.05$) (Table 4).

Table 4
Contents of serum IL-6 (ng/L) in patients with different RP grades.

Time	RP Grade II	RP Grade III	RP Grade IV	*P*
Before radiotherapy	46.4 ± 6.1	44.1 ± 7.7	44.8 ± 7.3	> 0.05
Week 2 after radiotherapy	51.2 ± 7.6	58.5 ± 9.1	64.2 ± 10.2	< 0.05
Week 4 after radiotherapy	70.4 ± 8.9	78.3 ± 10.8	84.7 ± 11.6	< 0.05
Week 6 after radiotherapy	79.7 ± 10.3	98.7 ± 11.3	110.3 ± 14.3	< 0.05
Week 8 after radiotherapy	100.3 ± 15.4	118.4 ± 19.3	139.4 ± 28.5	< 0.05
Week 10 after radiotherapy	116.7 ± 23.1	135.1 ± 20.5	149.5 ± 24.1	< 0.05

4. Discussion

In the aforementioned study, we analyzed the correlation between the serum contents of TGF-β1 and IL-6 and acute RP in patients with non-small cell lung cancer in the process of radiotherapy. Acute RP is a common complication occurring in patients with non-small cell lung cancer in the process of radiotherapy, with an incidence rate of 13%–37%, as well as the important factor to limit radiotherapy dose and affect radiotherapeutic effect[7–9]. At present, the means of diagnosis for RP are quite limited, which mainly required to combine clinical symptoms and image examinations to conducted diagnosis. Clinical symptoms of RP are mainly low-grade fever, anhelation, continuous dry cough, chest distress and other non-specific symptoms, which cannot provide powerful grounds for the clinical diagnosis. Although image examinations can provide bases for the diagnosis of the disease after the clinical symptoms aggravated, most patients missed the most ideal time for effective treatment, which means the risk of developing into respiratory failure greatly increases and the prognosis becomes poor[10,11].

The pathological feature of RP is the inflammatory response caused by radiation damage. Locally compounded and secreted inflammatory mediators and cell factors can lead to epithelium damage in pulmonary alveoli and induce pulmonary interstitial fibrosis, which, in turn, leads to the occurrence of respiratory symptoms and the damage of lung function[12–14]. TGF-β1 is widely distributed in multiple organs and tissues in the body, which regulate the process of organizing fibrosis by combining with TGF-βII. In the development process of RP, TGF-β1 can stimulate the synthesis of fibrocyte, secrete matrix protein and increase interstitial matrix protein and collagen deposition as well as aggravate pulmonary fibrosis and lung injury[15,16]. Under the physiological conditions, locally generated TGF-β1 is mainly limited in lung tissues, which will not enter the blood circulation. Under the effect of radioactivity, the synthesis and secretion of TGF-β1 increases and the local tissues were damaged, then the overproduced TGF-β1 enters blood circulation[17,18]. We detected the content of serum TGF-β1 in patients with non-small cell lung cancer and discovered that the occurrence of RP would lead to an abnormal increase in the content of serum TGF-β1 in the process of radiotherapy. Besides, the higher the RP grade was, the more the content of serum TGF-β1 would become.

IL-6 is a cell factor with multiple biological activities. Mononuclear macrophages and lymphocytes in local lung tissues can synthesize and secrete IL-6[19]. Under the acute state of pulmonary injury caused by radioactive rays, the synthesis and secretion of IL-6 in local tissues increase obviously and have strong inflammation-causing effects, which can promote the recruitment and activation of various inflammatory cells in pulmonary alveoli, as well as increase the secretion of tumor necrosis factor-α, IL-1β, IL-8 and other inflammatory factors, and then mediate the cascade amplification of inflammatory responses in lung tissues[20,21]. We tested the contents of serum IL-6 in patients with non-small cell lung cancer and discovered that the contents of serum IL-6 at weeks 2, 4, 6, 8 and 10 after treatment in patients of the RP group were obviously higher than those in the non RP group. The higher the RP grade is, the more the content of serum IL-6 becomes. It can be concluded that the occurrence of RP in the process of radiotherapy will lead to the abnormal increase in the content of serum IL-6 in the patients, and the higher the RP grade is, the more the content of serum IL-6 will be.

In conclusion, the increase of the serum contents of TGF-β1 and IL-6 is closely related to the occurrence of acute RP in patients with lung cancer in the process of radiotherapy.

Conflict of interest statement

The authors report no conflict of interest.

References

[1] Kerner GS, van Dullemen LF, Wiegman EM, Widder J, Blokzijl E, Driever EM, et al. Concurrent gemcitabine and 3D radiotherapy in patients with stage III unresectable non-small cell lung cancer. *Radiat Oncol* 2014; **9**: 190.

[2] Su S, Li T, Lu B, Wang X, Li J, Chen M, et al. Three-dimensional radiation therapy to the primary tumor with concurrent chemotherapy in patients with stage iv non-small cell lung cancer: results of a multicenter phase 2 study from PPRA-RTOG, China. *Int J Radiat Oncol Biol Phys* 2015; **93**(4): 769-77.

[3] Yao B, Wang YD, Liu QZ. Radiation pneumonitis in non-small-cell lung cancer patients treated with helical tomotherapy. *Niger J Clin Pract* 2016; **19**(1): 25-9.

[4] Briere TM, Krafft S, Liao Z, Martel MK. Lung size and the risk of radiation pneumonitis. *Int J Radiat Oncol Biol Phys* 2016; **94**(2): 377-84.

[5] Holgersson G, Bergström S, Liv P, Nilsson J, Edlund P, Blomberg C, et al. Effect of increased radiotoxicity on survival of patients with non-small cell lung cancer treated with curatively intended radiotherapy. *Anticancer Res* 2015; **35**(10): 5491-7.

[6] Ochiai S, Nomoto Y, Yamashita Y, Murashima S, Hasegawa D, Kurobe Y, et al. Radiation-induced organizing pneumonia after stereotactic body radiotherapy for lung tumor. *J Radiat Res* 2015; **56**(6): 904-11.

[7] Ling DC, Hess CB, Chen AM, Daly ME. Comparison of toxicity between intensity-modulated radiotherapy and 3-dimensional conformal radiotherapy for locally advanced non-small-cell lung cancer. *Clin Lung Cancer* 2016; **17**(1): 18-23.

[8] Giridhar P, Mallick S, Rath GK, Julka PK. Radiation induced lung injury: prediction, assessment and management. *Asian Pac J Cancer Prev* 2015; **16**(7): 2613-7.

[9] Choy H, Gerber DE, Bradley JD, Iyengar P, Monberg M, Treat J, et al. Concurrent pemetrexed and radiation therapy in the treatment of patients with inoperable stage III non-small cell lung cancer: a systematic review of completed and ongoing studies. *Lung Cancer* 2015; **87**(3): 232-40.

[10] Giuliani ME, Lindsay PE, Kwan JY, Sun A, Bezjak A, Le LW, et al. Correlation of dosimetric and clinical factors with the development of esophagitis and radiation pneumonitis in patients with limited-stage small-cell lung carcinoma. *Clin Lung Cancer* 2015; **16**(3): 216-20.

[11] Dang J, Li G, Zang S, Zhang S, Yao L. Comparison of risk and predictors for early radiation pneumonitis in patients with locally advanced non-small cell lung cancer treated with radiotherapy with or without surgery. *Lung Cancer* 2014; **86**(3): 329-33.

[12] Pu X, Wang L, Chang JY, Hildebrandt MA, Ye Y, Lu C, et al. Inflammation-related genetic variants predict toxicity following definitive radiotherapy for lung cancer. *Clin Pharmacol Ther* 2014; **96**(5): 609-15.

[13] Wen J, Liu H, Wang Q, Liu Z, Li Y, Xiong H, et al. Genetic variants of the LIN28B gene predict severe radiation pneumonitis in patients with non-small cell lung cancer treated with definitive radiation therapy. *Eur J Cancer* 2014; **50**(10): 1706-16.

[14] Yang S, Zhang M, Chen C, Cao Y, Tian Y, Guo Y, et al. Triptolide mitigates radiation-induced pulmonary fibrosis. *Radiat Res* 2015; **184**(5): 509-17.

[15] Xiao Y, Yuan X, Qiu H, Li Q. Single-nucleotide polymorphisms of TGFβ1 and ATM associated with radiation-induced pneumonitis: a prospective cohort study of thoracic cancer patients in China. *Int J Clin Exp Med* 2015; **8**(9): 16403-13.

[16] Lu Z, Ma Y, Zhang S, Liu F, Wan M, Luo J. Transforming growth factor-β1 small interfering RNA inhibits growth of human embryonic lung fibroblast HFL-I cells *in vitro* and defends against radiation-induced lung injury *in vivo*. *Mol Med Rep* 2015; **11**(3): 2055-61.

[17] Li J, Mu S, Mu L, Zhang X, Pang R, Gao S. Transforming growth factor-beta-1 is a serum biomarker of radiation-induced pneumonitis in esophageal cancer patients treated with thoracic radiotherapy: preliminary results of a prospective study. *Onco Targets Ther* 2015; **8**: 1129-36.

[18] He J, Deng L, Na F, Xue J, Gao H, Lu Y. The association between TGF-β1 polymorphisms and radiation pneumonia in lung cancer patients treated with definitive radiotherapy: a meta-analysis. *PLoS One* 2014; **9**(3): e91100.

[19] Fu ZZ, Peng Y, Cao LY, Chen YS, Li K, Fu BH. Correlations between serum IL-6 levels and radiation pneumonitis in lung cancer patients: a meta-analysis. *J Clin Lab Anal* 2016; **30**(2): 145-54.

[20] Sohn SH, Lee JM, Park S, Yoo H, Kang JW, Shin D, et al. The inflammasome accelerates radiation-induced lung inflammation and fibrosis in mice. *Environ Toxicol Pharmacol* 2015; **39**(2): 917-26.

[21] Westbury CB, Haviland J, Davies S, Gothard L, Abdi BA, Sydenham M, et al. Cytokine levels as biomarkers of radiation fibrosis in patients treated with breast radiotherapy. *Radiat Oncol* 2014; **9**: 103.