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Lung and Pleural Malignancies

Association of Chronic *Chlamydia pneumoniae* Infection and the Risk of Lung Cancer

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Abstract

Study Objectives: *Chlamydia pneumoniae*, a respiratory tract pathogen, have tendency to cause chronic infections and suspected to play a role in the development of lung cancer. In this study, we aimed to evaluate the association between chronic *C. pneumoniae* infection and the risk of lung cancer. **Patients and Methods:** Sixty newly diagnosed lung cancer patients and 35 healthy controls were included in this case-control study. IgA and IgG antibodies to *C. pneumoniae* were measured with commercial kits. **Results:** The prevalence of *C. pneumoniae* specific IgA and IgG seropositivity were significantly higher in lung cancer patients compared to healthy controls. IgA seropositivity was detected in 45% of lung cancer patients and 14.3% of control cases (p=0.002). IgG seropositivity was detected in 70% of lung cancer patients and 28.6% of control cases (p=0.0001). *C. pneumoniae* specific IgA and IgG antibodies increased the risk of lung cancer 4.6 and 5.3 fold respectively, independent from age, sex, and smoking history. *C. pneumoniae* specific IgA antibody was significantly more common in non small-cell lung cancer patients. **Conclusions:** *C. pneumoniae* specific IgA and IgG antibodies were independently associated with risk of lung cancer. This finding suggested the contribution of chronic *C. pneumoniae* infection to malignant transformation.

Keywords: chlamydia pneumoniae, chronic infection, inflammation, lung cancer, serology

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INTRODUCTION

Chlamydia pneumoniae was first described as a respiratory tract pathogen by Grayston et al. [1] in 1986. It is an obligate intracellular bacterium that causes various acute or chronic respiratory infections including pneumonia, bronchitis, sinusitis, pharyngitis or asymptomatic infection world-wide [2]. *C. pneumoniae* has also been associated with several other non-respiratory diseases, such as atherosclerosis [3], Alzheimer's disease [4], and multiple sclerosis [5]. Several seroepidemiological studies have shown that the prevalence of humoral antibodies against *C. pneumoniae* increases with age [6]. Antibodies are more common in males than females, and smokers have a higher seroprevalence than non-smokers [7]. The seroprevalence of *C. pneumoniae* infection in adult population is as high as 80%, indicating that nearly everybody acquires the infection at least once during their life-time and that re-infections often occur [6].

Chronic inflammation associated with various persistent infections has been considered as a risk factor in the development of malignancies. For example *Helicobacter pylori*, a common bacterium causing chronic inflammation of the gastric wall, has been associated with gastric carcinoma [8]. Chlamydial species have tendecy to cause chronic infections and were suspected to play a role in the development of malignancies. Antilla et al.

reported the seroepidemiological evidence of an association between chronic chlamydial infections and lymphomas [9]. *C. trachomatis* has been associated to rectal [10] and cervical cancer [11]. There are also a few studies indicating the association between chronic *C. pneumoniae* infection and lung cancer [12-16]. In all of these studies chronic ***C. pneumoniae*** infection were found to increase the risk of lung cancer. In this study, we also aimed to evaluate the association between chronic *C. pneumoniae* infection and the risk of lung cancer.

MATERIAL AND METHODS

Patients

Sixty patients who were diagnosed as lung cancer in our clinic during a six month period (July 1st 2004 – December 31st 2004) and 35 healthy controls were included in this case-control study. The diagnosis of lung cancer was confirmed by tissue biopsy specimens obtained by fiberoptic bronchoscopy, toracotomy, mediastinoscopy, and transthoracic needle biopsy. Control group was chosen from healthy hospital staff, relatives of the patients, and blood donors with similar age, sex, and smoking history.

Patients with a history of myocardial infarction, hypertension, and other cardiovascular diseases and prior history of bronchitis, pneumonia, sinusitis, and pharyngitis within the preceding 3 months were excluded from the study. The study was approved by the local ethics committee of our hospital and informed concent was obtained from all the subjects enrolled in the study.

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Table 1. Characteristics of the lung cancer patients and controls

	Lung cancer patients (n=60)	Controls (n=35)	p
Mean age (years)	55±10 (Range:33-74)	52±7 (Range:42-65)	0.1
Sex (Male/female)	56/4	32/3	0.7
Smokers/non-smokers	57/3	34/1	1.0
Current smokers n (%)	31 (51.7%)	24 (68.6%)	0.1
Age of starting smoking (years)	19±6	19±4	0.6
Smoking history (mean pack-years)	43.9±28.9	34.2±22.3	0.09

Serological tests

The blood samples (6ml) were collected at the time of diagnosis from lung cancer patients and at the time they were selected from healthy controls. Blood samples were centrifuged and sera were stored at -40°C until testing. IgA and IgG antibodies to *C. pneumoniae* were measured with commercial kits [*Anti-Chlamydia pneumoniae* IIFT-IgG, *Anti-Chlamydia pneumoniae* IIFT-IgA (Euroimmun, Germany)] using microimmunofluorescence method. Titers ≥1/100 were used as a cut-off level for positivity in sera. All assays were performed by a single observer who was unaware of the case or control status of the specimens.

Statistical Analysis

Statistical analysis was carried out using SPSS programme version 12.0 (SPSS Inc. Chicago IL, USA). Chi-Square and Fisher's exact test were used to compare differences in proportions of categorical variables. Student's t test was used to compare differences in means of continuous variables. Spearman rank correlation coefficient test was used to examine the association between variables. Logistic regression test was used for multivariate analysis. Age, sex, and smoking status were used as covariates. There was not multicollinearity in the final reggression model. There was not interaction between independent variables. The fitness of the model was tested by Hosmer-Lemeshowl test. Any p value less than or equal to 0.05 was considered statistically significant.

RESULTS

Sixty (56 male, 4 female) lung cancer patients with a mean age of 55±10 years and 35 (32 male, 3 female) healthy controls with a mean age of 52±7 years were enrolled in the study. The histological diagnosis of lung cancer cases were 44 non-small cell carcinoma (19 squamous cell carcinoma, 13 adenocarcinoma, 12 non-small cell carcinoma) and 16 small cell carcinoma. Mean ages, sex distribution, smoking status of lung cancer patients and control cases are presented in Table 1. Lung cancer patients and control cases were similar by age, sex, and smoking status. The prevalence of *C. pneumoniae* specific IgA and IgG seropositivity were significantly higher in lung cancer patients compared to control cases (Table 2). IgA seropositivity was detected in 45% of lung cancer patients and 14.3% of control cases (p=0.002). IgG seropositivity was detected in 70% of lung cancer patients and 28.6% of control cases (p=0.0001).

Table 2. Prevalence of *C. pneumoniae* specific IgA and IgG seropositivity in lung cancer and control group

	Lung cancer patients (n=60)	Controls (n=35)	p
C. pneumoniae specific IgA positivity n (%)	27 (45%)	5 (14.3%)	0.002*
C. pneumoniae specific IgG positivity n (%)	42 (70%)	10 (28.6%)	0.0001*

*Statistically significant

The relationship between clinicopathological features and seropositivity of *C. pneumoniae* specific IgA and IgG antibodies are presented in Table 3. IgA seropositivity was significantly in higher proportion in non-small cell lung cancer patients (p=0.014). Both IgA and IgG seropositivity were in higher proportion in left sided lung cancer patients (p=0.045 and p=0.02 respectively). IgA seropositivity was in higher proportion in lung cancer patients without distant metastases (p=0.007).

In multivariate analysis controlling for age, sex, and smoking history (pack-years), *C. pneumoniae* specific IgA (OR, 4.64; 95% CI, 1.51-14.21) and IgG (OR, 5.31; 95% CI, 2.04-13.80) antibodies were independently associated with the risk of lung cancer (Table 4).

DISCUSSION

The present study provided a serological evidence on the association between chronic *C. pneumoniae* infection and lung cancer. The prevalence of *C. pneumoniae* specific IgA and IgG seropositivity were significantly higher in lung cancer patients compared to healthy controls. Independent from age, sex, and smoking history, the presence of *C. pneumoniae* specific IgA and IgG antibody increased the risk of lung cancer 4.6 and 5.3 fold respectively. Additionally *C. pneumoniae* specific IgA antibody was significantly more common in non-small cell lung cancer patients.

Exposure to *C. pneumoniae* usually occurs in childhood and increases with age often resulting from re-infection several times during a lifetime [6]. The majority of adult population have *C. pneumoniae* specific IgG antibodies as an indication of earlier exposure. After an acute infection with *C. pneumoniae*, IgG antibody titres rise and decrease

Table 3. The relationship of clinicopathological features with seropositivity of *C. pneumoniae* specific IgA and IgG antibodies

Variables	*C. pneumoniae* specific IgA positivity n (%)	*C. pneumoniae* specific IgG positivity n (%)
NSCLC	24 (54.5%)	32 (72.7%)
SCLC	3 (18.8%)	10 (62.5%)
P	0.014*	0.5
Right lung	11 (33.3%)	19 (57.6%)
Left lung	16 (59.3%)	23 (85.2%)
p	0.045*	0.02*
Central	23 (45.1%)	36 (70.6%)
Peripheral	4 (44.4%)	6 (66.7%)
p	1.0	1.0
Metastatic	7 (25.9%)	26 (78.8%)
Non-metastatic	20 (60.6%)	16 (59.3%)
p	0.007*	0.1

*Statistically significant, NSCLC: Non small cell lung cancer, SCLC: Small cell lung cancer

Table 4. Estimated odds ratio of lung cancer associated with *C. pneumoniae* specific IgA and IgG seropositivity

	OR	95% CI
C. pneumoniae specific IgA	4.64	1.51-14.21
C. pneumoniae specific IgG	5.31	2.04-13.80
C. pneumoniae specific IgA and IgG	3.55	1.06-11.89

OR: Odds ratio, CI : Confidence interval

slowly [17]. The half life of IgG antibodies is 23-28 days [18]. In re-infection, IgA response is the predominant feature [19]. IgA antibodies tend to disappear more rapidly. The half-life of IgA antibodies is 5-6 days. Persistant elevated IgA titres are considered as a reliable marker of chronic infection with *C. pneumoniae* [20].

Chronic inflammation associated with various persistent infections has been considered as a risk factor in the development of malignancy [21]. Viruses induce malignancy by means of their oncogenic effects on cells [22]. But the role of bacterial infections is less clear. It is thought that activated inflammatory cells, which release inflammatory mediators, nitric oxide, and other free radicals may induce genetic damage and neoplastic transformation [21, 23].

In vitro infection of human alveolar macrophages and peripheral blood mononuclear cells with *C. pneumoniae* has been demonstrated to induce secretion of cytokines, TNF-α, IL-1β, and IL-8 [24]. It has been reported that *C. pneumoniae* downregulate apoptosis of infected cells by induction of IL-10 [25] and that it will induce production of IL-8 [26]. IL-8 is an angiogenic factor which acts as a promotor of tumor growth for human non-small cell lung carcinomas through its angiogenic properties [27,28]. Also liberation of nitric oxide has been demonstrated in chlamydial infections [29]. Thus, it is plausible to consider induction of an inflammatory response as a potential mechanism in the development of lung cancer associated with *C. pneumoniae* infection.

Laurila et al. [12] were the first who investigated the presence of *C. pneumoniae* infection in 230 lung cancer cases and age, locality, and smoking history matched controls selected from the cohort of ATBC study. ATBC study was a randomized, double blind, placebo controlled prevention trial that examined whether supplementation with alpha-tocopherol (AT), beta-carotene (BC) or both reduce the incidence of lung cancer in male smokers of Finnish population. Chronic *C. pneumoniae* infection defined by stable elevated IgA and immune complex titres was diagnosed in 52% of the cases and 45% of the controls. The infection increased the overall risk of lung cancer 1.6 times. The risk was 2.9 times higher among persons under 60 years of age.

Koyi et al. published a preliminary report of the possible association between chronic *C. pneumoniae* infection and lung cancer in a 1-year prospective study of patients referred for bronchoscopy because of suspected lung cancer [14]. They studied *C. Pneumoniae* specific IgA, IgG, and IgM antibodies in sera. The results of extended investigation, 2-year prospective study, suggested an association between chronic *C. pneumoniae* infection, specific IgA antibodies in particular, and risk of lung cancer [15].

Jackson et al. have reported an association between increased *C. pneumoniae* specific IgA levels and lung cancer in smoking males under 60 years of age [13]. Similar to the findings of Jackson et al., Kocazeybek reported that chronic C. pneumoniae infections were seen more often in male patients with carcinoma who were under 55 years of age, supporting the idea that chronic *C. pneumoniae* infection increases the risk of lung carcinoma [16].

In the present study, both IgG and IgA antibodies positivity were related with the increased risk of lung cancer similar to the findings of Anttila et al. They studied *C. pneumoniae* specific immune complexes, IgA and IgG antibodies in sera of female lung cancer cases and found that all three parameters were associated with the risk of lung cancer [30].

In this study we also investigated the relationship of clinicopathological features with seropositivity of *C. pneumoniae* specific IgA and IgG antibodies. Our study population were mostly males and smokers that we could not compare males with females and smokers with non-smokers. There is only one study in the literature comparing male and female lung cancer patients. In that study, male lung cancer patients had significantly higher levels of IgG and/or IgA antibodies than female lung cancer patients [15].

Laurila et al. [12] compared different histological cell types and observed a higher relative risk in the combined group of small cell and squamous cell carcinomas (1.7 times) than in the other cancer types. Stratification by age indicated a particularly high relative risk for all cancer types in cases younger than 60 years. Kocazeybek et al. [16] observed a higher relative risk in small cell lung cancer patients. There are also two studies indicating no significant difference among the histological subgroups [13, 30]. In the present study, IgA seropositivity was significantly in higher proportion in non-small cell lung cancer patients compared to small-cell lung cancer patients. Because of low number of cases, we could not make statistical analysis considering each histological subtype.

In the study of Jackson et al. [13], IgA seropositivity rates did not vary significantly by stage and location of the malignancy. In this study, we unexpectedly found that IgA and IgG seropositivity were in higher proportion in left sided lung cancer patients and IgA seropositivity was in higher proportion in non-metastatic lung cancer patients. We think that these findings may be accidental because of low number of cases.

In conclusion, the present study provided a serological evidence on the association between chronic *C. pneumoniae* infection and lung cancer. The determinants of chronic *C. pneumoniae* infection, *C. pneumoniae* specific IgA and IgG antibodies were significantly higher in lung cancer patients compared to healthy controls. Independent from age, sex, and smoking history, the presence of *C. pneumoniae* specific IgA and IgG antibody increased the risk of lung cancer 4.6 and 5.3 fold respectively. Additionally *C. pneumoniae* specific IgA antibody was significantly more common in non-small cell lung cancer patients. These findings together with the findings of other studies were consistent with the hypothesis that chronic inflammation resulting from persistant *C. pneumoniae* infection, may be an etiological factor in the occurence of lung cancers. But the pathogenesis needs further investigation.

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