

COTININE LEVELS IN SERUM AND BRONCHOALVEOLAR LAVAGE FLUID

Özlem Erçen Diken¹, Serap Unculu¹, Demet Karnak¹, Osman Çağlayan²,
Julide Sedef Göçmen³ and Oya Kayacan¹

¹Department of Chest Diseases, Ankara University School of Medicine, Ankara;
²Department of Biochemistry, ³Department of Microbiology, Kirikkale University
School of Medicine, Kirikkale, Turkey

Abstract. Cotinine is a major metabolite of nicotine. This study was planned to investigate the relationship between bronchoalveolar lavage (BAL) fluid cotinine levels and serum cotinine levels in smokers and nonsmokers with various pulmonary diseases and to investigate whether these levels are affected by passive smoking. Serum and BAL fluid cotinine levels were measured in 27 patients. BAL cotinine levels were measured using a sensitive ELISA kit produced to measure cotinine in saliva. Plates were read by μ Quant (BioTek, USA) micro plate reader. All patient serum cotinine levels were detectable except for one nonsmoker patient. However, BAL fluid cotinine levels were measurable in only 6 patients (two of them were nonsmokers). A significant positive correlation was seen between serum and BAL fluid cotinine levels ($r=0.726$; $p=0.000$). Serum cotinine levels were significantly higher in present smokers than non-smokers (21.0 ± 16.01 ; 5.35 ± 7.65 ; $p=0.004$). However, there were no significant differences in BAL fluid cotinine levels between smokers and nonsmokers. Passive smoking can increase nicotine metabolites in serum and other body fluids, including BAL fluid. Since BAL fluid and serum cotinine levels were well correlated, there is no need to use invasive procedures, such as bronchoscopy and expensive, time consuming BAL fluid analyses. Serum cotinine levels can give a rough idea of smoking status. BAL fluid cotinine measurements should be done for only scientific reasons.

Key words: cotinine, nicotine, bronchoalveolar lavage fluid

INTRODUCTION

Nicotine is an alkaloid absorbed from tobacco smoke with spreads to the body through the blood stream in seconds. In

humans the major elimination pathway of nicotine is oxidation to cotinine by hepatic cytochrome P2A6 and aldehyde oxidase. In humans, 40-60% of nicotine is eliminated as trans-3 hydroxicotinine, 22-35% as cotinine and cotinine glucuronide, and 8-10% as nicotine (Raunio *et al*, 2008).

Correspondence: Prof Demet Karnak, Chest Disease Department, Ankara University School of Medicine, 06100-Cebeci-Ankara, Turkey.

Tel: +90 312 5956572; Fax: +90 312 3190046

E-mail: karnak@medicine.ankara.edu.tr,

demet.karnak@gmail.com

Cotinine, the major metabolite of nicotine, is generally regarded as the best biomarker for monitoring tobacco exposure in both actively and passively exposed individuals (Benowitz, 1996). The

most common sources for cotinine assays are serum, urine, and saliva (Bernert *et al*, 2000). Smokers generally have plasma cotinine levels above 15 ng/ml (85 nmol/l), while heavy smokers (>25 cigarettes/day) usually have levels higher than 300 ng/ml (1,704 nmol/l) (Woodward *et al*, 1991). Due to rapid elimination, plasma and urine levels of both nicotine and cotinine mainly reflect exposure to cigarette smoke within 24 hours. Nicotine and cotinine appear in hair and nails later, 3 months after tobacco smoke exposure (Metz-Favre *et al*, 2005).

To evaluate cigarette smoke exposure in infants during and after pregnancy, cotinine is measured in cord blood samples (Pichini *et al*, 2000), meconium (Köhler *et al*, 2007) and colostrum (Paszowski and Wojewoda, 2001). It is also studied in seminal fluid (Macaron *et al*, 1997) and cervical mucus (McCann *et al*, 1992). But there is no information about the cotinine content of bronchoalveolar lavage (BAL) fluid in the literature.

BAL sampling was done to analyze cellular and acellular components of the alveolar spaces (Benowitz and Brunetta, 2005). Different substances in serum, such as immunoglobulins (Karnak *et al*, 2001), cytokines (Balamugesh *et al*, 2006), proteases (Cosgrove and du Bois, 2008), prostaglandins (Estella *et al*, 2008), leukotrienes (Kowal-Bielecka *et al*, 2005) and angiotensin converting enzyme (Huang *et al*, 2006) are measurable in BAL fluid. These components cross over into the alveolar space from blood through capillaries by extravasation and diffusion. Therefore, the amount of a substance in BAL fluid correlates with serum levels (Haslam and Baughman, 1999). Determining the level of cotinine in BAL fluid could be an indicator of tobacco smoke exposure affecting the lungs. This study investigated the relationship between bronchoalveolar lav-

age (BAL) fluid and serum cotinine levels in patients undergoing bronchoscopy for various diseases and also investigated whether or not these cotinine levels can be affected by passive cigarette smoking.

MATERIAL AND METHODS

Patients

Twenty-seven patients undergoing bronchoscopy for various reasons were included in the study. Informed consent was obtained from all patients. Institutional review board and local ethical committee approval was obtained before the study was performed.

Methods

BAL was carried out with flexible bronchoscopy (BFT240 Olympus) of the middle lobe with four 50-ml aliquots of normal saline, totalling 200 ml. At least 70% of instilled fluid was removed and the BAL samples and simultaneous blood samples were centrifuged and the BAL supernatant and blood serum were stored at -70°C until studied.

The serum and BAL fluid cotinine levels were measured by a sensitive micro ELISA kit (DRG, Germany) developed to measure saliva cotinine levels.

Data analysis

The data were analyzed with SPSS software version 11.0. Results were given as mean \pm SD. Mann-Whitney *U*, chi-square and Spearman correlation tests were used for data analyses. A *p*-value < 0.05 was considered significant.

RESULTS

Fifteen of the 27 patients were males with a mean age of 53.2 \pm 16.8 years. The patient diagnoses are summarized in Table 1. Seven patients with several diseases

also had chronic obstructive pulmonary disease (COPD).

The primary diagnoses of the patients with COPD were tuberculosis in 2 patients, pneumonia in 1, interstitial lung disease in 2, pneumoconiosis in 1 and bronchiectasis in 1 patient. Seven patients were present smokers. Ten patients had never smoked and the rest had quit smoking at least one month before bronchoscopy.

Table 1
Patient diagnoses.

Diagnosis	n (%)
Tuberculosis ^a	9 (33.3)
Pneumonia ^a	6 (22.2)
Interstitial lung disease ^a	3 (11.1)
Lung nodule	1 (3.7)
Others ^{a,b}	8 (29.6)
Total	27 (100)

^aCOPD in addition to the primary pathology. Primary pathologies: tuberculosis (*n*:2), pneumonia (*n*:1), interstitial lung disease (*n*:2), pneumoconiosis (*n*:1), bronchiectasis (*n*:1)

^bOthers: Bronchiectasis (*n*:3), pulmonary thromboembolism (*n*:2), pneumoconiosis (*n*:2), bronciolitis obliterans (*n*:1).

Measurable amounts of cotinine were detected in the sera of 26 cases and in BAL fluid of 6 cases. The mean serum and BAL fluid cotinine levels in these individuals were 9.56 ± 12.4 ng/ml (range=0.33-43.87) and 7.78 ± 6.17 ng/ml (range=1.95-17.14), respectively. Measurable cotinine levels were detected in the sera of all subjects except in one case who was a non-smoker. There were measurable levels of cotinine in BAL fluid samples of 4 out of 7 smoking patients and 2 out of 20 nonsmoking patients. The ratio of patients with a measurable cotinine level was significantly higher in smokers (4 out of 7 subjects) than non-smokers (2 out of 20 subjects) ($p=0.024$) (Table 2). Serum cotinine levels were significantly higher in present smokers than non-smokers (21.0 ± 16.01 ng/ml vs 5.35 ± 7.65 ng/ml; $p=0.004$). But the difference in BAL cotinine levels was not significant (Table 2). Serum cotinine levels in individuals with measurable cotinine in BAL fluid samples ranged from 16.27 to 43.87 ng/ml. If serum cotinine levels were at least 16.27 ng/ml, cotinine levels became measurable in BAL fluid. No differences in BAL or serum cotinine levels, were seen between COPD patients and others.

Table 2
Comparison of serum and BAL fluid cotinine levels with smoking history (ng/ml).

Smoking history	Serum cotinine levels		BAL cotinine levels	
	Patients with measurable cotinine level	Cotinine levels (Mean \pm SD)	Patients with measurable cotinine level	Cotinine levels (Mean \pm SD)
Nonsmoker (<i>n</i> =20)	19	5.35 ± 7.65	2	7.71 ± 8.14
Smoker (<i>n</i> =7)	7	21.0 ± 16.01^a	4 ^b	7.82 ± 6.43

^aSignificantly higher than non-smokers ($p=0.004$)

^bMeasurable BAL fluid cotinine levels were higher in smokers (4/7 vs 2/20) ($p=0.024$).

There was a significant correlation between serum cotinine levels and cigarette consumption in present smokers ($r=0.948$; $p=0.000$). A significant positive correlation was also observed between serum and BAL cotinine levels ($r=0.726$; $p=0.000$).

DISCUSSION

Tobacco smoking is one of the leading public health problems in the world. When a cigarette is smoked nicotine is absorbed from the tobacco smoke and spreads throughout the body via the blood stream in seconds. Cotinine is a major metabolite of nicotine with a 10 times longer half-life than nicotine (Benowitz *et al*, 1983); it reflects both active and passive tobacco exposure (Benowitz, 1996; Faught *et al*, 2009; Yue *et al*, 2010).

Non-smokers can be exposed to and affected by tobacco smoke. This can be detected by measuring cotinine levels in non-smoker serum samples (Benowitz, 1996; Faught *et al*, 2009). Average plasma cotinine levels in smokers are reported to be above 15 ng/ml (Woodward *et al*, 1991). Plasma cotinine levels lower than 15 ng/ml are suggestive of environmental tobacco smoke exposure in the absence of smoking (Cummings and Richard, 1988). In the current study, current smoker serum cotinine levels were significantly higher than non-smokers. However, nearly all non-smokers had measurable serum cotinine levels, and two of them had levels equal to smokers (16.27 ng/ml), suggesting intense passive smoke exposure. These findings probably reflect side-stream cigarette smoke as an environmental air pollution. In many studies cotinine levels correlated well with lung cancer risk (de Waard *et al*, 1995; Paolo *et al*, 2006).

The ratio of patients with measurable cotinine levels in BAL samples was signifi-

cantly higher in smokers (4/7, 57%) than non-smokers (2/20, 10%). BAL cotinine measurement can detect smoke exposure. However, BAL sampling is invasive and measurement is time consuming due to diluted specimen. Measurement decisions can be made based on the individual case.

Cotinine has been studied in many body fluids. To the best of our knowledge this is the first study to examine BAL fluid cotinine levels (Merrill *et al*, 1985; Karnak *et al*, 2001). Detectable BAL fluid cotinine was only found when the serum level reached 16.27 ng/ml. We believe this is due to transmission of cotinine from the serum into the alveolar space. Cotinine most probably diffuses by extravazation into the alveolar space similar to other substances in previous studies (Karnak *et al*, 2001; Küpeli *et al*, 2008). The good positive correlation between serum and BAL cotinine is evidence for this. Some clinical trials found BAL fluid correlates well with serum levels (Haslam and Baughman, 1999). Therefore, measurement of serum cotinine can give an idea of tobacco exposure.

The dilution effect of BAL may account for lower or negative results (Haslam and Baughman, 1999). To overcome this dilution effect, sensitive measurement methods need to be used (Marrone *et al*, 2010). Measurement of saliva or urine drug levels is easier and less invasive compared to BAL.

Steady-state cotinine level has been found to be linearly and directly related to daily nicotine intake, with an increase in the correlation coefficient directly related to an increase in tar and nicotine (Rosa *et al*, 1992; Muscat *et al*, 2009). Our study also found a significant positive correlation between daily tobacco consumption and serum cotinine levels. In our study, cotinine levels did not differ between COPD cases and non-COPD cases,

probably due to the small number of cases.

Environmental cigarette smoke exposure can increase cotinine levels in the serum and also BAL fluid. Measuring cotinine levels is valuable to estimate cancer risk due to passive cigarette smoke exposure. This is the first study to examine cotinine levels in BAL fluid. In this study, the correlation between BAL fluid and serum cotinine levels and its relationship between active smoking and amount of smoking were determined. We believe passive smoking results in nicotine metabolites in the serum and other body fluids, including BAL fluid. It is clear cotinine can passively diffuse from the serum into BAL fluid. Since BAL is invasive and processing is difficult, it should only be done for scientific reasons.

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