RESEARCH ARTICLE

Can Urinary Cotinine Predict Nicotine Dependence Level in Smokers?

Hyun-Suk Jung[1], Yeol Kim[2]*, Jungsik Son[3], Young-Jee Jeon[4], Hong-Gwan Seo[2], So-Hee Park[5], Bong Ryul Huh[2]

Abstract

Background: Although nicotine dependence plays a role as a main barrier for smoking cessation, there is still a lack of solid evidence on the validity of biomarkers to determine nicotine dependence in clinical settings. This study aimed to investigate whether urinary cotinine levels could reflect the severity of nicotine dependence in active smokers. Materials and Methods: Data regarding general characteristics and smoking status was collected using a self-administered smoking questionnaire. The Fagerström test for nicotine dependence (FTND) was used to determine nicotine dependence of the participants, and a total of 381 participants were classified into 3 groups of nicotine dependence: low (n=205, 53.8%), moderate (n=127, 33.3%), and high dependence groups (n=49, 12.9%). Stepwise multiple linear regression model and receiver operating characteristic (ROC) curves analyses were used to determine the validity of urinary cotinine for high nicotine dependence. Results: In correlation analysis, urinary cotinine levels increased with FTND score (r=0.567, P<0.001). ROC curves analysis showed that urinary cotinine levels predicted the high-dependence group with reasonable accuracy (optimal cut-off value=1,000 ng/mL; AUC=0.82; P<0.001; sensitivity=71.4%; specificity=74.4%). In stepwise multiple regression analysis, the total smoking period (β=0.042, P=0.001) and urinary cotinine levels (β=0.234, P<0.001) were positively associated with nicotine dependence, whereas an inverse association was observed between highest education levels (>16 years) and nicotine dependence (β=-0.573, P=0.034). Conclusions: The results of this study support the validity of using urinary cotinine levels for assessment of nicotine dependence in active smokers.

Keywords: Cotinine - biological markers - smoking - tobacco use disorder

Asian Pacific J Cancer Prev, **13 (11)**, 5483-5488

Introduction

Cigarette smoking can cause various cancers and cardiovascular disease, and smoking cessation can reduce the risk of these diseases (USDHHS, 2004). Many smokers experience withdrawal symptoms while attempting to quit smoking; therefore, nicotine dependence should be considered as one of the substantial barriers to success in smoking cessation (Fagerstrom and Furberg, 2008; Hagimoto et al., 2010). The Fagerström test for nicotine dependence (FTND), a short, 6-question, convenient, self-report measure, has been widely used to assess the degree of nicotine dependence (Heatherton et al., 1991). However, there is insufficient evidence for application of objective measurements, such as urinary cotinine levels, to assess nicotine dependence.

Although nicotine is a desirable biomarker for smoking status, there are limitations to its utility: a short half-life, complex measurement, and variability in plasma levels (which might be higher in subjects with impaired kidney excretion function) (Molander et al., 2000). Cotinine, a metabolite of nicotine, can be measured in saliva, plasma, and urine. Cotinine has relative advantages over other biomarkers—a longer half-life (about 18-20 hours), convenience of measurement, and little daily variability in chronic smokers; therefore, its level has been regarded as an useful objective measure for smoking status or exposure (Jarvis et al., 1987; Lerman et al., 1993). Particularly, measurement of cotinine in the urine is easier and less invasive than of cotinine in the plasma. Furthermore, unlike nicotine, urinary excretion of cotinine is rarely affected by the amount or pH of the

*[1]Total Healthcare Center, Kangbuk Samsung Hospital, Sungkyunkwan University College of Medicine, Seoul, [2]Center for Cancer Prevention and Detection, [5]The Korea Central Cancer Registry, National Cancer Center, Goyang, [3]Department of Family Medicine, INHA International Medical Center, Inchon, [4]Department of Family Medicine, Haeundae Paik Hospital, Inje University School of Medicine, Busan, Korea *For correspondence: drheat10@gmail.com*

urine (Verification., 2002).

Nicotine dependence could refer to the result of long habituation of cigarette smoking. Interestingly, several studies found that levels of cotinine, a metabolite with limited residual period, were associated with some specific smoking behaviors reflecting the severity of nicotine dependence. The amount of time before smoking the first cigarette after waking in the morning, one item of the FTND, was associated with cotinine levels in daily smokers (Heatherton et al., 1991; Muscat et al., 2009; Fu et al., 2012). How quickly to smoke after awakening in the morning has been regarded as a strong marker of nicotine dependence, because highly dependent smokers have lower tolerability to depleted plasma nicotine levels on waking (Heatherton et al., 1991; Baker et al., 2007). Other reports showed a dose-dependent relationship between cotinine levels and the number of cigarettes smoked per day, another measure of the FTND (Law et al., 1997; Blackford et al., 2006; Fu et al., 2012). Moreover, several previous reports showed a significant relationship between the degree of nicotine dependence (as assessed by the total FTND score) and cotinine levels (Heatherton et al., 1991; Payne et al., 1994; Pomerleau et al., 1994). However, as those studies primarily focused on a simple linear correlation, there is a lack of data showing how cotinine levels independently predict severity of nicotine dependence after adjustment of potential confounding factors. Furthermore, the standard cut-off values for cotinine levels as an indicator of high nicotine dependence have not been set yet, whereas there have been mainly several reports on urinary cotinine cut-off points discriminating non-smokers/smokers or passive/active smokers (Zielinska-Danch et al., 2007).

Therefore, the aim of this study is to investigate if the urinary cotinine levels are independently associated with the degree of nicotine dependence, and to determine the validity of using urinary cotinine levels to predict high nicotine dependence in active smokers.

Materials and Methods

Study population

We collected health data from smokers who had reported for a check-up at the Center for Cancer Prevention and Detection in the National Cancer Center, Republic of Korea from June 1 to December 31, 2007. Study inclusion criteria were as follows: adult ages ≥18; current smoker with total number of

Table 1. General Characteristics, FTND Score, and Urinary Cotinine Levels (n=381)

Variable	no (%)	FTND score	P*	Urinary cotinine levels (ng/mL)	P*
Age (year)	45.4±7.8				
Sex			0.44		0.09
Male	352 (92.4)	3.5±2.4		800.9±582.7	
Female	29 (7.6)	3.1±2.2		610.4±465.7	
Socioeconomic status					
Marital status			0.21		0.02
Married	368 (96.6)	3.4±2.4		773.0±570.0	
Not married, divorced, separated, or bereaved†	13 (3.4)	4.3±2.9		1165.4±647.3	
Education (year)			0.06		0.89
≤ 12	116 (30.4)	3.8±2.4		800.1±539.3	
13-16	201 (52.8)	3.5±2.5		787.6±601.6	
> 16	64 (16.8)	2.9±2.2		757.7±567.8	
Average monthly income (million won‡)			0.18		0.04
< 2	18 (4.7)	3.8±2.1		861.9±494.2	
2-4	92 (24.1)	3.2±2.5		798.0±583.7	
4-7	135 (35.4)	3.8±2.4		875.1±599.0	
> 7	136 (35.7)	3.3±2.4		680.4±546.2	
Smoking status					
Cigarette per day (cigarette)	19.4±9.7				
Total smoking period (year)	24.9±8.1				
Total lifetime cigarettes (pack year)	24.8±16.3				
Age at smoking initiation (year)	20.8±4.4				
History of smoking cessation			0.07		0.096
Yes	4 (1.0)	1.3±1.5		308.5±189.4	
No	377 (99.0)	3.5±2.4		791.4±577.0	
Intention to quit smoking			0.01		0.002
Begin within near 6 months	141 (37.0)	3.0±2.4		666.1±527.8	
Someday, but after 6 months	213 (55.9)	3.7±2.4		837.0±585.1	
None	27 (7.1)	4.2±2.4		1015.0±639.7	
Partner's smoking			0.69		0.83
Yes	44 (11.5)	3.6±2.2		768.8±542.4	
No	337 (88.5)	3.5±2.5		788.7±581.3	

FTND, the Fagerström test for nicotine dependence; *P was obtained by comparison of mean FTND scores or mean urinary cotinine levels using the Student t-test or One-way ANOVA test; †Not married (n=1), divorced (n=8), separated (n=2), and bereaved (n=2); ‡Won, a unit of Korean currency which the exchange rate is approximately 1120 won on the US dollar as of January, 2011

lifetime cigarettes smoked ≥400; total lifetime smoking period ≥6 months; and absence of serious physical or mental illness, such as malignancy, chronic obstructive pulmonary disease, chronic kidney disease, depression, and other psychiatric diseases with a high probability of substance abuse. Smoking status and nicotine dependence were determined using self-administered smoking questionnaires that assessed the number of cigarettes smoked per day, total smoking period, total number of lifetime cigarettes smoked, age at smoking initiation, history of smoking cessation, intent to quit smoking, and the presence of a partner that smoked, as well as the measures in the FTND. Based on the FTND score, nicotine dependence status of all study subjects were classified into 3 grades: low (0-3), moderate (4-6), and high (7-10) dependence. This study was approved by the Institutional Review Board at the National Cancer Center at Goyang, Korea.

Measurement of urinary cotinine

Urine specimens were collected in plastic containers and stored in a refrigerator at 4°C. On the day of collection, cotinine levels were assessed using a Cotinine Enzyme Immunoassay (Diagnostic Reagents Inc., CA, USA) on a 502X Multiple Chemistry Unit automated analyzer (A & T Co., Tokyo, Japan) according to manufacturer's instructions. Briefly, 35 μL of urine or calibrator was added to 125 μL of reagent, which included a cotinine-specific monoclonal antibody and enzyme substrate. Next, 125 μL of cotinine-labeled enzyme (glucose-6-phosphate dehydrogenase) was administered and incubated at 37°C. Spectrophotometric measurements were obtained at 340 nm.

Statistical analysis

All statistical analyses were performed using the Statistical Package PASW 18.0 (Chicago, IL, USA). Continuous variables were expressed as mean±standard deviation (SD). To compare urinary cotinine levels of each group, the Student t-test or one-way analysis of variance (ANOVA) were performed. The intergroup differences of median urinary cotinine levels were assessed by the Mann-Whitney U test. Pearson's or Spearman's correlation coefficients were used to investigate correlations between smoking status profile, the FTND score, and urinary cotinine levels. To assess the association between urinary cotinine levels and other variables with high nicotine dependence, stepwise multiple linear regression model was used with adjustment for age, sex, smoking characteristics, and socioeconomic status. Using receiver operating characteristic (ROC) curves, the accuracy and optimal cut-off values of urinary cotinine in the prediction of high nicotine dependence were assessed by the area under the curve (AUC) range of 0.5-1. All confidence intervals (CIs) were set at 95% and statistical significance was determined as $P<0.05$.

Results

A total of 381 subjects were included in the final study: 352 men (92.4%) and 29 women (7.6%). The mean age was 45.4±7.8 years. The mean FTND score and urinary cotinine level of all subjects were 3.5±2.4 and 786.3±576.3 ng/mL, respectively. General and socioeconomic characteristics and smoking status of the study subjects are summarized in Table 1. Furthermore, the mean FTND score and urinary cotinine level for each subgroup based on characteristics and smoking status are shown. Subjects with little or no intention to quit smoking, who were unmarried, or had been divorced, separated, or bereaved, had significantly higher FTND scores and urinary cotinine levels.

Table 2 shows the results of the correlation analysis among age, education, average monthly income, smoking status, FTND scores, and urinary cotinine levels. Particularly, the number of cigarettes smoked per day showed a marked correlation with the FTND scores and

Table 2. Correlation Analysis among Age, Education, Average Monthly Income, Smoking Status, FTND Score, and Urinary Cotinine Levels

Variable	FTND score		Urinary cotinine levels (ng/mL)	
	Coefficient	P	Coefficient	P
Age (year)	0.07	0.17	-0.04	0.48
Education (≤ 12, 13-16, and > 16 years)	-0.13	0.01	-0.04	0.46
Average monthly income (<2, 2-4, 4-7, and >7 million won*)	-0.02	0.70	-0.12	0.03
Cigarette per day (cigarette)	0.69	<0.001	0.55	<0.001
Total smoking period (year)	0.17	<0.001	0.05	0.33
Total lifetime cigarettes (pack year)	0.59	<0.001	0.42	<0.001
Age at smoking initiation (year)	-0.16	0.002	-0.13	0.01
Intention to quit smoking (begin within near 6 months, someday, but after 6 months, and none)	0.15	0.003	0.18	<0.001
Urinary cotinine levels (ng/mL)	0.57	<0.001	N/A	N/A

FTND, the Fagerström test for nicotine dependence; N/A, not available; Correlation coefficient of continuous variable was obtained by the Pearson's correlation analysis, while that of categorical variable was obtained by the Spearman correlation analysis; *Won, a unit of Korean currency which the exchange rate is approximately 1120 won on the US dollar as of January, 2011

Table 3. Stepwise Multiple Linear Regression Model for Nicotine Dependence Measured by FTND Score (n=381, R2=0.348, P<0.001)

Variable	Coefficient (B)	Standardized Beta	P value	95% CI for coefficient		VIF
				Lower bound	Upper bound	
Education (year)						
≤ 12	reference					
> 16	-0.573	-0.089	0.034	-1.102	-0.043	1.001
Total smoking period (year)	0.042	0.141	0.001	0.018	0.067	1.004
Urinary cotinine levels (hundred ng/mL)	0.234	0.557	<0.001	0.200	0.268	1.003

FTND, the Fagerström test for nicotine dependence; VIF, the Variance Inflation Factor; Performing a stepwise multiple regression analysis, age, marital status, education (13-16 years), average monthly income, age at smoking initiation, history of smoking cessation, intention to quit smoking, and partner's smoking were excluded in the final model

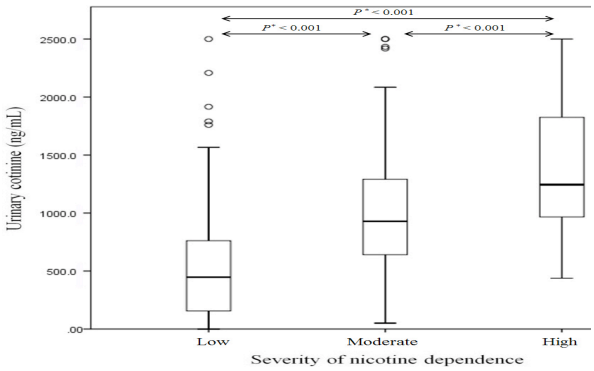

Figure 1. Urinary Cotinine Levels by Nicotine Dependence. Median urinary cotinine levels with interquartile range (25-75 percentile) by nicotine dependence groups (low, FTND score=0-3; moderate, FTND score=4-6; and high, FTND score=7-10). Urinary cotinine levels significantly increased as the degree of nicotine dependence. *P was obtained by the Mann-Whitney U test

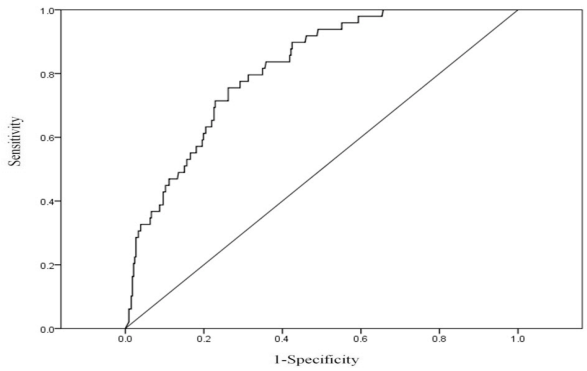

Figure 2. Receiver Operating Characteristics (ROC) Curves Analysis of Urinary Cotinine Levels for High Nicotine Dependence. ROC curves analysis showed that urinary cotinine levels were reasonably accurate for prediction of the high nicotine dependence group (high nicotine dependence is defined as FTND score≥7) (area under curve=0.82; 95% confidence interval=0.76-0.87; P<0.001; sensitivity=71.4%; specificity=74.4%; positive predictive value=58.2%; and negative predictive value=83.9%)

urinary cotinine levels (r=0.693, P < 0.001; r=0.546, P < 0.001, respectively). There was a positive correlation between the FTND scores and urinary cotinine levels (r=0.567, P < 0.001).

Of the 381 subjects, 205 (53.8%) were classified into the low, 127 (33.3%) into the moderate, and 49 (12.9%) into the high nicotine dependence groups. Urinary cotinine levels significantly increased with the degree of nicotine dependence (P < 0.001): low=523.3±449.0 ng/mL; moderate=982.6±510.4 ng/mL; and high=1378.3±577.7 ng/mL nicotine dependence group (Figure 1). In a post-hoc analysis, there were significant differences between the comparisons of urinary cotinine levels of each nicotine dependence groups.

In ROC analysis (Figures 2), urinary cotinine levels were reasonably accurate for prediction of the high nicotine dependence group (AUC=0.82; 95% CI=0.76-0.87; P < 0.001). Using this analysis, the optimal cut-off value of urinary cotinine levels for high nicotine dependence was identified as 1,000 ng/mL. For this cut-off value, the sensitivity, specificity, positive predictive value, and negative predictive value (%) for prediction of the high nicotine dependence were 71.4, 74.4, 58.2, and 83.9 respectively.

In the stepwise multiple linear regression model (Table 3), age, marital status, education (13-16 years), average monthly income, age at smoking initiation, history of smoking cessation, intention to quit smoking, and the presence of a smoking partner were excluded. Finally, education (>16 years), total smoking period, and urinary cotinine levels were included with a satisfactory explanation power (R2=0.348, P < 0.001). The results of the collinearity diagnostics on those variables were acceptable, because their variance inflation factor (VIF) ranged from 1.001 to 1.003. The total smoking period (β=0.042, P=0.001) and urinary cotinine levels (β=0.234, P < 0.001) were positively associated with nicotine dependence, whereas an inverse association between highest education levels (>16 years) and nicotine dependence was observed (β=-0.573, P=0.034).

Discussion

Our results indicate that the level of urinary cotinine is independently associated with the degree of nicotine dependence and that cotinine is a valid biomarker for prediction of high nicotine dependence. To the best of our knowledge, this study is the first to suggest an available cut-off value of urinary cotinine as an indicator

of high nicotine dependence. Because cotinine is an alkaloid found in tobacco and a metabolite of nicotine, its levels in the body have predominantly been used to verify smoking status in individuals. However, our data potentially suggest an extended application of urinary cotinine levels, particularly for assessment of nicotine dependence. We suggest that the optimal cut-off value for the urinary cotinine level that can predict high nicotine dependence is 1,000 ng/mL (sensitivity: 71.4%, specificity: 74.4%).

Urinary cotinine levels and the FTND scores showed similar patterns of change with respect to the socioeconomic status of smokers and smoking-related factors. In the correlation analysis, urinary cotinine levels were associated with both the number of cigarettes smoked per day and the FTND scores, which was consistent with previous studies (Heatherton et al., 1991; Payne et al., 1994; Law et al., 1997; Caraballo et al., 1998; Park et al., 2004; Blackford et al., 2006). In contrast to previous reports that showed that the duration of smoking was not associated with the FTND scores (John et al., 2003; Park et al., 2004), the degree of nicotine dependence increased according to the total smoking period in our study. This is an interesting finding because FTND scores have been thought to reflect mainly physical dependence, and the scale did not include any component related to smoking persistence. Furthermore, this result could partly support the "hardening hypothesis" that more dependent smokers maintain smoking, despite the overall decline of smoking prevalence (Fagerstrom and Furberg, 2008).

Our results also showed an inverse correlation between the age of smoking initiation and the FTND scores, consistent with previous findings (Breslau et al., 1993; Park et al., 2004). A possible explanation for this is that an earlier onset of smoking could induce the development of nicotine habituation and tolerance in a younger age, contributing to subsequent higher nicotine dependence and difficulty in smoking cessation (Breslau and Peterson, 1996; Khuder et al., 1999). However, this inverse correlation was not observed in the step-wise multiple regression analysis.

As observed in previous research (Kraft et al., 1998; Siahpush et al., 2006; Cheah and Naidu, 2012), higher education levels had an inverse association with nicotine dependence. This finding could explain the observation that low education levels could be a barrier for smoking cessation (Breslau and Peterson, 1996; Khuder et al., 1999), although the causal relationship should be confirmed in further prospective research. Consistent with a recent study that showed that lower intention to quit smoking was associated with higher nicotine dependence (Feng et al., 2010), our study found that willingness to quit smoking was inversely correlated with FTND scores and urinary cotinine levels, respectively. This finding could further support our study hypothesis that urinary cotinine levels reflect nicotine dependence status in active smokers because increased intention to quit smoking often leads to a substantial increase of smoking cessation attempts. Thus, more comprehensive approach to regard the association of urinary cotinine levels with the socioeconomic status and smoking behaviors of smokers might be required in future studies.

Serum cotinine is a reliable indicator of nicotine intake. However, previous reports have shown that nicotine is eliminated in the serum, urine, and saliva at a similar rate and suggested that cotinine levels in the urine could provide a comparable indication to serum levels of nicotine intake (de Weerd et al., 2002; Shin et al., 2002). In another study that investigated the association of urine and serum cotinine levels with the amount of time prior to smoking the first cigarette of the day, a similar parallel tendency and association of urinary and plasma cotinine levels with the smoking behavior was observed (Muscat et al., 2009). Therefore, considering the simplicity and non-invasiveness of obtaining urine samples, measurement of urine cotinine levels might be a preferable alternative to measurement of plasma cotinine levels in clinical settings.

There are several limitations to this study. First, because the study was retrospective, the major findings should be confirmed by additional studies with higher evidence levels. Second, the mean age of the study subjects was 45.4 years, and the percentage of women in the study was low (7.6%). The high average age of subjects could be attributed to their selection from a regular health check-up program in a single cancer prevention center. Consequently, these data should be interpreted with caution and not generalized to other populations, especially women and young smokers. All information pertaining to smoking status was acquired by self-administered smoking questionnaires; therefore, the potential recall bias should be considered. Finally, because serum cotinine levels in cigarette smokers have been shown to be dependent on race and ethnicity (Caraballo et al., 1998), the application of the cut-off value for the urinary cotinine level in this study should be restricted to Asians. Accordingly, determination of an optimal cut-off value to detect high nicotine dependence for other races should only follow sufficient consideration of results from additional inter-racial comparative studies.

The present study suggested that urinary cotinine levels could reflect not only smoking status, but also the degree of nicotine dependence. In addition, an available optimal cut-off value of the urinary cotinine to determine high nicotine dependence was also assessed, which further support a clinical availability of urinary cotinine measurement as a screening biomarker ahead of smoking cessation treatment in active smokers. Further studies are needed for a more broad utilization of urinary cotinine measurement.

References

Baker TB, Piper ME, McCarthy DE, et al (2007). Time to first cigarette in the morning as an index of ability to quit

smoking: implications for nicotine dependence. *Nicotine Tob Res*, **9**, 555-70.
Blackford AL, Yang G, Hernandez-Avila M, et al (2006). Cotinine concentration in smokers from different countries: relationship with amount smoked and cigarette type. *Cancer Epidemiol Biomarkers Prev*, **15**, 1799-804.
Breslau N, Fenn N, Peterson EL (1993). Early smoking initiation and nicotine dependence in a cohort of young adults. *Drug Alcohol Depend*, **33**, 129-37.
Breslau N, Peterson EL (1996). Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences. *Am J Public Hlth*, **86**, 214-20.
Caraballo RS, Giovino GA, Pechacek TF, et al (1998). Racial and ethnic differences in serum cotinine levels of cigarette smokers: Third National Health and Nutrition Examination Survey, 1988-1991. *JAMA*, **280**, 135-9.
Cheah YK, Naidu BM (2012). Exploring factors influencing smoking behaviour in Malaysia. *Asian Pac J Cancer Prev*, **13**, 1125-30.
de Weerd S, Thomas CM, Kuster JE, et al (2002). Variation of serum and urine cotinine in passive and active smokers and applicability in preconceptional smoking cessation counseling. *Environ Res*, **90**, 119-24.
Dhavan P, Bassi S, Stigler MH, et al (2011). Using salivary cotinine to validate self-reports of tobacco use by Indian youth living in low-income neighborhoods. *Asian Pac J Cancer Prev*, **12**, 2551-4.
Fagerstrom K, Furberg H (2008). A comparison of the fagerstrom test for nicotine dependence and smoking prevalence across countries. *Addiction*, **103**, 841-5.
Feng G, Jiang Y, Li Q, et al (2010). Individual-level factors associated with intentions to quit smoking among adult smokers in six cities of China: findings from the ITC China Survey. *Tob Control*, **19**, 6-11.
Fu M, Martinez-Sanchez JM, Agudo A, et al (2012). Nicotine depedence and salivary cotinine concentration in daily smokers. *Eur J Cancer Prev*, **21**, 96-102.
Hagimoto A, Nakamura M, Morita T, et al (2010). Smoking cessation patterns and predictors of quitting smoking among the Japanese general population: a 1-year follow-up study. *Addiction*, **105**, 164-73.
Heatherton TF, Kozlowski LT, Frecker RC, et al (1991). The fagerstrom test for nicotine dependence: a revision of the fagerstrom tolerance questionnaire. *Br J Addict*, **86**, 1119-27.
Jang SY, Kim JH, Lim MK, et al (2012). Relationship between BMI, body image, and smoking in Korean women as determined by urine cotinine: results of a nationwide survey. *Asian Pac J Cancer Prev*, **13**, 1003-10.
Jarvis MJ, Tunstall-Pedoe H, Feyerabend C, et al (1987). Comparison of tests used to distinguish smokers from nonsmokers. *Am J Public Hlth*, **77**, 1435-8.
John U, Meyer C, Hapke U, et al (2003). The fagerstrom test for nicotine dependence in two adult population samples-potential influence of lifetime amount of tobacco smoked on the degree of dependence. *Drug Alcohol Depend*, **71**, 1-6.
Khuder SA, Dayal HH, Mutgi AB (1999). Age at smoking onset and its effect on smoking cessation. *Addict Behav*, **24**, 673-7.
Kraft P, Svendsen T, Hauknes A (1998). Intention to stop smoking among Norwegian smokers: the role of nicotine dependence, type of cigarettes, and age at onset of daily smoking. *Addict Behav*, **23**, 133-7.
Law MR, Morris JK, Watt HC, et al (1997). The dose-response relationship between cigarette consumption, biochemical markers and risk of lung cancer. *Br J Cancer*, **75**, 1690-3.
Lerman C, Orleans CT, Engstrom PF (1993). Biological markers in smoking cessation treatment. *Semin Oncol*, **20**, 359-67.
Molander L, Hansson A, Lunell E, et al (2000). Pharmacokinetics of nicotine in kidney failure. *Clin Pharmacol Ther*, **68**, 250-60.
Muscat JE, Stellman SD, Caraballo RS, et al (2009). Time to first cigarette after waking predicts cotinine levels. *Cancer Epidemiol Biomarkers Prev*, **18**, 3415-20.
Oh IH, Yoon SJ, Yoon TY, et al (2012). Health and economic burden of major cancers due to smoking in Korea. *Asian Pac J Cancer Prev*, **13**, 1525-31.
Park SM, Son KY, Lee YJ, et al (2004). A preliminary investigation of early smoking initiation and nicotine dependence in Korean adults. *Drug Alcohol Depend*, **74**, 197-203.
Payne TJ, Smith PO, McCracken LM, et al (1994). Assessing nicotine dependence: a comparison of the Fagerstrom Tolerance Questionnaire (FTQ) with the Fagerstrom Test for Nicotine Dependence (FTND) in a clinical sample. *Addict Behav*, **19**, 307-17.
Pomerleau CS, Carton SM, Lutzke ML, et al (1994). Reliability of the Fagerstrom Tolerance Questionnaire and the Fagerstrom Test for Nicotine Dependence. *Addict Behav*, **19**, 33-9.
Shin HS, Kim JG, Shin YJ, et al (2002). Sensitive and simple method for the determination of nicotine and cotinine in human urine, plasma and saliva by gas chromatography-mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci*, **769**, 177-83.
Siahpush M, McNeill A, Borland R, et al (2006). Socioeconomic variations in nicotine dependence, self-efficacy, and intention to quit across four countries: findings from the International Tobacco Control (ITC) Four Country Survey. *Tob Control*, **15**, 71-5.
USDHHS (2004). The Health Consequences of Smoking: A Report of the Surgeon General.
Verification (2002). Biochemical verification of tobacco use and cessation. *Nicotine Tob Res*, **4**, 149-59.
Zielinska-Danch W, Wardas W, Sobczak A, et al (2007). Estimation of urinary cotinine cut-off points distinguishing non-smokers, passive and active smokers. *Biomarkers*, **12**, 484-96.