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Urinary Nicotine and Cotinine Levels in Smokers and Nonsmokers Related to Smoking Habit in Korea

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ABSTRACT : This study was conducted to determine the urinary nicotine and cotinine concentration in 126 smokers and 143 nonsmokers. While urine samples were being collected, personal characteristics related to smoking habit such as sex, age, number of years since a person has been a smoker, average number of cigarettes consumed per day, and number of smokers in the family were surveyed. Urinary nicotine and cotinine concentration were analyzed by GC/NPD. The smokers smoked an average 17.0 cigarettes per day and the average concentration of nicotine and cotinine was 3.88 μg/ml and 3.64 μg/ml, respectively. The average number of smokers in the family was 0.72 persons and the average concentration of nicotine and cotinine were 0.11 μg/ml and 0.02 μg/ml in the urine of non-smokers, respectively. The concentration of nicotine and cotinine in smoker's urine was dependent on the number of cigarettes smoked per day($p<0.01$). The number of years since a person had been a smoker, and the number of smokers in the family were not associated with the concentration of nicotine and cotinine. Also there was no significant effects of passive smoking on the family of smokers by the level of nicotine and cotinine concentration. We describe the relationship between smoking habit as assessed by urinary nicotine and cotinine excretion. This study provides an evidence for the assessment of active and passive exposure to tobacco smoke.

Key words **: urinary nicotine, cotinine, ETS**

More than 4000 compounds have been identified in tobacco smoke of which nicotine is a principal alkaloid. The main metabolities of nicotine are cotinine, trans-3-hydroxycotinine, nicotine-1-N-oxide, and 3-pyridylcarbinol. An increase in urinary nicotine and cotinine is interpreted to indicate that an individual has recently experienced increased exposure to nicotine agents. Previous investigators who have examined the urinary nicotine and cotinine from smokers and non-smokers generally concluded that smokers urine had more nicotine and cotinine than those of non-smokers. Under selected and controlled field conditions, investigators have demonstrated that the quantities of nicotine and its major metabolites in the physiological fluid appears proportional to the degree of exposure (Margolis et al. 1997; Hansen et al., 2001, Doolittle, 1989). However, it is probably premature to employ nicotine and its metabolites as

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a direct quantitative indicator of ETS exposure. Jarvis(1989) has estimated that, based on physiological levels of nicotine and cotinine, non-smokers exposed to ETS receive a dose of nicotine ranging from 0.5% to 2% that of a heavy smoker. However, many workers investigation of the potential health effects of exposure to ETS believe that nicotine or cotinine is the best currently available biomarker for ETS exposure(Oddoze er al., 1999; Benowitz. 1999; Cummings et al., 1990; Jarvis, 1989). Cotinine concentration in biological fluids may be useful for classifying persons as active smokers or non-smokers.

The use of personal monitoring has been a common practice in the industrial hygiene field for many years, but only recently the analytical methodology has been refined sufficiently to allow ETS measurements by this approach. It is important to determine how well ETS exposure can be predicted by questionnaire or by measurements of urinary nicotine and cotinine, since these approaches are also used as an alternative to direct measurements of exposure (Phillips et al., 1994). Most of the information about the quantities of smoke constituents to which non-smokers may be exposed is based on fixed-site measurements of ETS levels in locations such as homes, offices, and restaurants to gether with assumptions about the time people spend in these locations (Guerin et al., 1992).

This study was carried out to determine the personal effects of tobacco smoke by measurements of the concentration of nicotine and cotinine in the urine. Further, it was the purpose to provide data on biological variation including variation within and between subjects for passive smoking.

MATERIALS AND METHODS

Subjects

Sex and smoking status distribution for study subjects are listed in Table 1. 126 smokers and 143 non-smokers participated in this study; 126 male smokers, 101 male non-smokers and 42 female non-smokers. All subjects were asked detailed questions such as age, sex, the number of years since a person has been a smoker, the average consumption number of cigarettes per day(smoking amount), and the number of smoker in family. All subjects seemed to be in normal health for their age, and lived in Seoul and Taejeon, Korea. Misclassification occurred in this study when smokers reported themselves to be non-smokers or vice versa. In this study, subjects who were regarded as misclassified, they were excluded from personal exposure evaluation.

Table 1. Sex and smoking status distribution for study subjects

Sex	Male		Female	
Smoking status	Smoker	Non-smoker	Smoker	Non-smoker
Sample No	126	101	0	42
Total	227		42	

Urine collection

Total 269 urine samples were collected from May 1, 1996 to June 30, 1998 for this study. Each samples were collected in a 100ml sterile urine collection bottle with NaOH to increase the pH over 11. Subjects were provided with coolers and sufficient reusable ice substitute to keep urine cold throughout the collection period and were kept frozen(-20℃) until analysis.

Nicotine and cotinine analysis

The method of Teeuwen et al.(1989) was modified. A 50 ml aliquot of urine was used for nicotine and cotinine analysis. All analytical works were completed within 2 weeks after sampling. Gas chromatographic analyses were performed on a Hewlett-Packard Model 5880A gas chromatography. A fused silica capillary column (15m x 0.32 mm i.d.) with a 1.0 μm film thickness (DB-5, 5% phenyl-95% methylpolysiloxane bonded phase) was used for separation

of nicotine and cotinine. Calibration curves for nicotine and cotinine were developed by linear regression of the peak area v. known standard concentrations. Standards and samples were injected in triple and the results were averaged.

RESULTS AND DISCUSSION

The concentrations of nicotine and cotinine generally found in the urine of smokers were clearly much higher than those found in non-smokers. Average values and standard deviation for the overall distibutions are shown in Table 2 and Table 3. The average urinary concentration of nicotine and cotinine were 3.88 μg/ml, 3.64 μg/ml in smokers and 0.11 μg/ml, 0.02 μg/ml in non-smokers, respectively. As the nicotine concentration of smoker was similar to the cotinine concentration in urine samples, the nicotine concentration of non-smoker was different from the concentration of cotinine in urine samples. The concentration of nicotine of smokers was approximately thirty-fold higher than the concentration of non-smokers. There was no significant difference of urinary nicotine and cotinine levels among the age group. Also, the smoking amounts assessed through age shows no significant difference among the age group. It means that the absorption and metabolism of nicotine in the human body were similar in the different age group. The range of urinary nicotine values on 126 smokers was 0.2 to 38.9 μg/ml. Nicotine and cotinine levels found in this study were similar with those reported in other studies, including a study by Feyerabend(1982) and Wilcox (1979). The ranges of urinary nicotine and cotinine of smokers reflected the variation in smoking amount, puff frequency, and depth and duration of inhalation. In non-smokers, the detection of nicotine and cotinine in urine could be influenced by proximity to the source of smoke and ventilation in the environment. The average urinary concentration of nicotine and cotinine were 0.07 μg/ml, 0.008 μg/ml in female non-smokers and 0.121 μg/ml, 0.01 μg/ml in male non-smokers, respectively. The different level of nicotine and cotinine between female and male shows a little, but it was not significant. The range of urinary nicotine and cotinine values on 143 non-smokers was 0.0 to 0.28μg /ml, and 0.0 to 0.55 μg/ml, respectively. The 53 samples, and 128 samples over 143 non smoker urine samples shows zero in nicotine and cotinine concentration, respectively. The source of the nicotine and

Table 2. Smoking amount, nicotine and cotinine concentration in the urine of smoker assessed through age

Age	Smoking amount (Cig./day)	Nicotine (μg/ml)	Cotinine) (μg/ml)
Under 20	17.1	5.23±2.11	3.51±0.53
21 - 30	16.0	3.31±0.57	3.04±0.38
31 - 50	17.6	5.18±1.33	3.78±0.58
Over 50	19.1	2.65±0.47	5.36±1.15
All age	17.0	3.88±1.12	3.64±0.66

Table 3. Urinary nicotine and cotinine concentration of non-smokers assessed through age and sex

Age range	Nicotine(μg/ml)		Cotinine(μg/ml)	
	Male	Female	Male	Female
Under 20	0.113±0.126	-	0.021±0.106	-
21 - 30	0.106±0.131	0.034±0.058	0.011±0.035	-
31 - 40	0.102±0.110	-	0.063±0.103	-
Over 40	0.184±0.263	0.095±0.175	0.002±0.012	0.024±0.049
All ages	0.121±0.159	0.069±0.126	0.010±0.059	0.008±0.030

cotinine found in non-smoker's urine poses an interesting question. Davis et al.(1991) demonstrated the presence of nicotine in foods and beverages common to the diet and discussed the implications of this finding for studies related to ETS. The daily intake of nicotine from food would be 8.8 μg. The resulting urinary cotinine concentration is estimated to be 0.6 μg/ml. Maximal food and tea consumption results in an approximately ten-fold increase in nicotine intake (100 μg/day) and an estimated urinary cotinine concentration of 6.2 ng/ml(Davis et al, 1991). Greenberg et al.(1984) suggested that urinary cotinine excretion appeared to be a good indicator of passive smoking by young children. However, its sensitivity, specificity, and range of predictive values need to be established in subjects representing the full spectrum of exposures found in the general population.

Figure 1 shows the relationship between smoking amount and the urinary nicotine and cotinine levels. This figure shows significant

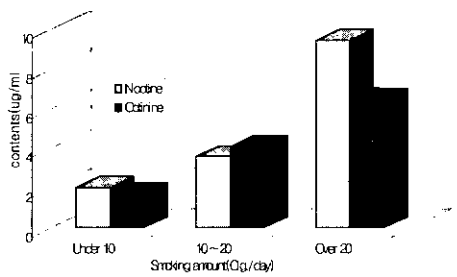

Fig. 1. Changes of urinary nicotine and cotinine concentration assessed through smoking amount

difference from urinary nicotine and cotinine by smoking amount. There was an isolated statistical difference ($p<0.01$, $p<0.005$) in the urinary nicotine and cotinine content among smoking amount. As increasing smoking amount, urinary nicotine and cotinine levels of smoker positively increased. The correlation between measured nicotine and cotinine levels, and smoking amount were determined. A significant, direct relation was found in each of these. Smoking amount was highly correlated with nicotine ($R=0.95$), and cotinine ($R=0.99$) in urine of smokers. This results suggest that urinary nicotine and cotinine excretion may be an useful indicator of exposure to tobacco smoke.

Table 4 shows the summary of analytical data for urinary nicotine and cotinine concentration associated with the number of smoker in family. The level of nicotine and cotinine slightly increased as increasing the number of smoker in family. But, there was no statistical difference ($p>0.1$) in the urinary nicotine and cotinine levels among smoker number in family. The concentration of nicotine and cotinine in the group of no smoker in family was twice higher than those of other smoker number in family. But, because the urinary nicotine and cotinine levels of smoker were mostly affected by smoker's habit of smoking, those difference were not important in this study. Phillips et al.(1996) reported that the highest levels found from smoking house who were exposed to median concentrations of 1.1 $\mu g/m^3$ for nicotine. These levels equated to annualized exposures of 5.8 mg of nicotine for the highest exposed and 0.15 mg

Table 4. Urinary nicotine and cotinine concentration associated with sex and the number of smoker in family

No. of smoker in family	Nicotine(μg/ml)		Cotinine(μg/ml)	
	Male	Female	Male	Female
0	0.094±0.024	0.015±0.010	0.005±0.003	0
1	0.140±0.021	0.061±0.020	0.019±0.087	0.007±0.007
2	0.171±0.063	0.161±0.069	0.014±0.014	0.152±0.304

of nicotine for the worker in non-smoking workplaces. In this study, the average concentration of urinary nicotine measured was 0.07 μg /ml for non-smokers, no appreciable differences among smoker number in family were apparent. The results obtained from this study indicate that the number of smoker in family had no effect on increasing nicotine and cotinine values in the urine of non-smoker. The urinary nicotine and cotinine levels were directly affected on the smoking amount of smoker. Any questionnaire information did not clearly explain the changes of urinary nicotine and cotinine levels of non-smoker.

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