Journal of the Korean Society of
Tobacco Science Vol. 19, No.1, 40~45(1997)
Printed in Republic of Korea

Measurement of Urinary Nicotine and Cotinine Values in Smokers and Non-smokers

Moon-Soo Rhee, Do-young Ra and Keon-Joong Hwang*
Korea Ginseng and Tobacco Research Institute, Taejon 305-345, Korea
(Received May 15, 1997)

흡연자 및 비흡연자의 뇨중 니코틴 및 코티닌 함량

이문수, 나도영, 황건중*
한국인삼연초연구원 분석부
(1997년 5월 15일 접수)

ABSTRACT : This study was conducted to evaluate the personal effects of tobacco smoke and environmental tobacco smoke(ETS) by measuring the concentration of nicotine and cotinine in the urine. While 129 urine samples were being collected, personal characteristics such as sex, age, number of years since a person has been a smoker, average consumption number of cigarettes per day, and number of smoker in family were also surveyed. Collected urine samples were used for analysis of nicotine and cotinine by GC/NPD after passing the extrelut column. In the urine of the smoker, the average contents of nicotine and cotinine were 5.38 μg/ml and 3.14 μg/ml, respectively. The average contents of nicotine and cotinine were 0.18 μg/ml and 0.07 μg/ml in the urine of male non-smoker, respectively. The contents of nicotine and cotinine in the non-smoker's urine were dependent on sex and age. On the other hand, the contents of nicotine and cotinine in smoker's urine were dependent on average consumption amount of cigarettes per day. Also, there was a direct relation between nicotine levels in the smoker's urine and the average consumption number of cigarettes per day of smoker. The possible sources of nicotine and cotinine in the non-smoker's urine seemed to be caused by food, beverage and ETS. Our results indicate that the number of smoker in family had no effect on increasing nicotine and cotinine contents in the urine of non-smoker.

Key words : urinary nicotine, cotinine, ETS marker

Measurement of urinary nicotine and its major metabolite, cotinine is widely used to evaluate recent human exposure to tobacco smoke and environmental tobacco smoke(ETS) since their subsequent contribution to indoor air are still continuing. 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

* Corresponding Author : *Korea Ginseng and Tobacco Research Institute, 302 Shinseong-Dong, Yuseong-Ku, Taejon, 305-345, Korea*
* 연락저자 : 305-345, 대전광역시 유성구 신성동 302번지, 한국인삼연초연구원

the quantities of nicotine and its major metabolites in the physiological fluid appears proportional to the degree of exposure (Jarvis et al., 1984; Roussel et al., 1991; Henderson et al., 1989; Doolittle, 1989). However, it is probably premature to employ nicotine and its metabolites as 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(Cummings et al., 1990; Jarvis, 1989; Wall et al., 1988). The usefulness of nicotine as a biomarker for ETS is limited, because of its short half-life. On the other hand cotinine has a much longer half-life (Benowitz et al., 1986). The presence of cotinine can act as an indicator of chronic exposure to tobacco products, whereas that of nicotine provides information about recent exposure (Greenberg, 1984). Cotinine has been used as a biomarker for exposure to nicotine because 1) it is one of the major metabolites of nicotine, 2) it is eliminated by the kidney with little influence of urinary flow and pH, 3) it is easy to measure in biological fluids (Benowitz et al., 1983; Curval et al., 1990). Cotinine concentration in biological fluids may be useful for classifing persons as active smokers or non-smokers. Cotinine was shown to be superior to the other biomarker compounds used by Jarvis et al. (1987).

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 undertaken to evaluate the personal effects of tobacco smoke and ETS by measuring the concentration of nicotine and cotinine in the urine. Subjects were asked detailed questions about their smoking history and exposure to ETS, and urinary samples were taken for nicotine and cotinine measurements.

MATERIALS AND METHODS

Subjects. Sex and smoking status distribution for study subjects are listed in Table 1. 49 smokers and 80 non-smokers participated in this study; 49 male smokers, 50 male non-smokers and 30 famale non-smokers. All subjects were asked detailed questions such as age, sex, the number of years since a person has been a smoker(smoking period), the average consumption number of cigarettes per day(smoking strength), and the number of smoker in family. All subjects seemed to be in normal health for their age, and lived in Taejeon city, Korea. Misclassification occured 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 ETS exposure evaluation.

Table 1. Sex and smoking status distribution for study subjects

Sex	Smoking status	Subjects
Male	Smoker	49
	Non-smoker	50
Female	Non-smoker	30
Total		129

Urine collection. Total 129 urine samples were collected from July 14 to September 30, 1996 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. A 50 ml aliquot of urine was used for nicotine and cotinine analysis. All analytical works were completed within

2 weeks after sampling.

Nicotine and cotinine analysis. The method of Teeuwen et al.(1989) was modified. Gas chromatographic analyses were performed on a Hewlett-Packard Model 5880A gas chromatograph. 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 number of subjects, smoking period, smoking strength, and the smoker number in family assessed through age are shown in Table 2. The total number of subjects, average smoking period of smoker, average smoking amount of smoker, and average smoker number in family were 49 person, 11.9 year, 19.8 cig., and 1.6 person, respectively.

There was a significant relationship between smoking period and age. This results were most likely due to the continuity of smoking after a person had been a smoker. As increasing an age, smoker number in family was decreased. But the decreasing trend of smoker number in family by age was inclined after 40 year age. When the age range were compared, smoking amount was the highest, and smoker number in family was the lowest in the 31 to 40 year age range. However, in one's thirties, a person smoked the maximum number, 23.9 cigarettes per day, became a heavy smoker.

Table 2. Smoking period, smoking amount, and number of smoker in family assessed through age.

Age range	Subjects	Smoking period (Year)	Smoking amount (Cig./Day)	No. of smoker. in family
Under 20	12	2.5±1.6	16.9±6.3	2.3±1.8
21 - 30	14	6.4±2.7	20.6±6.3	1.9±1.7
31 - 40	13	14.9±4.9	23.9±7.7	1.0±0.0
Over 40	10	23.8±6.8	18.0±7.9	1.2±1.4
All ages	49	11.9±8.9	19.8±7.3	1.6±1.8

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 3. The average urinary concentration of nicotine and cotinine were 5.38 μg/ml, 3.14 μg/ml in smokers and 0.18 μg/ml, 0.07 μg/ml in male non-smokers, respectively. In case of female non-smoker, the average urinary concentration of nicotine and cotinine were .0.05 μg/ml, and 0.09 μg/ml, respectively. The nicotine concentration was about twice as the cotinine concentration of smokers. The concentration of nicotine in smokers was approximately thirty-fold greater than the concentration of non-smokers. When the age range were compared, urinary nicotine level of non-smoker were the highest in the over 40 year age range.

These differences in urinary nicotine and cotinine

Table 3. Urinary nicotine and cotinine concentration of smokers and non-smokers by age

Age range	Nicotine(μg/ml)			Cotinine(μg/ml)		
	Smokers	Non-smokers		Smokers	Non-smokers	
		Male	Female		Male	Female
Under 20	5.91±3.95	0.13±0.13	-	2.94±1.47	0.03±0.01	-
21 - 30	5.38±3.12	0.20±0.17	0.11±0.34	3.42±2.04	0.01±0.04	0.20±0.57
31 - 40	5.34±2.60	0.14±0.12	0.02±0.04	3.09±1.99	0.33±0.92	0.04±0.17
Over 40	4.88±1.96	0.33±0.34	0.02±0.04	3.15±1.59	0.02±0.02	0.05±0.20
All ages	5.38±2.95	0.18±0.20	0.05±0.20	3.14±1.77	0.07±0.37	0.09±0.36

levels of non-smokers by age may be indicative of a higher expose of that age group to ETS and nicotine containing foods, such as black tea, cauliflower.. Nicotine and cotinine levels found in this study were similar with those reported in other studies, including a study by Feyerabend et al. (1982). in which measured urinary nicotine in both smokers and non-smokers. The range of urinary nicotine values in 82 smokers was 0.032 to 6.4 μg/ml. The mean urinary nicotine values in 58 active smokers in study by Wilcox et al.(1979) was reported as 13.3± 1.9 μg/ml. Wilcox et al. also measured the cotinine values in 29 smoker who has a constant daily consumption of cigarettes. The approximate range was 0.156 to 5.14 μg/ml. The range of urinary nicotine and cotinine of smoker 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 source of the nicotine and 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 mg/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. In an early study, Feyerabend et al. (1982) reported urinary nicotine level in a group on non-smoking adults, and found significant increases after experimental exposure in a smoke-filled room. Greenberg et al.(1984) also 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.

Group averages and standard deviation for smoking amount, smoker number in family, and urinary nicotine and cotinine levels assessed through smoking period are presented in Table 4. There was no apparent difference in the smoking amount and smoking period. When the smoking period were compared, smoking amount were the highest in the 11 to 15 year smoking period. The next highest smoking amount was in the over 20 year smoking period. These differences in smoking amount by smoking period may be indicative of the habit of smoking. The urinary nicotine and cotinine levels were not showed clear trend by increasing smoking period. An overview of Table 4 suggested a small variation in urinary nicotine and cotinine levels by smoking period.

Table 4. Smoking amount and urinary nicotine and cotinine concentration assessed through smoking period.

Smoking period (Year)	Subjects	Smoking Amount (Cig./Day)	Nicotine (μg/ml)	Cotinine (μg/ml)
Under 5	21	18.2±5.9	5.62±2.76	3.11±1.68
6 - 10	6	15.8±4.9	4.68±3.28	2.52±2.05
11 - 15	8	27.5±7.1	5.35±3.00	3.77±1.76
16 - 20	8	18.8±8.4	5.54±3.29	3.82±1.76
Over 20	6	21.7±7.5	5.54±1.43	3.45±1.32

The average smoking period, smoker number in family, and urinary nicotine and cotinine levels by smoking amount listed in Table 5. This table showed significant difference from urinary nicotine and cotinine by smoking amount. There was an isolated statistical difference ($p<0.025$, and $p<0.0005$) in the urinary nicotine content among smoking amount. There was another isolated statistical difference ($p<0.005$, and $p<0.025$) in the urinary cotinine content

Table 5. Smoking period and urinary nicotine and cotinine concentration assessed through smoking amount.

Smoking amount (Cig./Day)	Subjects	Smoking period (Year)	Nicotine (μg/ml)	Cotinine (μg/ml)
Under 20	13	10.3±9.9	2.39±1.02	1.71±0.96
20	25	10.3±8.7	4.40±3.07	3.52±2.04
Over 20	11	15.4±7.8	9.50±4.98	5.21±2.63

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 determimed. A significant, direct relation was found in each of these. Smoking amount was highly correlated with nicotine (R=0.88), and cotinine (R=0.78) in urine of smokers. This results suggest that urinary nicotine and cotinine excretion may be an useful indicator of exposure to tobacco smoke. No obvious relationship could be seen between smoking period and smoking amount.

Table 6 shows the summary of analytical data for urinary nicotine and cotinine concentration associated with smoker number in family. The level of nicotine with one smoker in family was the highest measured in this study. This level was two-fold higher than those of other smoker number in family. But, because the urinary nicotine levels of smoker were mostly affected by smoker's habit of smoking, those difference were not important in this study. There was no statistical difference ($p>0.1$) in the urinary nicotine and cotinine levels among smoker number in family. The ETS exposures of individuals in smoking and non-smoking environments have extensively investigated as part of this study. The possible source of nicotine in non-smoker's urine seemed to be caused by food, beverage, and ETS. 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 highest exposed and 0.15 mg of nicotine for the worker in non-smoking workplaces. For the housewives living in smoking homes, the subjects with the highest exposure in this study, the median cotinine level was 2.9 ng/ml. In this study, the average concentration of urinary nicotine measured was 0.18 μg/ml for non-smokers. In the urinary nicotine levels of non-smoker, no appreciable differences among smoker number in family were apparent. Our results indicate that the smoker's number in family had no effect on increasing nicotine and cotinine values in the urine of non-smoker.

Table 6. Urinary nicotine and cotinine concentration associated with number of smoker in family.

No. of smoker in family	Nicotine(μg/ml)		Cotinine(μg/ml)	
	Smoker	Non-smoker	Smoker	Non-smoker
0	3.99±2.64	0.20±0.31	2.82±1.95	0.11±0.34
1	8.05±5.21	0.17±0.17	4.75±2.85	0.03±0.04
2	5.24±3.44	0.19±0.25	3.21±1.65	0.00±0.00

요 약

본 실험은 담배연기 및 환경담배연기(ETS)에 의한 개인적인 영향을 평가하기위하여 urine중의 nicotine과 cotinine 함량을 조사하였다. 129명의 urine 시료를 채취하면서 각 개인의 성별, 나이, 흡연년수, 흡연강도 및 가족중 흡연자 유무 등을 조사하였다. Urine중의 nicotine 및 cotinine 분석은 extrelut column을 이용하여 시료를 정제하고, GC/NPD로 분석하였다. 흡연자의 뇨중에는 ml당 5.38 μg의 nicotine과 3.14 μg의 cotinine이 검출되었으며, 남성 비흡연자의 뇨중에는 ml당 평균 0.18 μg의 nicitine과 0.07 μg의 cotinine이 검출되었다. 비흡연자군에서의 urine중 nicotine 및 cotinine 함량은 개인의 성별 과 나이에 따라 다소의 차이가 있었으며, 흡연자군에서는 개인의 흡연강도에 따라 유의한 차이가 있었다. 비흡연군에서 urine중의 nicotine 및 cotinine의 검출은 음식물, 음료수 및 ETS등에 의한 영향으로 사료되며, 가족중의 흡연자 유무와는 관련이 없는 것으로 나타났다.

REFERENCES

1. Benowitz, N. L., F. Kuyt, P. Jacob, and A. Osman (1983) Cotinine disposition and effects. *Clinical Pharmacology and Therapeutics* 34:604-611.
2. Benowitz, N. L. (1986) The human pharmacology of nicotine. *In*: research advances on alcohol and drug problems. 9:1-52, Plenum Press, New York, USA.
3. Cummings, K. M., S. J. Markello, M. Mahoney, A. K. Bhargava, P. D. McElroy, and J. R. Marchall (1990) Measurement of current exposure to environmental tobacco smoke. *Archives of Envir. Health* 45:74-79.
4. Curvall, M., C. Elwin, E. Kazemi-Vala, C. Qarholm, and C. R.. Enzell (1990) The pharmaco-

kinetics of cotinine in plasma and saliva from non-smoking healthy volunteers. *European J. of Clinical Pharmacology* 38:281-287

5. Davis, R. A., M. F. Stiles, J. D. Eebethizy, and J. H. Reynolds (1991) Dietary nicotine: A source of urinary cotinine. *Fd. Chem. Toxic.* 29:821-827.
6. Doolittle, D. J., C. A. Rahn, G. T. Burger, R. Davis, C. K. Lee, and A. W. Hayes (1989) Human urine mutagenicity study comparing cigarettes which burn or only heat tobacco. *Mutation Research.* 223:221-232.
7. Feyerabend, C., T. Higenbottam, and M. A. H. Russell (1982) Nicotine concentrations in urine and saliva of smokers and non-smokers. *British Medical J.* 284:1002-1004.
8. Greenberg, R. A., N. J. Haley, R. A. Etzel, and F. A. Loda (1984) Measuring the exposure of infants to tobacco smoke. The New *England J. of Med.* 310:1075-1078.
9. Guerin, M. R., R. A. Jenkins, B. A. Tomkins (1992) The chemistry of environmental tobacco smoke: composition and measurement. p.137-158, Ann Arbor, Lewis Publishers, USA.
10. Henderson, F. W., H. F. Reid, R. Morris, O. L. Wang, and S. K. Hammond (1989) Home air nicotine levels and urinary cotinine excretion in preschool children. *Am. Rev. Respir. Dis.* 140: 197-201.
11. Jarvos, M. J., H. Tunstall-Pedoe, C. Feyerabend, C. Vesey, and Y. Salloojoo (1984) Biochemical markers of smoke absorption and self reported exposure to passive smoking. *J. of Epidemiology and Community Health* 38:335-339.
12. Javis, M., H. Tunstall-Pedoe, C. Feyerabend, C. Vesey, and Y. Saloojee (1987) Comparison of tests used to distinguish smokers from non-smokers. *American J. of Public Health* 77:1435-1438.
13. Jarvis, M. J. (1989) Application of biochemical intake markers to passive smoking measurement and risk estimation. *Mutation Research* 222:101-110.
14. Phillips, K., D. A. Howard, D. Browne, and J. M. Lewsley (1994) Assessment of personal exposures to environmental tobacco smoke in british nonsmokers. *Envir. International* 20:693-712.
15. Phillip, K., M. C. Bentley, D. A. Howard, and G. Alvan (1996) Assessment of air quality in stockholm by personal monitoring of nonsmokers for respirable suspended particles and environmental tobacco smoke. *Scand. J. Work Envir. Health* 22:1-24
16. Roussel, G., L. E. Quang, M. L. Migueres, D. Roche, and O. G. Ekindjian (1991) An interpretation of urinary cotinine values in smokers and non-smokers. *Rev. Mal. Resp.* 8:225-232.
17. Russel, M. A. H.., M. J. Jarvis, and R. J. West (1986) Use of urinary nicotine concentrations to estimate exposure and mortality from passive smoking in non-smokers. *British J. of addiction* 81:275-281.
18. Teeuwen, H. W. A., R. J. W. Aalders, and J. M. Van Rossum (1989) Simultaneous estimation of nicotine and cotinine levels in biological fluids using high-resolution capillary column gas chromatography combined with solid phase extraction work-up. *Molucular Biology Reports* 13:165-175.
19. Wall, M. A., J. Johnson, P. Jacob, and N. L. Benowitz (1988) Cotinine in the serum, saliva, and urine of nonsmokers, passive smokers, and active smokers. *American J. of Public Health* 78: 699-701.
20. Wilcox, R. G., J. Hughes, J. Roland (1979) Verification of smoking history in patients after infarction using urinary nicotine and cotinine measurements. *British Med. J.* 2:1026-1028.