〈原　　著〉

Relationship Between Urinary Cotinine Levels of Schoolchildren and Their Parental Smoking Habits

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Ninety-two children (38 males, 54 females) of sixth grade in a primary school. 86 of their fathers and 85 of their mothers were subjected to determination of cotinine in spot urine samples in the early morning by using ion pair HPLC. in ng/mg creatinine.

The habitual smoker among fathers was 62.8%, and that among mothers was 4.7%, and 3.3% of children smoked occasionally. In all families, 65.2% were smoking families where one or more members smoked. A statistically significant difference was noticed between fathers' urinary cotinine levels (mean±S.D.) of 1412.0±871.0 for smokers and 12.2±9.1 for nonsmokers, while no difference between urinary cotinine levels of children in smoking families and nonsmoking families. Moreover, no significant difference was noticed for their respective relationships between paternal smoking doses and children's urinary cotinine levels, and between fathers' and children's urinary cotinine levels, although significant relationship was clear between paternal smoking doses and their urinary cotinine levels. There was also significant relationship between fathers' and

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mothers' urinary cotinine levels.

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Introduction

The health hazards of passive smoking have been well known all over the world[1)~5)]. Since several measures against smoking have been taken recently, the prevalence of smokers has gradually fallen with the exception of young females in Japan[3)]. On the other hand, space sharing between nonsmokers and smokers is getting on. Lately the numbers of nonsmoking railway carriages have considerably increased in a train, and this seems to receive wider support from the general passengers.

The physical effects of passive smoking tend to be realized when some subjective symptoms, such as sneezing, nose running and eye irritation, are developed[6)]. But when people breathe environmental tobacco smoke (ETS) and inhale a certain quantity of nicotine, carbon monoxide (CO) and other harmful constituents of it for a long period of time, they will suffer for some chronic untoward effects of ETS, even if they are unaware of any physiological symptoms. Biomarkers of ETS exposure usually refer to cotinine levels obtained from body fluids such as blood, saliva and urine. Cotinine is a major nicotine metabolite and it is regarded as a marker of active inhalation of tobacco smoke as well as passive inhalation of ETS because of its long biological half-life (about 24 hours), chemical stability and comparatively high concentration in urine[7)8)].

The first aim of this study is to evaluate the extent of passive smokig of schoolchildren by measuring urinary cotinine levels, and the second is to clarify the relation of parental smoking to their children's urinary cotinine levels.

Subjects and Methods

Ninety-five children and their parents who lived in the same households together were requested to provide their urine and to answer a questionnaire. Children were in the sixth grade of a public primary school in Nishio city, which is located in the central part of Japan. Ninety-two children (38 males, 54 females). 86 of their fathers and 85 of their mothers had accepted as to be subjects of this study.

To determine cotinine concentration spot urine samples in the early morning were carefully collected from all of the subjects on one Monday of October in 1994. Urine samples were kept frozen on the spot until the time of assay. Urinary cotinine levels were quantitated by using ion pair reversed-phase HPLC. Urine. 5N NaOH and water saturated with $CHCl_3$ were mixed and shaken. After the liquid-liquid extraction and centrifugation, $CHCl_3$ layer was obtained, evaporated and dissolved in distilled water. As a mobile phase, a mixed solution of 20 mM KH_2PO_4 containing 3 mM sodium 1 decane-sulfonate and CH_3CN (85 : 15) was used. The analyses were performed on TOSOH-HPLC components. The details of determination have been described elsewhere by Takeda and coworkers[9)]. The detectable limit was 1 ng/ml. To adjust urine dilution, the cotinine levels (ng/ml) were standardized by the creatinine levels which were determined by the Jaffe reaction at the same time, so we indicated cotinine levels in ng/mgCr. In addition, CO levels in expired air of children were measured using Smokerlyzer® Micro II (Bedfont. U.K.).

Parents filled out the questionnaire about their smoking status and cigarette consumption, the number of smokers in their households and their child-

ren's respiratory diseases i.e. asthma, emphysema or other respiratory disorders.

Statistical analyses were performed according to t-test and correlation coefficient calculation.

Results

The parental smoking status is shown in Table 1. The rate of habitual smokers among fathers was 62.8%, including occasional smokers. Nonsmoking ratio was 37.2% including exsmokers. Almost all mothers were nonsmokers, and very few mothers were daily smokers. Among children 3 of them (3.3%) were occasional smokers. In this paper cotinine and CO levels of these three children were rejected for calculation. Habitual smokers among fathers consumed 24.8 cigarettes (S.D.=10.9) a day, and those among mothers 16.7 cigarettes (S.D.=5.8) as shown in Table 2.

Table 3 shows the distribution of smokers in the family. About half of the subjects' families had one smoker, and about one third of them no smoker. In this paper "smoking family" denotes the family in which one or more members have smoking habit, and "nonsmoking family" the family in which nobody smokes. In smoking families parents, grandparents and/or siblings smoke, but almost all the smokers were fathers.

Children's CO levels in their expired air are shown in Table 4. The average value of expired CO levels in children from smoking families was almost same with the value in children from nonsmoking families. Thus there was no difference between the CO levels in children from smoking and from nonsmoking families.

Table 5 shows the mean urinary cotinine levels in children according to the familial smoking conditions. No difference existed between urinary cotinine levels of children from smoking families (7.8±11.9 ng/mgCr) and from nonsmoking families (8.6±13.4ng/mgCr), although maximum concentration of urinary cotinine such as 65.0 ng/mgCr and 47.5 ng/mgCr was recognized. respectively, over measurements in both groups.

Table 6 shows the mean urinary cotinine levels in fathers and mothers according to their own smoking states. A statistically significant difference ($p<0.01$)

Table 1 Smoking Status. N (%)

Smoking habits	Fathers		Mothers		Children
Daily smokers	52(60.5)	54(62.8)	3(3.5)	4(4.7)	0(0.0)
Occasional smokers	2(2.3)		1(1.2)		3(3.3)
Ex-smokers	18(20.9)	32(37.2)	5(5.9)	81(95.3)	0(0.0)
Never-smokers	14(16.3)		76(89.4)		89(96.7)
Total	86(100.0)	86(100.0)	85(100.0)	85(100.0)	92(100.0)

Table 2 Habitually Smoking Parents' Daily Cigarette Consumption.

Parents	N	Mean	S.D.	Min	Max
Fathers	51	24.8	10.9	10	70
Mothers	3	16.7	5.8	10	20

Table 3 Distribution of Smokers in the Family.

Numbers of smokers	N (%)	N (%)
None	32 (34.8)	32 (34.8)
1 person	51 (55.4)	60 (65.2)
2 persons	8 (8.7)	
3 or more persons	1 (1.1)	
Total	92 (100.0)	92 (100.0)

Table 4 CO Levels in Expired Air of Children in Relation to Smoking Conditions in the Family. (ppm)

Children	N	Mean	S.D.	Min	Max
Children in smoking families	55	6.7	1.4	5.0	12.0
Children in nonsmoking families	32	6.7	1.2	5.0	9.0

Table 5 Urinay Cotinine Levels of Children in Relation to Smoking Conditions in the Family. (ng/mg/Cr)

Children	N	Mean	S.D.	Min	Max
Children in smoking families	56	7.8	11.9	0.5	65.0
Children in nonsmoking families	31	8.6	13.4	0.5	47.5

Table 6 Urinary Cotinine Lelels of Parents in Relation to Their Smoking Status. (ng/mg/Cr)

Fathers	N	Mean		S.D.	Min	Max
Smokers	52	1412.0	**	871.0	126.8	4406.3
Nonsmokers	28	12.2		9.1	0.6	34.8
Mothers	**N**	**Mean**		**S.D.**	**Min**	**Max**
Smokers	2	2484.6		2792.3	510.1	4459.0
Nonsmokers	73	14.4		33.2	0.3	218.0

(** : $p<0.01$)

was noticed between fathers' urinary cotinine levels (mean±S.D.) of 1412.0±871.0 ng/mgCr for smokers and 12.2±9.1 ng/mgCr for nonsmokers. Moreover, a significant correlation was clearly noticed between paternal smoking doses (X) and their own urinary cotinine levels (Y) such as $Y=148.10+51.947X$ ($R=0.71$, $p<0.01$), as shown in Figure 1. On the other hand, no statistical significance was noticed for their respective relationships between paternal smoking doses (X) and children's urinary cotinine levels (Y), $Y=6.64+0.103X$ ($R=0.11$), in Figure 2, and between fathers' (X) and children's (Y) urinary cotinine levels, $Y=7.24+9.205e^{-4X}$ ($R=0.07$), in Figure 3, Concerning maternal urinary cotinine levels (Y), Figure 4 shows a low but statistically significant correlation with paternal cotinine levels (X) such as $Y=-21.208+0.148X$ ($R=0.24$, $p<0.05$). There was no significant correlation between cotinine levels in children and in mothers ($R=0.04$),

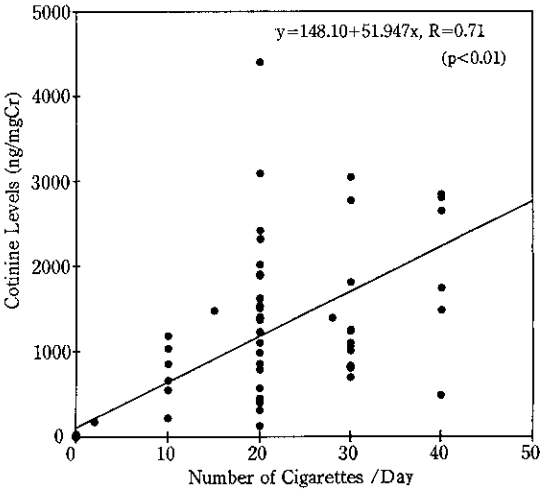

Figure 1 Fathers' urinary cotinine levels in relation to their reported cigarette consumption.

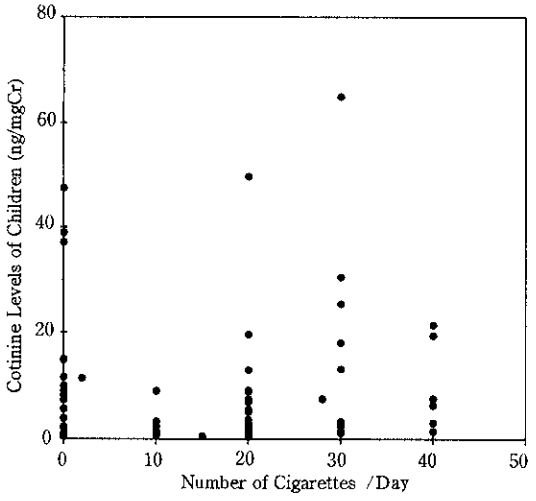

Figure 2 Children's cotinine levels in relation to fathers' daily cigarette consumption.

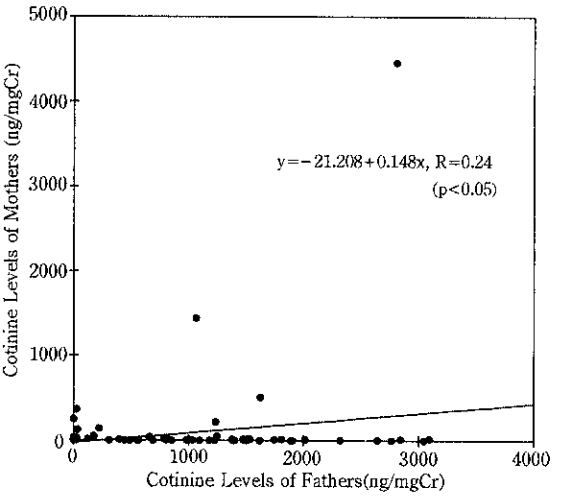

Figure 3 Relationship between urinary cotinine levels of children and fathers.

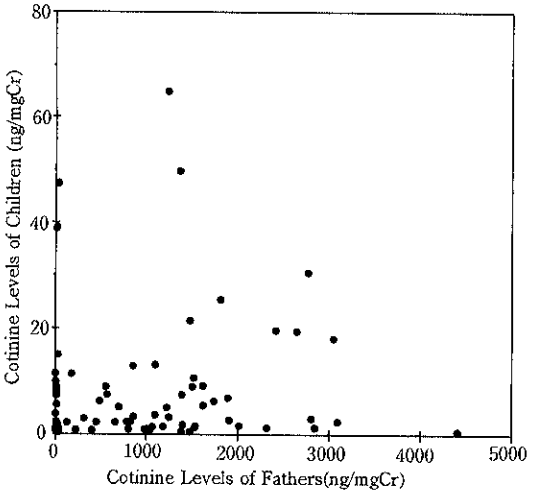

Figure 4 Relationship between urinary cotinine levels of fathers and mothers.

though not shown in figure.

Table 7 shows the influence of passive smoking among nonsmoking parents in relation to smoking status in their families. Although the mean value of urinary cotinine levels of nonsmoking fathers in smoking families was rather larger than that in nonsmoking families, and among nonsmoking mothers the difference between the two of mean values was much larger, any statistical significance was not noticed among these apparent differences, because of the wide distribution. We have not made any description about parental passive smoking in their work places, but it will be able to reflect on their cotinine levels.

Table 8 shows the incidence rates of children's respiratory diseases answered by their parents. We didn't notice any statistically significant differences between children from smoking and those from nonsmoking families.

Discussion

Cotinine, which is a major metabolite of nicotine, has been regarded as an appropriate "biomarker" of passive smoking[10)-13)] because its half life is relatively long. In the results obtained, the relationship between the urinary cotinine levels and the dose of active smoking of fathers is consistent with the well-documented knowledge as described formerly. To the contrary, in the present study, the fact that no association of urinary cotinine levels of children was evidenced with those of their fathers in the

Table 7 Urinary Cotinine Lelels of Nonsmoking Parents in Relation to Smoking Status in the Family. (ng/mg/Cr)

Nonsmoking fathers	N	Mean	S.D.	Min	Max
Nonsmoking fathers in smoking families	5	14.5	11.5	0.6	29.0
Nonsmoking fathers in nonsmoking families	28	11.7	8.8	0.8	34.8
Nonsmoking mothers	**N**	**Mean**	**S.D.**	**Min**	**Max**
Nonsmoking mothers in smoking families	44	17.1	38.4	0.3	218.0
Nonsmoking mothers in nonsmoking families	29	10.2	22.3	0.4	124.3

Table 8 Numbers and Percentages of Children Suffering from Respiratory Diseases in Smoking Families and Nonsmoking Families.

N (%)

	Children in smoking families	Children in nonsmoking families
None disease	36 (62.1)	25 (78.1)
Some diseases	22 (37.9)	7 (21.9)
Total	58 (100.0)	32 (100.0)

same households may indicate at first sight that the urinary cotinine is not a valid surrogate for children passively exposed to ETS in household. A study[14] using urinary cotinine has reported highly positive correlation between cotinine levels and parental, especially maternal, smoking. Jarvis[15] also measured saliva cotinine and stated that saliva cotinine concentrations in nonsmoking schoolchildren were strongly related to the smoking habits of their parents. To establish a stable profile of exposure to ETS in the home, multiple measurements are needed[16]. In this paper children's CO levels in expired air also showed no relationship with parents' smoking. What led the present results different from those of other reports? There might be a few causes, such as air leakage or ventilation of the housing, personal contact in the family, and knowledge about effects of active and passive smoking among parents.

Recently in Japan the warning against the health effects of active and passive smoking has been well communicated to the general public through the mass media. Most parents participated in this study, even smokers appeared very likely to understand the hazards of smoking. So, we can speculate that smoking parents might smoke in thier houses, with paying attention not to diffuse the tobacco smoke over their family. Additionally in school education, the smoking-prevention programs for children have been much improved and practiced. The permeation of these procedures against smoking, that lead children to avoid expose to ETS, might be one of the reasons of no association of urinary cotinine levels of children with those of their fathers. The prevalence of exsmokers (20.9%) might also suggest the educational effects. Further, from the findings of this study that fathers' cotinine levels have the relationship not with those of their children but with those of their partners, we can guess that the present smokers in the households might be much less careful to smoke cigarettes near their wives than near the children.

To clarify the reason of this controversy, we should make researches on details of paternal smok-

ing behavior in a household by measuring the duration of fathers' smoking near their children. Additionally, from the result that the mean of urinary cotinine levels of children in nonsmoking families was almost the same as that of children in smoking families, we can guess that children might be exposed to ETS not only inside but also outside their households. In fact, habitual smokers in town tend to light up their cigarettes regardless of the presence of people around them in Japan.

The close relationship between passive smoking and the incidence of children's respiratory diseases, such as asthma, are well known[17)-19)].

Moreover, Casale[20)] reported that the greater the exposure to passive smoking, the lower the pulmonary function in schoolchildren. In the present study, nevertheless, we could not find any definite relation between childern's passive smoking and the incidence rates of their respiratory diseases as shown in Table 8. Although the previous reports[17)-19)] mentioned the hazardous effects of maternal smoking on children, we are also not able to clarify whether or not maternal smoking doses correlate with childern's cotinine levels, because of extremely low prevalence of mothers' smoking habit (4.7%).

Comparing the urinary cotinine levels in children and those in nonsmoking parents, the parents were much more exposed to ETS. This is most conceivable, because the parents might be exposed to smoke not only in the households but in their work places, where the smoking is not generally forbidden. In Great Britain[9)] ETS exposure in the household was estimated to account for an average of 70% of the total reported exposure for nonsmokers who lived with smokers while a corresponding figure for subjects not living with smokers was 13%. Other investigations[21)-23)] in U.S. and the Netherlands reported high indoor air pollution by smoking in the household, although a paper[24)] in Japan reported low air pollution by tobacco smoke. We must consider the difference not only of the air exchange of residences but also of the smoking behavior between Japan and other countries, in studying the health effect of passive smoking especially for children.

Conclusion

From the findings that urinary cotinine levels of children, even if they were members of nonsmoking families, varied in a wide range, we can strongly suggest that schoolchildren experiencing urban life are inevitably exposed somewhere to ETS regardless of their parents' smoking habits. In our country, smoke-free spaces have been gradually increased, whereas, most of the shops and restaurants where children often enter do not have non-smoking areas. They will provide, if any, not spaces but only seats, where nonsmokers may breathe ETS easily. To protect children's health against passive smoking, it must be necessary that the space where children often use is completely smoke-free.

References

1) U.S. Surgeon General: The Health Consequences of Involuntary Smoking; A report of the Surgoon General. Rockville, U.S. Department of Health and Human Services, *Public Health Services*, (CDC) 87-8398, 1986.
2) National Institute of Health: Respiratory Health Effects of Passive Smoking; Lung Cancer and Other Disorders, Smoking and Tobacco Control. NIH Publication No. 93-3605, 1993.
3) Koseishou (Ministry of Health and Welfare): Kitsuen to kenkou (Smoking and Health). Kitsuen to kenkoumondai ni kansuru houkokusho Dai 2 Han, KenkouTairyokuzukuri, Tokyo, 1993, (in Japanese)
4) Shimizu, Y., Takemoto, K., Kasuga, H.: Judoukitsuen ni kansuru kenkyu no genjou to mondaiten. Digest of Science of Labour, **43** (5), 19-25, 1988. (in Japanese)
5) Asano, M.: Environments and health effects of passive smoking, Respiration, **4** (5), 478-491, 1985. (in Japanese)
6) Muramatsu, T. Morita, J., Kojima, J., Muramatsu, S., Takahashi.K. and Itoh, A.: Parental Smoking

Habits and Their Children's Second Hand Tobacco Smoke, Jpn J School Health, **19**, 382-389, 1977. (in Japanese)

7) Tomita, M., Okada, M., Matsumoto, M., Yamaguchi, M., Hosoda, Y. and Mizuno, S.: Cigarette smoking and mortality among Japanese males in a prospective cohort study. Jpn J Public Health, **38**, 492-497, 1991. (in Japanese)

8) Haley, N.J., Sepkovic, D.W. and Hoffmann, D.: Elimination of cotinine from body fluids; Disposition in smokers and nonsmokers, *Am J Public Health*, **79**, 1046-1047, 1989.

9) Takeda, N., Jitsunari, H., Asakawa, F., Suna, S., Manabe, Y., Fukunaga, I. and Goto, A.: Simultaneous determination of cotinine and creatinine by high performance liquid chromatography, Jpn J Ind Health, **35**, 198-199, 1993.

10) Jarvis, M., Tunstall, P.H., Feyerabend, C., Vesey, C. and Salloojee, Y.: Biochemical markers of smoke absorption and self reported exposure to passive smoking, *J Epidemiol Commun Health*, **38**, 335-339, 1984.

11) Thompson, S.G., Stone, R. Nanchahl, K. and Wald N.J.: Relation of urinary cotinine concentrations to cigarette smoking and to exposure to other people's smoke, *Thorax*, **45**, 356-361, 1990.

12) Willeres, S., Attewell, R., Bensryd, I., Schutz, A., Skarping, G. and Vahter, M.: Exposure to environmental tobacco smoke in the household and urinary cotinine excretion, heavy metals retention, and lung function, *Arch Environ Health*, **47** (5), 357-363, 1992.

13) Matsukura, S., Taminato, T., Kitano, N., Seino, Y., Hamada, H., Uchihashi, M., Nakajima, H. and Hirata, Y.: Effects of environmental tobacco smoke on urinary cotinine excretion in nonsmokers, *New Eng J Medicine*, **311** (13), 828-832, 1984.

14) Schneider, J.M., Copolaghi, B., Briancon, S., Merlin, J.P.: Urinary cotinine as a marker of passive tobacco smoking in children, *Arch Fr Pediatr*, **50**, 567-571, 1993.

15) Jarvis. M.J. et al.: Passive Exposure to Tobacco Smoke; Saliva Cotinine Concentrations in a Representative Population Sample of Non-Smoking Schoolchildren, *Br Med J*, **291**, 927-929, 1985.

16) Coultas, D.B., Samet, J.M., Maccarthy J.F. and Spengler, J.D.: Variability of measures of exposure to environmental tobacco smoke in the home, *Am Rev Respir Dis*, **142** (3), 602-606, 1990.

17) Ehrlich, R., Kattan, M., Godbold, J., Saltzberg, D.S., Grimm, K.T., Landrigan, P.J. and Lilienfeld, D.E.: Childhood asthma and passive smoking, *Am Rev Respir Dis*, **145**, 594-599, 1992.

18) Wartenberg, D., Ehrlich, R. and Lilienfeld, D.: Environmental tobacco smoke and chilhood asthma: comparing exposure metrics using probability plots, *Environmental Research*, **64** (2), 122-135, 1994.

19) Martinez, F.D., Cline, M. and Burrows, B.: Increased incidence of asthma in children of smoking mothers, *Pediatrics*, **89**, 21-26, 1992.

20) Casale, R., Colantonio, D., Cialente, M., Clorizio, V., Barnabei, R. and Pasqualetti, P.; Impaired pulmonary function in schoolchildren exposed to passive smoking, *Respiration*, **58** (3-4), 198-203, 1991.

21) U.S. Department of Health and Human Services: The health consequcnces of involuntary smoking, a report of the Surgeon General. Rockvill, MD; U.S. Department of Health and Human Services, (DHHS publication no. [CDC] 87-8398), 1986.

22) Spengler, J.D., Dockery, D.W., Turner, W.A., Wolfson, J.M. and Ferris, B.G. Jr.: Long-term measurements of respirable sulfates and particles inside and outside homes, *Atoms Environ*, **15**, 23-30, 1981.

23) Brunekreef, B. and Boleij, J.S.M.: Long-term average suspended particulate concentrations in smokers' homes, *Int Arch Occup Environ Health*, **50**, 299-302, 1982.

24) Muramatsu, M., Umemura, S., Okada, T. and Tomita, H.: Estimate of personal exposure to tobacco smoke with a newly developed nicotine personal monitor, *Environ Res*, **35**, 218-227, 1984.