Active and Passive Exposure Status to Tobacco Smoke of Department Store Employees Measured by Cotinine ELISA

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Abstract

Quantitation of urinary cotinine, a major metabolite of nicotine, by an enzyme-linked immunosorbent assay (ELISA), was performed in parallel with questionnaires containing items on smoking status, such as active and/or passive smokers, the number of cigarettes smoked, and the presence or absence of active smokers in the surroundings in a department store (517 employees). The cotinine values corrected by creatinine (cotinine-creatinine ratios, CCRs) approximately conformed to the extent of self-recognition of their exposure status to tobacco-smoke, and were low in the order of active smokers, passive smokers and non-smokers who felt they were not exposed to tobacco-smoke. Occupational differences of the CCRs were not found in the employees. In the active smokers, the CCRs were increasing according to the number of cigarettes per day they smoked, and the values were nearly proportional to nicotine contents of cigarette in the moderate smokers who smoked 11-20 cigarettes per day. The CCRs of males were higher than those of females in the active smokers, which also agreed well with the numbers of cigarettes they smoked per day. In the passive smokers, the CCRs were remarkably and significantly higher in subjects who felt they were exposed to tobacco-smoke both in their workplaces and homes. Urinary CCRs measured by ELISA are thus found to be a reliable and excellent objective indicator of both active and passive exposure-status to tobacco-smoke.

Key words: Urinary cotinine, Exposure status to tobacco-smoke, Relationship between subjective recognition and cotinine values, ELISA, Department store employees

Introduction

In Japan, tobacco smoking prevalence was estimated at 52.7% (males), 10.6% (females) and 31.7% on average, according to the annual report on health and welfare by the Ministry of Health and Welfare in 1995. The report of Japan Tobacco Incorporated also reported that prevalence was estimated at 51.6% (males), 14.5% (females) and 34.6% on average in 1997 in Japan. The proportion of male smokers thus remains at about the same but female smokers are increasing gradually. In Japan, the smoking prevalence is higher in the young than in the old, and it is also higher when compared with other developed countries.

Smoking enhances the risk of suffering from cancers of lung and other organs, and from other various diseases such as ischemic heart diseases, pulmonary disorders, and gastrointestinal disorders. Some papers reported that mainstream smoke (smoke actively inhaled) and sidestream smoke (smoke released in ambient air from cigarettes between active puffs) had different composition and the latter contained higher amounts of tar and nicotine. However, by another report, both mainstream and sidestream smoke have been estimated to have similarities in composition. In any case, exposure to environmental tobacco smoke (ETS) has been linked to a number of adverse health effects in children and adults. Tobacco smoke furthermore produces an unpleasant smell, especially in public transportation, medical institutions, railroad stations and bus stops, schools, workplaces, and homes.

The majority of the estimates of exposure status to tabacco smoke in epidemiological studies has been based on questionnaire data. In the reports on the relationship between lung cancers and passive exposure to ETS in workplaces, Kabat and Wynder reported the existence of significant interrelationship only in male...
workers. However Lee et al.\textsuperscript{19} reported there was no increase in the relative risk of lung cancers by passive exposure to ETS. Furthermore, Fontham et al.\textsuperscript{20} reported there was a tendency to increase in the relative risk of lung cancers in nonsmoking women depending on the duration of exposure to ETS in the workplaces. These reports seem to show some discrepancies presumably due to lack of quantification in questionnaire data used in their epidemiological studies.

The present investigation was therefore undertaken to evaluate the extent of active and/or passive exposure status to tobacco-smoke by questionnaire and quantitating urinary cotinine, a major metabolite of nicotine\textsuperscript{18,19}, by using the enzyme-linked immunosorbent assay (ELISA) which we have developed\textsuperscript{24}. Considering its average biological half-life of 19 hours in blood\textsuperscript{20}, cotinine values may be an inaccurate biomarker in the case of unusual smokers who smoke only on the days when they drink alcohol or in the case of non-smokers who are exposed to ETS only in bars and/or game centers. Cotinine values, however, still offer several advantages over other biochemical markers as an objective indicator of nicotine intake or confirmation of nonsmoker status: it is a specific indicator of nicotine intake, its concentrations are not influenced by confounding factors such as diet or environment and its concentrations within a given individual varies by only 15 to 20\% over 24 hours\textsuperscript{24}. On the other hand, it is supposed to be difficult that non-smokers recognize quantitatively the real exposure status to ETS. This study also investigates the relationship between subjective recognition and objective measures concerning active and/or passive exposure to tobacco-smoke in a total of 517 employees in a department store. In this study, furthermore, we tried to elucidate whether an appropriate combination of questionnaires concerning smoking status and measuring cotinine or cotinine measurement alone improves epidemiological studies on passive smoking in particular.

Materials and Methods

1. Questionnaire concerning smoking status

Information collected on smoking status was as follows: the number of cigarettes smoked per day and on the day before the questionnaire was given out, brand names smoked in order to estimate nicotine contents, the presence or absence of active smokers in the surroundings in the workplace and/or at home, duration of exposure to tobacco-smoke, and whether they were exposed to tobacco-smoke on that day or not. Further information requested was age, gender and area of assigned work.

2. Study populations

Having obtained informed consent, a total of 517 employees (172 males: age 20 to 67 years with a mean of 29 years and 345 females: age 19 to 62 years with a mean of 34 years) was chosen from a department store. Each subject at a regular physical examination was given out a questionnaire as described above. After completing the questionnaire on the spot an aliquot of urine was collected. The urine samples were stored at -80°C until assayed. Except the 15 unusual smokers who smoked only on the days when they drank alcohol, the 502 study subjects were divided into 3 groups based on their exposure [A: 159 active smokers (105 males and 54 females), B: 192 passive smokers who were not active smokers but felt they were exposed to ETS in their workplaces and/or at their homes (31 males and 161 females), and C: 151 non-smokers who felt they were not exposed to any ETS (35 males and 116 females)].

3. ELISA of urinary cotinine and measurement of urinary creatinine

ELISA of urinary cotinine was performed with rabbit monospecific polyclonal anti-cotinine by using microtiter-plates (Becton Dickinson, NJ., USA) according to the method described by Yoshioka et al\textsuperscript{24}. The lower limit of sensitivity of this method was 1-2 ng cotinine/ml. Urinary creatinine was measured spectrophotometrically at 410 nm after adding 1\% (w/v) picric acid and 1 M NaOH to urine samples diluted 150 fold\textsuperscript{46}. In order to avoid the influence of the concentration (density) of urinary samples, cotinine values were corrected by dividing with creatinine values of the same urine samples and were represented as cotinine (ng)-creatinine (mg) ratios (CCR).

4. Statistical analysis

Since urinary cotinine values do not distribute normally (the values distribute with a positive skewness) in addition to the insufficient number in some study groups (less than 30), median, percentiles and the cumulative relative frequency were calculated according to the methods described by Dawson-Saunders and Trapp\textsuperscript{10}. Statistical comparison of means and medians among groups was performed by using Fisher-Behrens z test and the Wilcoxon rank-sum test\textsuperscript{10}, respectively.

Results

1. Distribution of the employees and their urinary CCRs

Excluding the 15 unusual smokers who smoked only on the days when they drank alcohol, the 502 study subjects were divided into sales assistants and sales representatives, and 22.9\% of the males and 48.2\% of the females were working as the formers and the remainder as the latters. Their urinary CCRs are shown according to their occupations in Table 1. Although CCRs were significantly higher in male than in female employees (p<0.01), no statistically significant differences were found between both occupations.

2. Comparison between urinary CCRs and self-recognition of exposure

In groups A (active smokers), B (passive smokers who felt

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### Table 1  Distribution of the employees and their urinary cotinine-creatinine ratios, CCRs (ng/mg).

<table>
<thead>
<tr>
<th></th>
<th>male</th>
<th>female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>sales assistant</td>
<td>sales representative</td>
</tr>
<tr>
<td>Number</td>
<td>64</td>
<td>107</td>
</tr>
<tr>
<td>Average age (range)</td>
<td>28 (20-51)</td>
<td>30 (20-67)</td>
</tr>
<tr>
<td>Urinary CCRs (ng/mg)</td>
<td>758 [133, 2449]</td>
<td>333 [27, 2300]</td>
</tr>
<tr>
<td>median [25% ile, 75% ile]</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
they were exposed to ETS) and C (non-smokers who felt they were not exposed to ETS), their urinary CCRs (ng/mg) are shown in Fig. 1.

The median, 25 and 75 percentile distribution of CCRs were 1568, 575 and 3634 for group A, 61, 9 and 168 for group B and 27, 5 and 86 for group C, respectively. Although 13.2% of the nonsmokers in group C showed higher than 100 ng/mg of CCRs and 25.5% of the passive smokers in group B showed lower than 10 ng/mg of CCRs, the median of CCRs group B was significantly higher than that of group C (p<0.01), and that of group A was also significantly higher than those of other groups (p<0.01). The CCRs of 33.8% of the non-smokers (group C) surpassed the median of the passive smokers (group B).

3. Comparison of urinary CCRs between both genders in active smokers

It was found that 105 out of 172 males and 54 out of 345 females smoked in this department store. The number of cigarettes smoked per day, the percentage of subjects who smoked cigarettes containing less than 0.5 mg, 0.5 to 0.8 mg, or more than 0.8 mg of nicotine per cigarette (based on information provided on packages by tobacco companies), and their urinary CCRs in these active smokers (group A) are shown in Table 2. The number of cigarettes smoked per day and on the very day before the questionnaire was given out (mean ± 1 standard deviation) were 19.3 ± 9.6 and 6.7 ± 4.4 for males and 9.8 ± 5.3 and 4.4 ± 3.2 for females, respectively. The number of cigarettes smoked was significantly higher in males than in females (p<0.01). Males smoked mostly cigarettes with high nicotine while females smoked mostly cigarettes with moderate nicotine, but such tendency was not significant. The median, 25 and 75 percentile distribution of urinary CCRs (ng/mg) were 1933, 666 and 3880 for males and 1108, 447 and 3351 for females, respectively, but such differences were not statistically significant.

4. Urinary CCRs of workers in smoking and in non-smoking areas

Their workplaces were divided into two areas: smoking area where all the staffs can smoke (offices, restaurants, cafeterias, galleries, etc.) and non-smoking area where smoking is prohibited (at sale areas, lifts and escalators for customer transportation, etc.). Active smokers (group A), passive smokers (group B) and nonsmokers who felt they were not exposed to ETS (group C) totaled 502 employees were classified into two classes based on the information given in the questionnaire as to where their assignment was to work (subjects who worked in the smoking area and those who worked in the non-smoking area), and their urinary CCRs are presented in Table 3. Medians of the CCRs of subjects who work in the smoking area were 1816 for group A, 50 for group B and 27 for group C. Medians of urinary CCRs in group A, B and C who worked in the non-smoking area were 1435, 67 and 30, respectively. There were no statistically significant differences between urinary CCRs of subjects who worked in the smoking area and those in the non-smoking area either in total, male, or female subjects.

5. Influence of exposure status in workplaces and/or homes on urinary CCRs

In 192 passive smokers (group B), 33 workers felt they were exposed to tobacco smoke passively both in their workplaces and at home (group H+W+), 113 workers felt they were exposed to tobacco smoke passively only in their workplaces (group H+W−), and 46 workers felt they were exposed to tobacco smoke only in their homes (group W+H−).
workplaces (group H-W-). Their urinary CCRs are presented in Fig. 2. Median, 25 and 75 percentile distribution of CCRs were 97, 41 and 201 for group H+W+, 48, 8 and 169 for group H+W- and 30, 9 and 142 for group H-W+, respectively. Incidentally, median, 25 and 75 percentile distribution of the urinary CCRs of 151 non-smokers who felt they were not exposed to any ETS (group C or group H-W-) were 27, 5 and 86, respectively. Median in group H+W+ was the highest among these groups, and was significantly higher in comparison with that in group H-W-(p<0.01) and with that in group H-W+ (p<0.05), but was not significantly different from that in group H+W-.

6. Relationship between estimated nicotine amounts inhaled and the urinary CCRs in active smokers

141 active smokers, who answered the questions on the numbers of cigarettes smoked per day and on that day before the questionnaire was given out, and the brand name of cigarettes smoked, were selected out of 159 active smokers (group A). They were divided into 9 groups based on the number of cigarettes smoked per day (10 or less (light smokers), 11 to 20 (moderate smokers), and 21 or more (heavy smokers)) and on the nicotine content per cigarette that they smoked usually [less than 0.5 mg (low nicotine), 0.5 to 0.8 mg (moderate nicotine), and more than 0.8 mg (high nicotine)]. The number of subjects of each group and medians of their urinary CCRs are presented in Fig. 3. The CCRs were increasing in the order of light, moderate and heavy smokers, and those of the heavy smokers were significantly higher in comparison with those of the light smokers at p<0.01, irrespective of nicotine contents of cigarettes they smoked. The CCRs of the heavy smokers, irrespective of nicotine contents of cigarettes they smoked, were also significantly higher in comparison with those of moderate smokers who smoked cigarettes with low or moderate (p<0.01) and with high nicotine contents (p<0.05). The CCRs of moderate smokers smoking high nicotine cigarettes deviated significantly from those of light smokers at p<0.01, irrespective of nicotine contents they smoked. In the moderate smokers, the CCRs were increasing in the order of nicotine contents in cigarettes they smoke, and that of the smokers smoking high nicotine cigarettes was significantly higher than that of the smokers smoking low nicotine cigarettes at p<0.01.

Table 3  Urinary CCRs (ng/mg) of staffs who worked in smoking and non-smoking areas.

<table>
<thead>
<tr>
<th></th>
<th>in smoking area</th>
<th>in non-smoking area</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
</tr>
<tr>
<td>Group A (active smokers)</td>
<td>1961 (63)</td>
<td>959 (14)</td>
</tr>
<tr>
<td>Group B (passive smokers)</td>
<td>36 (20)</td>
<td>63 (72)</td>
</tr>
<tr>
<td>Group C (non-smokers who feel they were not exposed to any tobacco smoke passively)</td>
<td>8 (24)</td>
<td>45 (29)</td>
</tr>
</tbody>
</table>

Urinary CCRs are presented as median.
Number in parentheses shows number of subjects.
Groups A, B and C: See details in the text.

Fig. 2  Three dimensional histogram of medians of urinary CCRs (ng/mg) of the passive smokers and the non-smokers who felt they were not exposed to any tobacco smoke passively.
H+W+ : non-smokers exposed to tobacco smoke in their workplaces and at their homes ; H+W- : non-smokers exposed to tobacco smoke only in their homes ; H-W+ : non-smokers exposed only in their workplaces ; H-W- : non-smokers who felt they were not exposed to any tobacco smoke either in their workplaces or homes.
The number attached on each column represents the number of subjects in that group.

Fig. 3  Three dimensional histogram of medians of urinary CCRs (ng/mg) of 141 active smokers. They are divided into 9 groups based on the number of cigarettes smoked per day and on nicotine contents per cigarette.
The number attached to each column represents the number of subjects in that group.
However, such tendency was not found in both the light smoker and the heavy smoker groups.

Discussion

In the present study, we quantified urinary cotinine using the ELISA method that we developed previously[46] in parallel with questionnaires on smoking status on a total of 517 employees of a department store, and showed that the CCRs were approximately consistent with the extent of self-recognition of their exposure status to ETS. Ranges of CCRs calculated (Fig. 1) were found to be 21-7629 with a median of 1568 for the active smokers (group A), 0.3-857 with a median of 61 for the passive smokers (group B), and 0.5-360 with a median of 27 for the non-smokers who felt they were not exposed to any ETS (group C). These values agreed well with those reported by Thompson et al.[40] who used radioimmunoassay and also with those by Langone et al.[19] who assayed with ELISA. The smoking prevalence in the department store, presented in this paper, was 61.0% for males and 15.7% for females (Table 2), which appeared to be slightly higher than that reported in the annual report of Japan Tobacco Incorporation in 1997. This paper also showed that the CCRs in the male active smokers were higher than those in the female active smokers (Table 2), which seemed to be more likely due to differences in the number of cigarettes smoked per day rather than due to those in nicotine content in a piece of cigarette they smoked usually based on statistical analysis. The experimental data in Fig. 3 seemed to have brought about similarly.

The reasons for the absence of statistically significant differences in the urinary CCRs between subjects who worked in the smoking and in the non-smoking areas, either in the active (group A), passive (group B), or non-smokers (group C) (Table 3), are unknown. Such situation of no effective differences of the exposure status to ETS between smoking and non-smoking areas must be improved in the near future in this department store. In order to clarify this problem, it requires further investigation in more detail into types and size of the workplaces, the efficiency of the ventilation system, the duration of smoking and not smoking, etc. It may be necessary to have some monitors introduced for ambient nicotine, which may be rather difficult because of the bigger sizes of the rooms in the department store.

In the present paper, the urinary CCRs of the passive smokers (group B) seemed to be more easily influenced by the presence or absence of ETS in their homes than in the workplaces (Fig. 2), which might be brought about by the smaller room size and/or a poorer method of ventilation at home than at the department store and also by the relatively slow metabolism of cotinine in vivo: its biological half-life in blood is 19 hours with a maximum concentration in 5 hours[19] after inhalation of ETS.

Although the CCRs were overall consistent with subjective recognition of active and/or passive inhalation of tobacco smoke as discussed above, there were considerable discrepancies between the extent of self-recognition on exposure to ETS and their actual urinary cotinine concentrations. In fact, 13.2% of the non-smokers who felt they were not exposed to any ETS (group C) showed higher than 100 ng/mg of CCRs and 25.5% of the passive smokers (group B) showed lower than 10 ng/mg of CCRs (Fig. 1). The ranges of CCRs of group B and C were considerably overlapped, which might be caused by uncertainties in self-recognition of exposure status to ETS. No proportional relationship was found between the CCRs and nicotine contents in the cigarette smoked in the heavy smoker and the light smoker groups (Fig 3). In the passive smokers, such discrepancies seemed to be caused by how sensitive subjects were to ETS. In the active smokers, such discrepancies seemed to be due to differences in the smoking status, such as the extent of inhalation of tobacco smoke (within the oral cavities and/or into the alveolar cavities) and the length of cigarettes they smoked (half or almost whole cigarette).

Since the subjective evaluation of active and/or passive inhalation of tobacco-smoke is thus uncertain, which is consistent with previous reports[20-23], and passive inhalation of ETS affects the health of non-smokers from fetuses to adults[23-26], the objective measurement of exposure status to ETS, such as the urinary CCR assay as presented in this paper, is of increasing importance in assessing both active and passive intake of nicotine for studies on effects of smoking on health. The present data may also suggest that measuring CCRs in parallel with appropriate questionnaires is one of the better epidemiological study methods on evaluating real exposure status to any tobacco-smoke.

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Cotinine: an Objective Indicator of Tobacco Smoke Exposure


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