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Assessment of Urinary Cotinine as a Marker of Nicotine Absorption from Tobacco Leaves: A Study on Tobacco Farmers in Malaysia

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Abstract: Assessment of Urinary Cotinine as a Marker of Nicotine Absorption from Tobacco Leaves: A Study on Tobacco Farmers in Malaysia: Mayumi ONUKI, *et al.* Department of Public Health, Graduate School of Medicine, The University of Tokyo—To assess dermal absorption of nicotine from tobacco leaves in relation to Green Tobacco Sickness (GTS), urinary cotinine concentrations were measured in 80 male tobacco-growing farmers and in 40 healthy males (controls) who did not handle wet tobacco leaves in Kelantan, Malaysia. Among non-smokers, urinary cotinine levels in farmers were significantly higher than those of controls; farmers with urinary cotinine of 50 ng/ml/m^2 or above showed eye symptoms more frequently than those below this level ($p<0.05$). Farmers who did not wear protective equipment had subjective symptoms more frequently than those who used the equipment ($p<0.05$); some of these symptoms were seen more frequently in organophosphate (Tamaron) users than in non-users. As tobacco farmers evidence a risk of nicotine poisoning from tobacco leaves, assessment including GTS together with effects of pesticides will be necessary.
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Key words: Green Tobacco Sickness, Biological monitoring, Urinary cotinine

Tobacco farmers are at risk of Green Tobacco Sickness (GTS), which is caused by dermal absorption of nicotine from wet tobacco leaves. GTS was first described in 1970 among 68 tobacco farmers in Florida, USA, by Weizenecker and Deal[1)] and was named by Stephen and Gehlbach[2)]. GTS is characterized by nausea, vomiting, weakness, prostration and dizziness, and occasionally by fluctuation in blood pressure or heart rate[1–22)]. Headache, increased salivation, abdominal cramps, difficulty in breathing, abdominal pain, and diarrhea have also been reported in recent studies of GTS[9–22)]. As GTS symptoms usually disappear rapidly (in 2–3 d), treatment is not always necessary, but symptoms are so severe in some cases that administration of dimenhydrinate (an antihistaminic and antiemetic agent) is required[7)].

Use of protective clothes or gear such as rubber gloves, boots and raincoats were reported to reduce the absorption of nicotine[2, 4, 6)]. As nicotine is a water-soluble alkaloid[23)] and liquates from leaves or sap to the dew on leaves[4)], working in wet conditions is considered to increase the risk of GTS[20–22)]. Smoking status is considered to decrease the risk of GTS by increasing tolerance for nicotine[2, 5, 11)], though some studies have pointed out that this tolerance would be no longer protective when customary nicotine intake was exceeded[12, 16)].

Quandt *et al*[20)] summarized factors affecting GTS. For example, farmers who have worked in tobacco 5 or more years show significantly lower incidence density of GTS than those who have worked in tobacco for 2 to 4 yr or who are in their first year[21)]. Absorption of chemical substances increases with skin damage or disease to levels far higher than those found in subjects with intact skin[20)]. Another considerable risk factor is the height of the tobacco plant. The flowering tops of tobacco plants are usually cut ("topping") when plants reach 4–6 feet in order to increase root growth, leaf weight and nicotine content; exposure to this stage of growth produces more

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cases of GTS.

GTS incidences have been studied in India, Japan, Italy and USA[2, 5, 8, 20–22, 24]. Although these cases are well known, only a few studies have reported levels of nicotine absorption[4–6, 24]. In the present study, urinary cotinine, a metabolite of nicotine, is measured in tobacco farmers in relation to their subjective symptoms and risk factors for GTS.

Methods

Subjects

Subjects examined were 80 male tobacco farmers in Kelantan, Malaysia, who were registered by the National Tobacco Board (NTB), Malaysia. Control subjects were 40 healthy male officers of NTB, who did not handle wet tobacco leaves. As described below, three farmers and two controls were excluded because of technical reasons. There were no significant differences in age, height, or weight between the 77 farmers and 38 controls (Table 1). None of the subjects consumed alcohol. All aspects of the study presented here were approved by the Research and Ethical Committee of the School of Medical Sciences, University Sains Malaysia. Written consent from each subject in the study was obtained after subjects were informed about the procedures and about the purely voluntary nature of their participation.

Interviews

During workdays in the period of August 3 to 30, 2001, subjects were interviewed by the staff of the Department of Community Medicine in the School of Medical Sciences, University Sains Malaysia. Topics of questions included subjects' age, smoking status, alcohol consumption, how many years working with tobacco, height of tobacco plants, names of pesticides used, and experience of various symptoms in the past month during or shortly after working. These symptoms included runny eyes, blurred vision, increased salivation, nausea, stomach cramps, bloating, cough, shortness of breath, nervousness, unusual tiredness, confusion, dizziness, headache, skin rash, numbness, and muscle weakness. Subjects were also asked about their working conditions, i.e. using rubber gloves, wearing boots, or working in wet conditions. Interviews were conducted in Malay in a quiet room at the department, on four farmers and two controls per day (5 d/wk).

Collection and analysis of urine samples

Urine samples were collected from all subjects in the morning on the day of their interview, and stored at –20°C until analyzed. Concentrations of cotinine in urine were measured by high-performance liquid chromatography (LC-10A Shimadzu, Japan) according to methods reported previously[25]. The detection limit was 15 ng/m*l*. A calibration curve (five points between 0 and 200, 0 and 2000 μg/m*l*) was drawn for each sample set (standard, control and patient). All chemical compounds were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Three samples from the exposed group and two from the controls were excluded because their urine contained too many impurities and did not yield a normal peak. All urine samples were analyzed at the Occupational Poisoning Center, Tokyo Rosai Hospital (Tokyo, Japan).

Correction of urinary cotinine levels by body surface area

As the body surface area (BSA) is known to be correlated with the half-life and metabolism of chemical substances and commonly used for adjusting their internal dosage[26, 27], urinary cotinine in each subject was adjusted for his BSA (an average of 1.65 m^2 with a standard deviation of 0.14 m^2 for all subjects included) estimated by the method previously reported[28]. In the present study, some non-smoking controls had cotinine excretion, suggesting that consideration of a passive smoking effect was needed. Urinary cotinine levels of 80 ng/m*l* or above were considered to reflect nicotine absorption higher than passive smoking effects[29], which was adjusted for BSA,

Table 1. Age, height, weight, years working with tobacco and smoking status in 77 tobacco farmers and 38 controls[a]

	Tobacco farmers	Controls	Differences p
Age (yr)[b]	45.0 (7.3)	45.1 (7.3)	>0.05[c]
Height (cm)[b]	162.3 (6.1)	163.2 (5.9)	>0.05[c]
Weight (kg)[b]	60.8 (10.4)	62.3 (9.6)	>0.05[c]
Years working with tobacco[d]			
0/1–5/6–10/11–15/16–20/>20	0/18/14/12/18/15	–	–
Smoking status (cigarettes/d)[d]			
0/1–5/6–10/11–15/16–20/>20	16/7/22/7/21/4	12/4/7/3/10/2	>0.05[e]

[a]: Three farmers and two controls were excluded from the initial 80 farmers and 40 controls for technical reasons; none of the farmers or controls was a drinker, [b]: Means with standard deviation, [c]: Student's t-test, [d]: Number of subjects, [e]: χ^2-test

i.e. 50 ng/ml/m^2 (approximately 80 ÷ 1.65), in the present study.

Statistical analyses

Differences between the exposed and control groups in urinary cotinine were examined by Mann-Whitney test. Relationships of frequency of symptoms to urinary cotinine levels, working conditions and pesticide use were tested by Fisher's exact probability test. All analyses were carried out with STATA 7.0 (Stata Corp., Stata Statistical S/W, College Station, TX).

Table 2. Differences in urinary cotinine (ng/ml/m^2) between 77 tobacco farmers and 38 controls by smoking status

Smoking status (cigarettes/d)	Tobacco farmers median (range), n	Controls median (range), n
0	17.7* (0[a]–289.9), 16	0[a,]*(0[a]–32.4), 12
1–10	948.3 (75.4–3778.5), 11	684.3 (23.3–2858), 29
>10	1119.2 (78.9–4725), 15	1194.5 (368.1–2724.2), 32

*: $p<0.05$ (Mann-Whitney test), [a]: Lower than detection limit

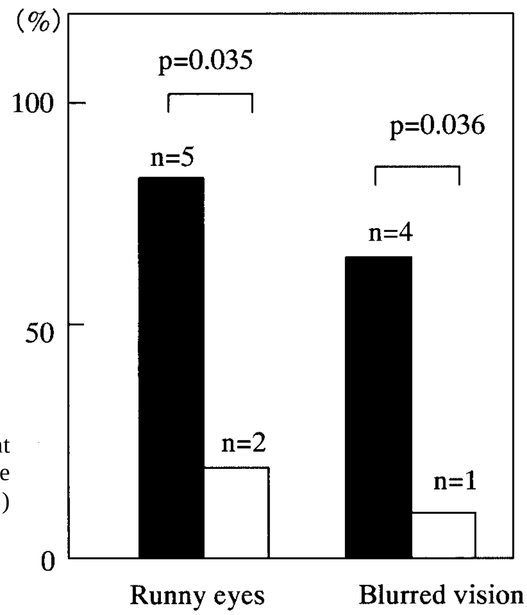

Fig. 1. Symptoms of which frequencies were significantly different between farmers with urinary cotinine of 50 ng/ml/m^2 or above (6 subjects, ■) and less than this level (10 subjects, □) (Fisher's exact probability test).

Table 3. Number of tobacco farmers with subjective symtoms during or shortly after work by daily use of protective equipment and by working conditions[a]: percentage in parenthesis

	Using glove			Using boots			Working under wet condition		
	Yes	No		Yes	No		Yes	No	
	n=37	n=40	p[b]	n=40	n=37	p[b]	n=47	n=30	p[b]
Nervousness	0 (0)	7 (17.1)	0.012	3 (8.1)	4 (10)	n.s.	2 (6.7)	5 (10.6)	n.s.
Dizziness	16 (42)	14 (36.6)	n.s.	10 (27.0)	20 (50)	0.009	13 (43.3)	17 (36.2)	n.s.
Pallor	2 (6)	2 (4.9)	n.s.	2 (5.4)	2 (5)	n.s.	4 (13.3)	0 (0)	0.02
Skin rash	8 (22)	7 (17.1)	n.s.	4 (10.8)	11 (27.5)	0.043	10 (63.3)	5 (10.6)	0.019
Numbness	12 (33)	8 (19.5)	n.s.	6 (16.2)	14 (35)	0.022	11 (36.7)	9 (19.1)	n.s.
Muscle weakness	6 (17)	14 (51.2)	0.06	8 (21.6)	12 (30)	n.s.	12 (40.0)	8 (17.0)	0.025

[a]: Only symptoms of which frequencies were significantly different between the groups are shown, [b]: Fisher's exact probability test (n.s.=p>0.05)

Table 4. Urinaly cotinine (ng/ml/m^2) by daily use of protective equipments and by working conditions (Table 3) in tobacco farmers[a]: median with range in parenthesis

	Using glove		Wearing boots		Working under wet condition	
	Yes	No	Yes	No	Yes	No
Non-smokers	17.2 (0[b]–289.9)	37.0 (0[b]–131.8)	29.3 (0[b]–147.9)	6.7 (0[b]–289.9)	0[b] (0[b]–131.8)	22.1 (0[b]–289.9)
Smokers	1118.6 (270.5–3778.5)	1045.9 (75.4–4735)	1107.4 (75.4–4143.1)	1105.4 (78.9–4735)	1032.7 (75.4–4143.1)	1107.4 (85.2–4735)

[a]: No significant differnces were abserved between "Yes" and "No", [b]: As in Table 2

Table 5. Number of farmers with subjective symptoms (Table 3) during or shortly after work by pesticides used: percentage in parenthesis

	Tamaron (organophosphate)			Fastac (pyrethroid)			Delsen (dithiocarbamate)		
	Yes n=26	No n=51	p[a]	Yes n=37	No n=40	p[a]	Yes n=14	No n=63	p[a]
Nervousness	3 (11.5)	4 (7.8)	n.s.	5 (13.5)	2 (5)	n.s.	1 (7.1)	6 (9.5)	n.s
Dizziness	15 (57.7)	15 (29.4)	0.016	14 (37.8)	16 (40)	n.s.	4 (28.6)	26 (41.3)	n.s.
Pallor	2 (7.7)	2 (3.9)	n.s.	1 (2.7)	3 (7.5)	n.s.	0 (0)	4 (6.3)	n.s.
Skin rash	8 (30.8)	7 (13.7)	n.s.	7 (18.9)	8 (21.6)	n.s.	2 (14.3)	13 (20.6)	n.s.
Numbness	9 (34.6)	11 (21.6)	n.s.	12 (32.4)	8 (21.6)	n.s.	3 (21.4)	17 (27)	n.s.
Muscle weakness	12 (46.2)	8 (15.7)	0.004	11 (29.7)	9 (24.3)	n.s.	1 (7.1)	19 (30.2)	n.s.

[a]: Fisher's exact probability test (n.s.=p>0.05)

Results

Among non-smokers, urinary cotinine levels in tobacco farmers were significantly higher than those of controls; on the other hand, no significant differences in urinary cotinine levels were observed between the smokers in either group (Table 2). Fig. 1 shows that subjects with urinary cotinine of 50 ng/m*l*/m^2 or above had runny eyes and blurred vision more frequently than those with a level below 50 ng/m*l*/m^2.

Tables 3 and 4 show subjective symptoms of farmers and urinary cotinine levels by use of protective equipment and by working condition, respectively. Farmers who did not use rubber gloves showed nervousness more frequently; similarly, those who did not wear boots experienced dizziness, skin rash and numbness. Those who did their work in wet conditions more often had pallor, skin rash and muscle weakness. Urinary cotinine levels were not significantly affected by daily use of protective equipment.

Table 5 shows subjective symptoms seen in farmers (Table 3) by use of pesticides. No significant differences were observed between farmers who did and did not use pesticides except for dizziness and muscle weakness, which normally accompany organophosphate exposure.

Discussion

It was observed that, among non-smokers, urinary cotinine levels of tobacco farmers were significantly increased, suggesting the absorption of nicotine from tobacco leaves. Furthermore, those subjects with higher levels of urinary cotinine had runny eyes and blurred vision. These symptoms have not been observed in other studies; but the fact that they are reportedly caused by nicotine[30–32] suggests that they are related to GTS. As the urinary cotinine levels of non-smoking farmers were less than those of smokers consuming 1–10 cigarettes/day (Table 2), these symptoms seems to be caused by very low levels of exposure, considering the short half-lives of nicotine and cotinine: 2 to 3 and 19.7 (range 11 to 37) hours, respectively[33, 34]. In the present study, no significant associations were observed between non-adjusted urinary cotinine levels and symptoms in farmers (data not shown). As excretion of cotinine is much influenced by urinary volume[35], adjustment for BSA seems useful.

Previous studies showed that using gloves, wearing boots or rubberized nylon rain-suits effectively reduces nicotine absorption[6, 17]. In the present study the observation that farmers who did not use boots or rubber gloves during their work and performed their work in wet conditions manifested subjective symptoms (nervousness, dizziness, pallor, skin rash, numbness and muscle weakness) more frequently agrees with these findings. By contrast, eye symptoms (Fig. 1) were not affected by the use of protective equipment or working conditions; more effective protective measures should be taken to prevent farmers from nicotine absorption. No significant relationship of urinary cotinine levels to the use of protective equipment or working conditions was observed. This is probably because urinary cotinine levels were influenced by exposure on the previous day[24]. Levels of exposure to nicotine in relation to working conditions should be examined in more detail.

For the above subjective symptoms related to the use of protective equipment or working conditions, no significant differences were found in the frequency of complaints between farmers who used and those who did not use dithiocarbamate or pyrethroid. Therefore, it does not appear that these pesticides caused the symptoms observed. By contrast, dizziness and muscle weakness were observed more frequently in those who used organophosphate. The eye symptoms observed here are also reported in sarin poisoning cases, suggesting that they were also organophosphate-related symptoms[36–38]. For assessment of pesticide absorption, measurement of urinary metabolites and/or serum cholinesterase activities would be necessary; the synergistic effects of nicotine and pesticides should be examined further.

In conclusion, our study confirmed that tobacco farmers are at risk of nicotine poisoning from tobacco leaves apart from or in addition to the effects of pesticides. A health assessment that includes both GTS and pesticide effects on tobacco farmers is needed, in addition to safe handling of pesticides as mentioned in our previous report[39].

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