Contents lists available at ScienceDirect

Safety and Health at Work

journal homepage: www.e-shaw.org

Original Article

Green Tobacco Sickness Among Tobacco Harvesters in a Korean Village

Sung-Jun Park, Hyun-Sul Lim, Kwan Lee, Seok-Ju Yoo*

Department of Preventive Medicine, Dongguk University College of Medicine, Gyeongju-si, Republic of Korea

A B S T R A C T

Background: Green tobacco sickness (GTS), an occupational disease in tobacco harvesters, is a form of acute nicotine intoxication by nicotine absorption through the skin from the wet green tobacco plant. We carried out a questionnaire survey and measured cotinine concentration, the metabolic product of nicotine, to determine the prevalence, incidence, and risk factors of GTS in Korean tobacco harvesters.
Methods: We measured cotinine concentrations, and administered a questionnaire survey to tobacco harvesters in Cheongsong-gun, Gyeongsangbuk-do, Korea. We repeatedly measured urine cotinine concentration five times with a questionnaire survey.
Results: Cotinine concentration at dawn was significantly higher than that at other times; it was significantly lower during the nonharvesting period than during the harvesting period. However, little change in cotinine concentration was detected in the daytime during the harvesting period. Study participants included 20 men and 20 women. The prevalence of GTS was 37.5% and was significantly higher in women than in men (55.0% vs. 20.0%, $p < 0.01$). GTS incidence according to number of workdays was 3.4 occurrences/100 person days.
Conclusion: In this study, nicotine exposure and metabolism were experimentally determined from the time of cotinine exposure, and biological monitoring was performed in each season. In the future, this information may be valuable for medical decision-making in GTS prevention.

1. Introduction

Green tobacco sickness (GTS), an occupational disease seen among tobacco harvesters, is a form of acute nicotine intoxication via the absorption of nicotine through the skin from the wet green tobacco plant [1]. Health issues in tobacco harvesters were first recorded in 1713; Ramazzini reported headaches and gastrointestinal disorders in Italian tobacco harvesters, and the occupational disease was first reported in 1970 by Weizenecker and Deal [2]. GTS mainly occurs when the clothes or tobacco leaves become wet with rain, dew, or sweat. The major symptoms are dizziness, headache, nausea, vomiting, and even seizure [3,4].

In Korea, there are an estimated 11,000 tobacco harvesters, and the production of tobacco leaves was 8.4 million kg in 2014 [5]. In the aspect of history and scope of tobacco leaf harvesting, there are many suspected GTS cases in Korea, and even more in other Asian countries including China and India, but studies on GTS have not been performed in Korea until now. GTS was mainly reported in American tobacco harvesters [4]. However, recently, cases have

been reported in India [3,6], Japan [7], Malaysia [8], Poland [9], Brazil [10], and Thailand [11]. In Korea, since Lim and Lee [12] reported the first GTS case, studies regarding the prevalence rate, incidence rate, risk factors, and preventive methods have been conducted [13,14].

To date, GTS has been known globally as a disease occurring by the absorption of nicotine through the skin [6,7,15–18]. However, Park et al. [19] and Yoo et al. [20] recently introduced the possibility of absorption through respiratory routes.

Regarding GTS in Korea, there are currently no national movements to use specific intervention measures for prevention, as nicotine poisoning among tobacco harvesters has only been vaguely understood. Additionally, because of the lack of awareness about GTS among medical personnel, many cases are misdiagnosed as pesticide poisoning or high temperature damage [1].

The aim of this study was to observe tobacco harvesters prior to and after working, and observe the temporal change in urine cotinine during tobacco harvesting and nonharvesting to propose an accurate diagnostic method for GTS.

* Corresponding author. Department of Preventive Medicine, Dongguk University College of Medicine, 123, Dongdaero, Gyeongju-si, Gyeongsangbuk-do 38066, Republic of Korea.
E-mail address: medhippo@hanmail.net (S.-J. Yoo).

2. Materials and methods

2.1. Participants

Our study was conducted in Cheongsong-gun, a rural city located in Gyeongsangbuk-do, Korea. Forty participants were enrolled; surveys and urine sampling for GTS were conducted in all participants. This study was approved by Dongguk University Hospital's clinical research review board prior to study commencement (Gyeongyak Article No. 08-14). Written informed consent was obtained from each participant prior to administering the survey.

2.2. Sampling

From July 20, 2008 to July 30, 2008, urine samples were obtained four times per day (immediately after waking, after working in the morning, after the afternoon work, after having dinner). After the samples were collected, they were immediately placed in the freezer. In the fields, during collection, the samples were placed in an icebox, and immediately after returning to the house, they were placed in the freezer. The following year (2009), urine was collected again from each participant during the non-harvesting period.

2.3. Analysis

High performance liquid chromatography (HPLC) assay was used to estimate cotinine concentration by modified Takeda methods. For extraction, 3 mL of urine was added to 2 mL of dichloromethane and 0.6 mL of 5M sodium hydroxide, and vortexed for 15 minutes; then, the mixture was centrifuged at 3,000 rev/min (5 minutes). The supernatant was dried under N_2 gas, and 10 μL of it was injected in the HPLC column; cotinine concentration values were read at a wavelength of 254 nm. The assay was performed using a reversed phase C_{18} column in an isocratic mode. The HPLC unit consisted of a pump (model 2695; Waters, Milford, MA, USA) and a variable-wavelength ultraviolet detector (model 2996; Waters, USA) with a deuterium lamp. We used a 250 mm × 4.6 mm XTerra column (Waters, USA) with a 5-μm particle size, and an injector with a 10-μL loop. The mobile phase used was a mixture of 85% dibasic phosphate (20 mmol of each per liter) containing 3 mmol of sodium 1-decanesulfonate and 150 mL of acetonitrile per liter (pH 4.5). The flow rate of the mobile phase was 1.0 mL/min, and the column pressure was 140.6 kgf/cm². Creatinine correction was used to measure the creatinine concentration with the Jaffe method, and the cotinine concentration per excreted creatinine 1 mol was calculated.

2.4. Surveys

The survey was administered to all participants; it was developed based on a summary of previous domestic research [13,14]. The presence of GTS was determined with the following criteria: (1) the presence of symptoms related to tobacco and harvesting tobacco, (2) headache or dizziness, and (3) nausea and vomiting. Complaints of symptoms were severe enough to warrant visiting a medical institution. Questionnaire items retrieved information on sex, age, smoking status, acreage (a), purchase amount (kg), harvesting time (hours), presence of symptoms during harvesting (headache, dizziness, nausea, and vomiting), previous hospitalization, and whether motion sickness pills were taken.

2.5. Statistical analysis

We used MS Excel for Windows to record survey items and SPSS for Windows (ver. 18.0; SPSS Inc., Chicago, IL, USA) for statistical analysis. The Friedman test was used to compare cotinine concentration over time (T1−T5), and survey information for risk factors associated with GTS was analyzed using the chi-square test. In analyzing GTS symptoms in farmers, a receiver operating characteristic analysis was performed using MedCalc Statistical Software version 16.1 (MedCalc Software bvba, Ostend, Belgium) to establish the cutoff value of urine cotinine concentrations.

3. Results

3.1. Concentrations of cotinine

Urine samples were collected at the following times: morning (T1), after morning work (T2), after afternoon work (T3), after dinner, prior to bedtime (T4), and the following year when the participant was not working (T5). As indicated, urine cotinine was measured a total of five times. The concentration was highest at T1 by 500.71 (geometric standard deviation, 4.67) ng/mg Cr, but there was no significant difference by time (T1−T4). The concentration in participants during the nonworking period [135.40 (1.73) ng/mg Cr; T5] was significantly lower than that seen when they were working ($p < 0.01$; Table 1).

3.2. Incidence of GTS from survey results

Among the cases that met the definition of GTS, the incidence was 15 out of 40 people (37.5%). By sex, women had a significantly higher incidence (55%) than men (20%; $p < 0.05$). There was no significant difference in age (Table 2). In addition, GTS incidence was significantly higher in nonsmokers than in smokers (57.7% vs. 0%, $p < 0.01$; Table 3).

GTS cutoff urine cotinine concentrations were 290.03 ng/mg Cr, 720.54 ng/mg Cr, 1,211.97 ng/mg Cr, and 1,022.49 ng/mg Cr at T1, T2, T3, and T4, respectively (Table 4).

4. Discussion

At present, cotinine has been shown to be the best available biomarker of nicotine exposure [21]. Cotinine is the major nicotine metabolite, and an average of 72% of nicotine was converted to cotinine [22]. The use of urine cotinine is illustrated in several circumstances where smoking status assessment is of interest. Such situations include evaluation of the impact of smoking cessation programs, monitoring of pregnancy and other groups at risk, assessment of occupational exposure to industrial pollutants, validation of phase I clinical trials, and the assessment of life insurance candidates [23].

Table 1
Time-phased urine cotinine concentration

Time [*]	No.	GM (GSD), ng/mg Cr
T1	39	500.71 (4.67)
T2	40	482.16 (5.26)
T3	40	465.15 (4.66)
T4	40	460.63 (4.44)
T5	39	135.40 (1.73)[†]

[*] T1, early morning; T2, after working A.M.; T3, after working P.M.; T4, prior to bedtime; T5, nonworking.
[†] By Friedman test.
GM, geometric mean; GSD, geometric standard deviation.

Table 2
Incidence of green tobacco sickness according to sex and age

Age (y)	Men			Women			Total		
	Total	No. of cases	%	Total	No. of cases	%	Total	No. of cases	%
<50	1	0	0.0	3	2	66.7	4	2	50.0
50−59	11	2	18.2	12	6	50.0	23	8	34.8
≥60	8	2	25.0	5	3	60.0	13	5	38.5
Total	20	4	20.0	20	11	55.0*	40	15	37.5

* $p < 0.05$ by Chi-square test.

Table 3
Incidence of green tobacco sickness in smokers (+) and nonsmokers (−)

Smoking (−)			Smoking (+)			Total		
Total	No. of cases	%	Total	No. of cases	%	Total	No. of cases	%
26	15	57.7*	14	0	0.0	40	15	37.5

* $p < 0.01$ by Chi-square test.

Nicotine or cotinine level measured in the urine or blood can be used to diagnose acute nicotine addiction, which can be present in GTS. Generally, the half-life of nicotine is 2−2.5 hours, and 4−5 hours when absorbed through the skin [17]. By contrast, cotinine has a half-life of 18−24 hours [24,25]. The diagnosis of GTS is preferred to be made by measuring cotinine, as the half-life is much longer [26].

Using urine samples to measure nicotine and cotinine rather than blood or saliva is ideal because it is easy to collect and the concentration is higher in urine than in serum or saliva [27,28]. Generally, the concentration in urine has been reported to be 10 to 100 times higher than that of serum and saliva [29]. In addition, whereas nicotine is affected by pH in the kidney when excreted in urine, cotinine is hardly affected by flow rate and pH; it is known to have the best biological exposure index and is not affected by diet or other factors [30,31]. Time-phased average cotinine concentration range of tobacco harvesters in this study were 460.63−500.71 ng/mg Cr, and the average maximum concentration in smokers was 2,951.30 ng/mg Cr. Lee et al. [32] studied patients with GTS in Korea and found urine cotinine concentrations of 73.1−2,574.3 ng/mL; other foreign studies found ranges of 1,170−3,340 ng/mL [6], 7,300−11,300 ng/mL [16], 81.9−108.8 ng/mL [17], and 3,400−10,300 ng/mL [18]. A recent epidemiological study on urine cotinine levels found an average of 432 ng/mL in tobacco harvesters in Southern Brazil [10]. Urine cotinine concentrations in this study are similar to those in domestic studies and recent studies in southern Brazil, whereas other studies in foreign countries generally found low levels of urine cotinine concentration. It is not reasonable to compare this study and other foreign studies because of the numerous differences in methodology. This is because urine cotinine might be lower as a result of less exposure, differences in race, urine collection timing, and smoking status, depending on factors such as the method used for analyzing urine cotinine.

Symptoms of GTS are dizziness and nausea within 15 minutes after contact of tobacco with the skin during the harvesting of tobacco leaves [33], with symptom presentation usually occurring after working for 3−17 hours; however, this may vary [34]. The median time to symptom expression in a domestic study in 2001 was 3.5 hours; it was 3 hours in 2002 [14]. Lee et al. [32] reported a median of 4.3 hours. The median time to the onset of symptoms in a foreign study was 10 hours [34]. GTS symptoms occurring during work have been reported most frequently. McKnight et al. [35] reported that symptoms occurred after work between 6 P.M. and 2 A.M. most often. In Korea, Gyeongsang-do farmers cultivate mainly flue-cured tobacco, whereas in Jeolla-do, the impact of burley tobacco GTS is estimated to be more severe, but the research has yet to be clarified on this topic.

Symptoms of GTS will vary depending on the type of work performed during tobacco harvesting [35]. GTS is reported to occur more and more in young people, and young people have not been trained to realize the extent of the exposure or that they are more sensitive to nicotine [4,35]. However, age and prevalence of GTS were not significantly associated in this study. We believe this is because the average age of Korean tobacco harvesters is high, and young tobacco harvesters comprise only a minority of the overall total.

The relationship between smoking and GTS has been reported to have a weak protective effect [1,4,36], but another report suggested no protective effect [37]. In Korea, research has focused significantly more on nonsmokers than on smokers, and the results on stratified analysis by sex are similarly significant in both sexes where smoking was determined to have a weak protective effect in GTS [14].

GTS in Korea is known to often occur during the harvesting season (spanning from the end of June to August). In this study, the incidence of GTS was 37.5% and the incidence density was 3.45 occurrences/100 person·working days. Gehlbach et al. [36] reported that a prevalence of 9% in North Carolina, USA, and Ballard et al. [4] found an incidence of 10 people per 1,000 in 1992, and 14 people per 1,000 in 1993. Quandt et al. [38] did not carry out preventive measures targeted at 144 Latino farmers and reported that 41% experienced GTS. Arcury et al. [39] studied 182 Latino farmers and found a prevalence of 24.2%.

To diagnose GTS using urine cotinine levels indicated at the cutoff value found in this study, if GTS symptoms occurred with a urine cotinine concentration between 700 and 1,000 ng/mg Cr more than that in a nonsmoker, GTS will be diagnosed.

An accurate diagnosis, treatment, and prevention plan for farmers is needed in Korea, as many cases are misdiagnosed and no prevention method has been developed. We could not match the control group because our study was initially planned to conduct

Table 4
Symptom presentation by GTS cutoff urine cotinine concentrations at different time points

Parameters	T1	T2	T3	T4
Area under the ROC curve (AUC)	0.851	0.783	0.801	0.785
95% Confidence interval	0.701−0.945	0.624−0.897	0.645−0.910	0.627−0.899
p value	<0.01	<0.01	<0.01	<0.01
Cut-off values	290.03	720.54	1,211.97	1,022.49
Sensitivity (%)	80.0	93.3	100.0	100.0
Specificity (%)	83.3	64.0	52.0	52.0

AUC, area under the curve; GTS, green tobacco sickness; ROC, receiver operating characteristic; T1, early morning; T2, after working A.M.; T3, after working P.M.; T4, prior to bedtime.

the survey with the tobacco harvesters to observe the temporal change in urine cotinine, and then we tried to propose an accurate diagnostic method for GTS with the result. In spite of this limitation, the result of this study can be used as the basic data to set the prevention plan and diagnostic criteria for GTS among tobacco harvesters in Korea.

Conflicts of interest

The authors declare no conflict of interest as related to the manuscript.

References

[1] Hipke ME. Green tobacco sickness. South Med J 1993;86:989—92.
[2] Weizenecker R, Deal WB. Tobacco cropper's sickness. J Fla Med Assoc 1970;57: 13—4.
[3] Ghosh SK, Parikh JR, Gokani VN, Kashyap SK, Chatterjee SK. Studies on occupational health problems during agricultural operation of Indian tobacco workers: a preliminary survey report. J Occup Med 1979;21:45—7.
[4] Ballard T, Ehlers J, Freund E, Auslander M, Brandt V, Halperin W. Green to-bacco sickness: occupational nicotine poisoning in tobacco workers. Arch Environ Health 1995;50:384—9.
[5] Korea Tobacco Growers Organization. In 2014, the tobacco leaf contract cur-rent status of actual results. [Internet]. 2014 [cited 2015 Feb 24]. Available from: http://www.ktgo.or.kr/.
[6] Ghosh SK, Gokani VN, Doctor PB, Parikh JR. Intervention studies against "green symptoms" among Indian tobacco harvesters. Arch Environ Health 1991;46: 316—7.
[7] Misumi J, Koyama W, Miura J. 2 cases of green tobacco disease among tobacco harvesters and percutaneous nicotine absorption in rats. Sangyo Igaku 1983;25:3—9.
[8] Onuki M, Yokoyama K, Kimura K, Sato H, Nordin RB, Naing L, Morita Y, Sakai T, Kobayashi Y, Araki S. Assessment of urinary cotinine as a marker of nicotine absorption from tobacco leaves: a study on tobacco farmers in Malaysia. J Occup Health 2003;45:140—5.
[9] Satora L, Goszcz H, Gomółka E, Biedroń W. Green tobacco sickness in Poland. Pol Arch Med Wewn 2009;119:184—6.
[10] Bartholomay P, Iser BP, de Oliveira PP, dos Santos TE, Malta DC, Sobel J, de Moura L. Epidemiologic investigation of an occupational illness of tobacco harvesters in southern Brazil, a worldwide leader in tobacco production. Occup Environ Med 2012;69:514—8.
[11] Saleeon T, Siriwong W, Maldonado-Pérez HL, Robson MG. Green tobacco sickness among Thai traditional tobacco farmers, Thailand. Int J Occup Environ Med 2015;6:169—76.
[12] Lim HS, Lee K. Cases of green tobacco sickness: occupational nicotine poisoning in tobacco harvesters in Korea. Korean J Rural Med 2001;26:7—14 [in Korean].
[13] Lee JS, Bae SH, Lim HS, Lee K. Epidemiological characteristics and changes of prevalence for green tobacco sickness among Korea tobacco harvesters. Korean J Epidemiol 2004;26:39—49 [in Korean].
[14] Lim HS, Lee K, Nam SH. Prevalence and risk factors of green tobacco sickness among Korean tobacco harvesters. Korean J Prev Med 2004;37:37—43 [in Korean].
[15] Arcury TA, Quandt SA, Preisser JS, Bernert JT, Norton D, Wang J. High levels of transdermal nicotine exposure produce green tobacco sickness in Latino farmworkers. Nicotine Tob Res 2003;5:315—21.
[16] National Institute for Occupational Safety and Health. HHE Report No. HETA-92-403-2329, Kentucky Cabinet for Human Resources. Kentucky (USA): NIOSH; 1992.
[17] D'Alessandro A, Benowitz NL, Muzi G, Eisner MD, Filiberto S, Fantozzi P, Montanari L, Abbritti G. Systemic nicotine exposure in tobacco harvesters. Arch Environ Health 2001;56:257—63.
[18] Doctor PB, Gokani VN, Kulkarni PK, Parikh JR, Saiyed HN. Determination of nicotine and cotinine in tobacco harvesters' urine by solid-phase extraction and liquid chromatography. J Chromatogr B Anal Technol Biomed Life Sci 2004;802:323—8.
[19] Park SJ, Kim JS, Kim JS, Lee K, Lim HS. Airborne nicotine concentrations in harvesting and the processing of tobacco leaves. J Korean Soc Occup Environ Hyg 2010;20:47—52 [in Korean].
[20] Yoo SJ, Park SJ, Kim BS, Lee K, Lim HS, Kim JS, Kim IS. Airborne nicotine concentrations in the workplaces of tobacco farmers. J Prev Med Public Health 2014;47:1—6 [in Korean].
[21] Benowitz NL, Jacob 3rd P. Metabolism of nicotine to cotinine studied by a dual stable isotope method. Clin Pharmacol Ther 1994;56:483—93.
[22] Benowitz NL. Cotinine as a biomarker of environmental tobacco smoke exposure. Epidemiol Rev 1996;18:188—204.
[23] Haufroid V, Lison D. Urinary cotinine as a tobacco-smoke exposure index: a minireview. Int Arch Occup Environ Health 1998;71:162—8.
[24] Benowitz NL, Kuyt F, Jacob 3rd P, Jones RT, Osman AL. Cotinine disposition and effects. Clin Pharmacol Ther 1983;34:604—11.
[25] Knight JM, Eliopoulos C, Klein J, Greenwald M, Koren G. Passive smoking in children. Racial differences in systemic exposure to cotinine by hair and urine analysis. Chest 1996;109:446—50.
[26] McBride JS, Altman DG, Klein M, White W. Green tobacco sickness. Tob Control 1998;7:294—8.
[27] Wall MA, Johnson J, Jacob P, Benowitz NL. Cotinine in the serum, saliva, and urine of nonsmokers, passive smokers, and active smokers. Am J Public Health 1988;78:699—701.
[28] Vine MF, Hulka BS, Margolin BH, Truong YK, Hu PC, Schramm MM, Griffith JD, McCann M, Everson RB. Cotinine concentrations in semen, urine, and blood of smokers and nonsmokers. Am J Public Health 1993;83:1335—8.
[29] Jarvis M, Tunstall-Pedoe H, Feyerabend C, Vesey C, Salloojee Y. Biochemical markers of smoke absorption and self reported exposure to passive smoking. J Epidemiol Community Health 1984;38:335—9.
[30] Miller NS, Cocores JA. Nicotine dependence: diagnosis, chemistry, and phar-macologic treatments. Pediatr Rev 1993;14:275—9.
[31] Samet JM, Marbury MC, Spengler JD. Health effects and sources of indoor air pollution: Part I. Am Rev Respir Dis 1987;136:1486—508.
[32] Lee K, Lim HS, Kim H, Nam SH. Urinary cotinine concentrations of cases with green tobacco sickness. Korean J Occup Environ Med 2004;16:413—21.
[33] Faulkner JM. Nicotine poisoning by absorption through the skin. JAMA 1933;100:1664—5.
[34] Centers for Disease Control and Prevention. Green tobacco sickness in tobacco harvesters—Kentucky, 1992. MMWR 1993;42:237—40.
[35] McKnight RH, Levine EJ, Rodgers Jr GC. Detection of green tobacco sickness by a regional poison center. Vet Hum Toxicol 1994;36:505—10.
[36] Gehlbach SH, Williams WA, Perry LD, Woodall JS. Green tobacco sickness: an illness of tobacco harvesters. JAMA 1974;229:1880—3.
[37] Ghosh S, Saiyed HN, Gokani VN, Thakker MU. Occupational health problems among workers handling Virginia tobacco. Int Arch Environ Health 1986;58: 47—52.
[38] Quandt SA, Arcury TA, Preisser JS, Norton D, Austin C. Migrant farmworkers and green tobacco sickness: new issues for an understudied disease. Am J Ind Med 2000;37:307—15.
[39] Arcury TA, Quandt SA, Preisser JS, Norton D. The incidence of green tobacco sickness among Latino farm workers. J Occup Environ Med 2001;43:601—9.