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ORIGINAL ARTICLE

SALIVARY COTININE LEVELS AS A BIOMARKER FOR GREEN TOBACCO SICKNESS IN DRY TOBACCO PRODUCTION AMONG THAI TRADITIONAL TOBACCO FARMERS

Thanusin Saleeon[1], *Wattasit Siriwong*[1,2*], *Héctor Luis Maldonado-Pérez*[3], *Mark Gregory Robson*[2,3]

[1] College of Public Health Science, Chulalongkorn University, Bangkok, Thailand
[2] Thai Fogarty ITREOH Center, Chulalongkorn University, Bangkok, Thailand
[3]School of Environmental and Biological Sciences, Rutgers University, New Jersey, USA

ABSTRACT

Background. Dry Thai traditional tobacco (*Nicotiana Tabacum* L.) production involves a unique process: (a) picking tobacco leaves, (b) curing tobacco leaves, (c) removing stems of tobacco leaves, cutting leaves and putting on a bamboo rack, (d) drying in the sun, reversing a rack, spraying a tobacco extract to adjust the tobacco's color, storing dried tobacco and packaging. These processes may lead to adverse health effects caused by dermal absorption of nicotine such as Green Tobacco Sickness (GTS).

Objectives. The aim of this study was to determine the correlation between GTS resulting from dry Thai traditional tobacco production and salivary cotinine levels among Thai traditional tobacco farmers in Nan Province, Thailand.

Materials and Methods. A prospective cohort study was conducted with 20 tobacco farmers and 20 non-tobacco farmers in Praputtabath Sub-District and Phatow Sub-District. The participants were randomly selected and interviewed using in person questionnaires with bi-weekly follow-up for 14 weeks. During each contact, the cotinine concentration was measured by NicAlert™ Saliva strip tests (NCTS). Descriptive statistics and *Spearman's* correlation (*Spearman's* rho) was used to examine the relationship between the variables at both 0.01 and 0.05 significant probability levels.

Results. This study indicated that GTS from dry tobacco production has the potential to be considered a common occupational disease. This study demonstrated the usefulness of salivary cotinine level measurements by NCTS. The levels were well correlated with farmers who were employed in the dry Thai tobacco production industry. Salivary cotinine levels were also significantly correlated with the prevalence of GTS in the group of tobacco farmers at any given time within a crop season. However, the production process of dry Thai traditional tobacco is different from that evaluated in our previous studies where GTS and salivary cotinine level were correlated in workers working in humid conditions.

Conclusions. The long-term effects of such exposure should be investigated and health education programs with health risk exposure studies to increase awareness amongst farmers is recommended.

Key words: *salivary cotinine levels, green tobacco sickness, dry Thai traditional tobacco*

INTRODUCTION

Tobacco farming is associated with the hazard for the so called green tobacco sickness (GTS). The disease is caused by nicotine which penetrates through the skin of the hands of the workers who cultivate and harvest tobacco [19]. GTS is an occupational illness reported in tobacco workers worldwide [6, 11, 14, 28]. The GTS morbidity affects nearly one quarter of tobacco workers. Typical symptoms include: nausea, vomiting, headache, abdominal cramps, breathing difficulty, abnormal body temperature, pallor, diarrhea, chills, fluctuations in blood pressure and heart rate, drenching sweats and increased salivation [9, 18, 19]. GTS is a type of acute nicotine poisoning caused by dermal absorption of nicotine from mature tobacco plants. The combined symptoms of acute nicotine poisoning are vomiting, nausea, headaches and dizziness [1, 3]. The Nan Province is one of the most popular areas for Thai traditional tobacco cultivation in the North of Thailand. Thai traditional tobacco (*Nicotiana Tabacum* L.) is known as a non-Virginia type tobacco. Mature leaves are thicker and contain 3-4 times more nicotine as compared to Virginia

*Corresponding author: Wattasit Siriwong, College of Public Health Science, Chulalongkorn University, Soi Chulalongkorn 62 Phyathai Rd., Bangkok 10330 , Thailand, Phone: +66 2 218 8184; Fax : +66 2 255 6046, e-mail: wattasit.s@chula.ac.th

type [14] and average 10% of all tobacco products and cultivation areas in Thailand. Furthermore, dried Thai traditional tobacco cultivation and final products involve many unique processes which are different from the western countries processes. A farmer works with and produces tobacco plants by himself or with help of family. From previous studies, it was found that farmers who were working with tobacco may develop adverse health effects due to nicotine poisoning (GTS) from raw tobacco leaves through skin absorption [1]. On the other hand, a person can be exposed to nicotine through inhalation, which is a possible route of exposure in dry Thai traditional tobacco production. There is close contact with vapors of nicotine from raw and dry tobacco and from working long hours each day. The gummy juice and sap from tobacco exude from the leaves produce a pungent odor supporting the idea of direct exposure the body by inhalation and collection in mucous membranes of the nasal ducts. Moreover, the use of bare hands in handling of tobacco dust may bring forth the health outcomes in GTS [27]. Such is the case with inappropriate personal protective equipment, supporting nicotine absorption as a potential mechanism involved in pathogenesis [22]. The processing of dry Thai traditional tobacco exposes the farmers to nicotine through contact with hands and inhalation with tobacco dust and can be considered a risk to acquiring nicotine poisoning. Measuring the concentration of nicotine in the body is a costly process and must be done in a laboratory in a remote location; this process has been reported in previous studies. Cotinine is the major metabolite of nicotine and has a relatively long half-life (ten times longer than that of nicotine) [8]. Cotinine measurements have been used to distinguish between tobacco users and non-users [13, 15, 16, 17] and can be detected in saliva [4]. *Quandt* et al. found that the level of salivary cotinine among the workers had a significant positive relationship to wet conditions, smoking, and work task (picking and topping (removing of the flower from the plant to induce plant growth and increase nicotine content) [24]. Salivary cotinine levels can be measured by NicAlert^{TM} Saliva strip tests (Nymox Pharmaceutical Cooperation, St.-Laurent, QC, Canada) which would provide the opportunity to classify cotinine levels between user and non-users of tobacco products. Because the Thai diagnostic criteria for GTS has not been established, the correlation between salivary cotinine levels and GTS in Thai traditional tobacco farmers has yet to be studied.

The purpose of this study is to determine correlation between GTS from dry Thai traditional tobacco production and salivary cotinine levels among Thai traditional tobacco farmers at Praputthabath Sub-District, Chiangklang District and Phatow Sub-District, Thawangpha District, in Nan Province, Thailand. Data from this study can be helpful to expand surveillance and prevention of GTS, defining GTS etiology for farmers and improving working conditions in this area.

MATERIAL AND METHODS

Subjects and sampling method

A prospective cohort study was conducted with twenty Thai traditional tobacco farmers and twenty non-tobacco farmers in Praputtabath Sub-District and Phatow Sub-District in Nan Province. The subjects were both male and female between 20 and 60 years of age. A total of 40 participants were randomly selected from tobacco farmers in this area. The participants were defined in two groups. The case study group was the Thai traditional tobacco farmers who picked tobacco leaves or produced dried tobacco during the regular season and a group of non-tobacco farmers as controls who live in the same area of the first group. They were generally healthy local agriculturalists that live in the study area (not mobile), with no reported fever or common cold symptoms, no diarrhea complications and no exposure to pesticides.

Measurement tool

The farmers were randomly selected and interviewed using face to face questionnaires that were modified from a previous study by *Arcury* et al. [1] and environmental surveys. The questionnaire is comprised of individual characteristics (gender, age, family status, level of education, current smoking status and alcohol consumption), work related conditions and the process of dry tobacco production which is consistent with: picking tobacco leaves, transferring tobacco from farm to home, curing tobacco leaves, removing stems of tobacco leaves, rolling a bundle of tobacco leaves, cutting tobacco leaves with a cutting machine, putting a slice of tobacco on a bamboo rack, bringing a rack of tobacco to dry in the sun, reversing a bamboo rack, spraying a tobacco extract for adjusting tobacco color, storing dried tobacco and packaging. The questionnaire also asked about personal protective equipment (PPE) use, hours worked in dried tobacco production, and GTS subjective health symptoms such vomiting, nausea, headaches or dizziness. Salivary samples were collected at each contact to measure cotinine concentration levels by NicAlert^{TM} Saliva strip test - NCTS.

Salivary cotinine evaluation

The salivary cotinine levels were evaluated using NicAlert^{TM} Saliva strip tests - an immunochromatographic assay using monoclonal antibodies (Nymox Pharmaceutical Cooperation, St.-Laurent, QC, Canada). The system provides a semi-quantitative measurement of cotinine in saliva for the purpose of determining whether an individual has been exposed to tobacco products within 48 hours. NicAlert™ Saliva strip zones range from level 0 (0-10 ng/mL, non-user of tobacco products) to level 6 (>1000 ng/mL, use of tobacco products). The cut-off concentration indicating a positive result, was 10 ng/mL (zones 1-6). The salivary cotinine concentration and the interpretation for each are shown in Table 1 [21].

Table 1. Cotinine concentration and its interpretation for each level of the NicAlert™ test

Level	Cotinine concentration (ng/mL)	Interpretation
0	0-10	non-user of tobacco products
1	10-30	user of tobacco products
2	30-100	user of tobacco products
3	100-200	user of tobacco products
4	200-500	user of tobacco products
5	500-1000	user of tobacco products
6	>1000	user of tobacco products

Salivary cotinine levels were recorded after squeezing eight drops from the saliva-containing tube that is wide at the top and narrow at the bottom (after bringing it to room temperature) directly onto the white padded end of the strip. Results were read after allowing the strip to develop by laying it on the marked area of the plastic laminated instruction card for 15 to 30 minutes. The lowest numbered zone displaying a red color was documented as the NicAlert™ Saliva trip test result [21].

Ethical approval

Written informed consent was obtained from all participants willing to join the study. This study was approved by Ethical consideration from the College of Public Health Sciences, Chulalongkorn University COA No.170/2012.

Data collection

The interview follow-up was conducted every two weeks between 08.00 a.m. and 07.00 p.m. from December 2012 to March 2013. The total number of visits were 7; December for 2 times, November for 2 times, January for 2 times and March for 1 time. The trained surveyors were responsible for conducting interviews for selected groups of Thai traditional tobacco farmers and non-tobacco farmers. They conducted interviews two times a month (bi-weekly), with one month for follow-up upon finishing the tobacco work. Personal interviews are used to collect information to measure the occurrence of the subjective health symptoms and risk factors for nicotine exposure, included smoking status, and process of tobacco work, personal protective equipment (PPE) use and hours worked in dried tobacco production. Salivary samples were collected at each contact and cotinine concentration levels were measured by using NCTS.

Data Analysis

All data were coded and processed using the Statistical Package for Social Sciences (SPSS) version 17. Statistical analyses were conducted using frequency and percentage to describe qualitative data. Mean and standard deviation were used to quantify data. The prevalence of GTS was stratified by the farmers' characteristics at each visit, amount of dry tobacco production and the GTS subjective health symptoms. A correlation between GTS, dry tobacco processing, PPE use and salivary cotinine levels were analyzed by *Spearman's* correlation (*Spearman's* rho) at both significant (0.01 and 0.05) probability levels (REPEAT).

RESULTS

There were 40 subjects that participated in the questionnaire interview who were 42 to 60 years of age; 50.0% male and 50.0% female. The average age (±SD) of the participants was 50.18 (±4.93) years. Most of participants (55.0%) were head of a family and 85.0% were educated at the primary school level. Almost all of the workers were exposed to tobacco in any given day for approximately 6 to 10 hours (60.0% of their day). Only one person was exposed for 5.0% of the day and was not living with a person who was smoking for 100% of the day, Farmers who had alcohol consumption were 10.0% of the group. The Body Mass Index (BMI) of the farmers was normal for 65.0% of the group. Characteristics of the study population are summarized in Table 2.

For all 7 times of sampling, the correlation of salivary cotinine levels between tobacco farmers and non-tobacco farmers was different ($p<0.05$). Moreover, this result was indicated that tobacco farmers have a higher saliva cotinine level than non-tobacco farmers. All of testing in seven times, the correlation of salivary cotinine levels on Thai traditional tobacco farmers was different between non-tobacco farmers and tobacco farmers ($p<0.05$). In the test of T1, T2, T3, T4, T5, T6 and T7 was found that in each time of testing, tobacco farmers group have a numbers of salivary cotinine exposure more than non-farmer that measured by NCTS strip test.

Test 1: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were twenty five persons (62.5%); farmer groups were seven persons (35.0%) and non-farmer group were eighteen persons (90.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) was seven persons (17.5%); farmers group were five persons (25.0%) and non-farmer group were two persons (10.0%). Total of testing found on Level 2 (30-100 ng/mL of cotinine concentration) was five persons; farmers group were five persons (25.0%) and none of non-farmer group.

Test 2: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were twenty seventeen persons (42.5%); farmer groups were four persons (20.0%) and non- farmer group were thirteen persons (65.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) were two persons (5.0%); farmers group was one person (5.0%) and non-farmer group was one person (5.0%). Total of testing found on Level 2 (30-100 ng/mL of cotinine concentration) were ten persons (25%); farmers group were five persons (25.0%) and five persons (25.0%) of non-farmer group. Total of testing found on Level 3 (100-200 ng/mL of cotinine concentration) were eleven persons (27.5%); farmers group were ten persons (50.0%) and one persons (5.0%) of non-farmer group.

Table 2. Characteristics of the study population (%)

Characteristics	Non-farmer (n=20)	Farmer (n=20)
Gender		
Male	10 (50.0)	10 (50.0)
Female	10 (50.0)	10 (50.0)
Age group (years)		
42 - 50	11 (55.0)	11 (55.0)
51 - 60	9 (45.0)	9 (45.0)
Mean age = 50.18, SD = 4.93, Min= 42, Max = 60		
Status in family		
Head of family	10 (50.0)	11 (55.0)
Housewife	10 (50.0)	9 (45.0)
Education level		
Primary school	20 (100)	17 (85.0)
Secondary High school	0 (0)	3 (15.0)
Smoking		
No	20 (100)	19 (95.0)
Yes	0 (0)	1 (5.0)
Living with smoking		
No	20 (0)	20 (100)
Yes	0 (0)	0 (0)
Alcohol consumption		
No	19 (95.0)	18(90.0)
Yes	1 (5.0)	2(10.0)
Work with tobacco (hours)		
0-5	0 (0)	8(40.0)
6-10	0 (0)	12(60.0)
Mean = 5.26, SD= 4.19, Min= 0, Max = 10		

Test 3: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were thirteen persons (32.5%); farmer group was one person (5.0%) and non-farmer group were twelve persons (50.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) were six persons (15.0%); farmers group were three person (15.0%) and non-farmer group were three persons (15.0%). Total of testing found on Level 2 (30-100 ng/mL of cotinine concentration) were eleven persons (27.5%); farmers group were six persons (30.0%) and five persons (25.0%) of non-farmer group. Total of testing found on Level 3 (100-200 ng/mL of cotinine concentration) were nine persons (22.5%); farmers group were nine persons (45.0%) and none of non-farmer group. Total of testing found on Level 4 (200-500 ng/mL of cotinine concentration) was one person (2.5%); farmers group was one person (5.0%) and none of non-farmer group.

Test 4: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were sixteen persons (40.0%); farmer group were three persons (15.0%) and non-farmer group were thirteen persons (65.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) were two persons (5.0%); none of farmers group and non- farmer group were two persons (10.0%). Total of testing found on Level 2 (30-100 ng/mL of cotinine concentration) were six persons (15.0%); farmers group were three persons (15.0%) and three persons (15.0%) of non-farmer group. Total of testing found on Level 3 (100-200 ng/mL of cotinine concentration) were thirteen persons (32.5%); farmers group were eleven persons (55.0%) and two of non- farmer group (10.0%). Total of testing found on Level 4 (200-500 ng/mL of cotinine concentration) were three persons (7.5%); farmers group were three persons (15.0%) and none of non-farmer group.

Test 5: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were eighteen persons (45.0%); farmer group were five persons (25.0%) and non-farmer group were thirteen persons (65.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) were nine persons (22.5%); three persons (15.0%) of farmers group and non-farmer group were six persons (30.0%). Total of testing found on Level 2 (30-100 ng/mL of cotinine concentration) were seven persons (17.5%); farmers group were six persons (30.0%) and one person (5.0%) of non-farmer group. Total of testing found on Level 3 (100-200 ng/mL of cotinine concentration) were five persons (12.5%); farmers group were five persons (25.0%) and none of non-farmer group. Total of testing found on Level 4 (200-500 ng/mL of cotinine concentration) was one person (2.5%); farmers group was one person (5.0%) and none of non- farmer group.

Test 6: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were seventeen persons (42.5%); none of farmer group and non-farmer group were seventeen persons (85.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) were six persons (15.0%); three persons (15.0%) of farmers group and non-farmer group were three persons (15.0%). Total of testing found on Level 2 (30-100 ng/mL of cotinine concentration) were six persons (15.0%); farmers group were six persons (30.0%) and none of non-farmer group. Total of testing found on Level 3 (100-200 ng/mL of cotinine concentration) were eleven persons (27.5%); farmers group were eleven persons (55.0%) and none of non-farmer group.

Test 7: totally of testing found on Level 0 (0-10 ng/mL of cotinine concentration) were thirty-nine persons (97.5%); nineteen persons (95.0%) of farmer group and non-farmer group were twenty persons (100.0%). Total of testing found on Level 1 (10-30 ng/mL of cotinine concentration) was one person (2.5%); one person (5.0%) of farmers group and none of non-farmer group. The results of the test of salivary cotinine levels was found that almost of farmer group have higher levels of cotinine concentration more than non-farmer group (Table 3).

Table 3. Distribution of salivary cotinine levels on Thai traditional tobacco farmers and non-farmers by time of testing (n=40)

Level	Cotinine concentration (ng/mL)	T1* n (%)			T2* n (%)			T3* n (%)			T4* n (%)		
		NF n=20	F n=20	Total N=40	NF n=20	F n=20	Total N=40	NF n=20	F n=20	Total N=40	NF n =20	F n=20	Total N=40
0	0-10	18(90.0)	7(35.0)	25(62.5)	13(65.0)	4(20.0)	17(42.5)	12(60.0)	1(5.0)	13(32.5)	13(65.0)	3(15.0)	16(40.0)
1	10-30	2 (10.0)	5 (25.0)	7 (17.5)	1(5.0)	1(5.0)	2(5.0)	3(15.0)	3(15.0)	6(15.0)	2(10.0)	0(0)	2(5.0)
2	30-100	0 (0)	5 (25.0)	5(12.5)	5(25.0)	5(25.0)	10(25.0)	5(25.0)	6(30.0)	11(27.5)	3(15.0)	3(15.0)	6(15.0)
3	100-200	0 (0)	3 (15.0)	3 (7.5)	1(5.0)	10(50.0)	11(27.5)	0(0)	9(45.0)	9(22.5)	2(10.0)	11(55.0)	13(32.5)
4	200-500	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(5.0)	1(2.5)	0(0)	3(15.0)	3(7.5)

Level	Cotinine concentration (ng/mL)	T5* n (%)			T6* n (%)			T7* n (%)		
		NF n=20	F n=20	Total N=40	NF n=20	F n=20	Total N=40	NF n=20	F n=20	Total N=40
0	0-10	13(65.0)	5(25.0)	18(45.0)	17(85.0)	0(0)	17(42.5)	20(100.0)	19(95.0)	39(97.5)
1	10-30	6(30.0)	3(15.0)	9(22.5)	3(15.0)	3(15.0)	6(15.0)	0(0)	1(5.0)	1(2.5)
2	30-100	1(5.0)	6(30.0)	7(17.5)	0(0)	6(30.00	6(15.0)	0(0)	0(0)	0(0)
3	100-200	0(0)	5(25.0)	5(12.5)	0(0)	11(55.0)	11(27.5)	0(0)	0(0)	0(0)
4	200-500	0(0)	1(5.0)	1(2.5)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)

*P<0.05, T=sequence of time for testing; T7= testing after tobacco work was finished 1 month
NF= non-farmer, F=farmer

These results indicate that there is a correlation between salivary cotinine levels and tobacco farming at every test time point, except in time point 7, which had no correlation (p>0.05). The data also indicates a strong correlation between the dry Thai traditional tobacco process of handling tobacco and salivary cotinine levels (p<0.01). According to the study findings, the correlation between four main symptoms of green tobacco sickness (GTS) including headache, nausea, vomiting, and dizziness and salivary cotinine levels were found. Simply put, tobacco farmers were likely to have a strong correlation with salivary cotinine levels as shown in six out of the seven tests conducted (T1-T6). Furthermore, it was found that headache was correlated with salivary cotinine levels at every test. On the other hand, vomiting was found to be correlated with salivary cotinine levels in three tests (T4, T5, and T6), whereas nausea was not found to have a correlation with salivary cotinine levels in all six tests (T1-T6). Finally, dizziness was strongly correlated with salivary cotinine levels only in the first test (T1). The correlation between tobacco farmers' use of personal protective equipment and salivary cotinine levels was highest in the sixth test. The correlation between PPE use of wearing a long-sleeved shirt, wearing gloves, and wearing a face mask was found to be high in all of the first six tests (T1-T6) with the p-value of 0.01 (Table 4).

Table 4. The salivary cotinine levels in Thai traditional tobacco farmers and non-farmers as a function of tobacco handling

Dry tobacco producing process	Salivary cotinine levels (ng/mL)						
	T1	T2	T3	T4	T5	T6	T7***
Tobacco Farmers (n =20)	0.591**	0.538**	0.680**	0.631**	0.539**	0.894**	0.160
Picking tobacco leaves	0.249	0.391*	0.680**	0.641**	0.539**	0.631**	NA
Transfer tobacco leaves	0.361*	0.244	0.311	0.476**	0.396*	0.435**	NA
Grading tobacco leaves	0.415**	0.402*	0.720**	0.414**	0.474**	0.433**	NA
Curing tobacco leaves	0.436**	0.324*	0.545**	0.371*	0.319*	0.303	NA
Removing stem tobacco leaves	0.525**	0.458**	0.616**	0.631**	0.397*	0.433**	NA
Rolling bundle tobacco leaves	0.429**	0.508**	0.616**	0.631**	0.397*	0.420**	NA
Cutting tobacco leaves	0.238	0.448**	0.465**	0.538**	0.273	0.420**	NA
Putting tobacco slice on rack	0.526**	0.203	0.355*	0.512**	0.159	0.518**	NA
Reverse bamboo rack	0.404**	0.513**	0.477**	0.364*	0.417**	0.523**	NA
Spraying tobacco exact	0.133	0.369*	0.231	0.121	NA	NA	NA
Keeping dried tobacco	0.214	0.419**	0.553**	0.744**	0.487**	0.620**	NA

*Significant at 0.05 probability level, **Significant at 0.01 probability level, ***T7= Control (after finished for 1 month)

There was a strong correlation between the four main kinds of GTS subjective health symptoms (headache, nausea, vomiting and dizziness) and salivary cotinine levels (T1-T6). Headache was correlated with salivary cotinine levels in each sampling timepoint. Conversely vomiting was correlated with salivary cotinine levels only in the last sample testing timepoints (T4, T5, T6) and nausea was not correlated with salivary cotinine levels in any sampling timepoint (T1-T6). Furthermore, dizziness was strongly correlated with salivary cotinine levels in only the first test (T1) (Table 5).

Table 5. The correlation between subjective health symptoms and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n=40)

Dry tobacco producing process	Salivary cotinine levels (R)						
	T1	T2	T3	T4	T5	T6	T7
Tobacco Farmers (n =20)	0.591**	0.538**	0.680**	0.631**	0.539**	0.894**	0.160
Headache	0.488**	0.399*	0.455**	0.413**	0.569**	0.504**	-0.053
Nausea	0.264	0.195	0.200	NA	NA	0.088	NA
Vomiting	0.232	0.059	0.303	0.384*	0.426**	0.416**	NA
Dizziness	0.554**	0.019	NA	NA	NA	0.001	NA

*Significant at 0.05 probability level, **Significant at 0.01 probability level, T=time to testing

The correlation between tobacco farmers and salivary cotinine levels had the highest correlation in test timepoint T6. PPE use and salivary cotinine levels was also strongly correlated with wearing a long sleeved shirt, gloves, and masks in all of the test timepoints (T1-T6) with a p-value of 0.01 (Table 6).

Table 6. The correlation between personal protective equipment (PPE) use and salivary cotinine levels among Thai traditional tobacco farmers and non-farmers (n=40)

PPE use in dry tobacco producing process	Salivary cotinine levels (R)						
	T1	T2	T3	T4	T5	T6	T7***
Tobacco Farmers (n =20)	0.591**	0.860**	0.680**	0.631**	0.539**	0.894**	0.160
Wearing long sleeved shirt	0.442**	0.692**	0.575**	0.471**	0.529**	0.494**	NA
Wearing long legged pants	0.549**	1.000	0.427**	0.342*	0.511**	0.510**	NA
Wearing rain coat	-0.120	0.186	NA	NA	NA	NA	NA
Wearing plastic apron	0.021	0.489**	0.368*	0.259	0.379*	0.304	NA
Wearing gloves	0.411**	0.692**	0.635**	0.631**	0.559**	0.690**	NA
Wearing boots	0.233	0.603**	0.349*	0.553**	0.575**	0.631**	NA
Mask	0.591**	0.860**	0.680**	0.631**	0.539**	0.894**	NA
Changing wet suit during work	0.034	0.440**	0.196	0.333*	0.261	0.496**	NA

* significant at 0.05 probability level, **significant at 0.01 probability level, *** T7= control (after finished for 1 month)

The correlation between GTS of the tobacco farmers and salivary cotinine levels had a strong correlation at all testing timepoints ($p<0.01$). The prevalence of GTS in T1 to T7 was found to increase in early testing (T2) and declined during T3 to T5. However, during T7 it was found that there was a 10% prevalence of GTS suggesting it to be from one of the tobacco farmers who smoked cigarettes and had symptoms that met definition of GTS.

DISCUSSION

The Thai traditional tobacco process produces a final product of dried tobacco. Almost all of the farmers are located in the northern part of Thailand. The unique method and process are carried out by the Thai traditional tobacco farmers with intensive hard-work and family labor. The demographic characteristics of the study populations are representative of rural areas in the northern part of Thailand. Almost all of the farmers graduated from primary school and followed farming as the traditional occupation. The average age of farmers was quite high which is a big proportion of agriculturalists at the present time in the rural areas. Thai traditional tobacco cultivation in this area is part of the culture and folk life of northern Thailand. From previous studies, health effects of tobacco cultivation were known to be caused by nicotine, which penetrates through the skin of the hands of workers who cultivate and harvest tobacco [9, 18, 19]. From this study it was shown that the prevalence of GTS among farmers increased in the early testing timepoints (T1 and T2) and declined during last testing timepoints (T3 to T5). This may support the results in the study conducted by Trapé-Cardoso et al. (2003) who found that nonsmokers were more likely than smokers to develop possible GTS symptoms and that nonsmokers may be especially vulnerable to GTS [4, 12]. The reason was presumably because smokers were tolerant to nicotine and therefore were less likely to have symptoms when exposed to additional nicotine [26]. Similarly to previous studies [4, 5, 11, 12, 13] the use of tobacco products (smoking or smokeless) appears to decrease absorption of nicotine and the dermal absorption variable "smoking tobacco" had a significant inverse relationship to GTS incidence [2]. On the other hand, from this study it was found that only one of farmers who smoked cigarettes had subjective health symptoms which met the definition of GTS.

This study aimed to test the hypothesis that there is a positive association between salivary cotinine levels and GTS among Thai traditional tobacco farmers. In parallel the second purpose was to determine the salivary cotinine levels of the tobacco farmers across the dry tobacco producing areas and to conduct follow up studies to determine whether or not Thai traditional tobacco farmers absorb nicotine from the tobacco leaves. Gas chromatography-nitrogen phosphorous detection (GC) is a valid, reliable, and commonly used quantitative method for measuring cotinine in urine or saliva [10]. However, GC is a time-consuming and relatively expensive method. There are many alternative methods to GC but in this study we chose the NicAlert^{TM} Saliva strip test (NCTS) because the test can detect as little as 10 ng/mL cotinine, requires minimal training to use reliably, can be used anywhere, and provides results in approximately 30 minutes. Moreover, providing a urine sample is often unacceptable for people and difficult to arrange in some settings, whereas providing a saliva specimen is likely to be more acceptable [23]. The diagnosis accuracy of NCTS has been tested in saliva with a sensitivity of 99% and a specificity of 96% [20]. NCTS can detect exposure to nicotine from all sources (e.g., nicotine replacement therapy, chewing tobacco, cigar, and second-hand smoke; SHS), not just from cigarettes [7]. From this study it was found that NCTS can detect cotinine level in both tobacco and non-tobacco farmers, whereas the correlation between salivary cotinine levels among tobacco farmers were different from non-farmers with statistical significance. In addition, NCTS maybe a physical tool for exposure surveillance among the non-farmers who do not work with tobacco, but smoke or live with smoking family members or are exposed to second-hand smoke (SHS). Our analysis showed that GTS prevalence at each timepoint measured to describe the internal dose of nicotine, estimated by salivary cotinine, accounted for the relationship between working in the processing of dry Thai traditional tobacco and GTS. This was corroborated by the study results of *Trikunakornwongs* et al. [27]. This study found that the nicotine dust exposure via the dermal route may promote the absorption of nicotine from dust more than inhalation because of the moisture from sweating in the summer in a similar way as moisture has promoted GTS among the harvesters [11]. In addition, those who work all day and every day may be exposed to nicotine dust through both inhalation and dermal contact for prolonged periods of time and may develop some symptoms [27] related to GTS. Moreover, from the tobacco working process, the correlation between improper use of personal protective equipment (PPE) and salivary cotinine level were considered risk factors for GTS. Similarly the previous study by *Arcury* et al. [1] found that the internal dose of nicotine, as estimated by salivary cotinine was correlated with the relationship between work behaviors and GTS [3]. The detection of nicotine poisoning from dried Thai traditional tobacco production via inhalation, absorption or dermal absorption must consider the specific effects of route of exposure. The results of this study found that those who had symptoms consistent with GTS did not appear to have any correlation between salivary cotinine levels and nausea. However, only the first test timepoints demonstrated correlation with dizziness and then did not have any correlation, possibly due to tolerance to nicotine poisoning [26]. Additionally the tobacco farmers who wore the necessary PPE to protect from the pungent odor from dried tobacco were able to reduce inhalation of the vapors.

GTS prevention should be based on methods to reduce nicotine absorption. This study showed that the high correlation between PPE and salivary cotinine levels were consistent when wearing long sleeved shirts, gloves and masks. However, in order to be accepted, protective suits and gloves should be lightweight and comfortable allowing the equipment to be used in hot climates [11, 25].

LIMITATION

Some limitations of this study should be noted. First, individual variability in the metabolism and clearance of cotinine and nicotine can affect the levels of cotinine detected in saliva. Second, it is also possible that we overestimated the occurrence of GTS because the symptoms of GTS are nonspecific, and some individuals with other subjective health symptoms such as heat stress or dehydration could have been mistakenly included. The numbers reported in the individual studies depend on the case definitions applied and health belief included the awareness of stakeholders that the condition GTS exists.

CONCLUSION

This analysis indicated that GTS continues to be a common occupational disease among Thai traditional tobacco farmers who cultivate and produce dry Thai traditional tobacco. It is the first analysis to examine the correlation between salivary cotinine which was measured by NCTS strip test and dry Thai tobacco production, the use of personal protective equipment, and the occurrence of GTS. The NCTS is both a valid and reliable test compared with the GC saliva test. In addition, measuring cotinine in saliva by NCTS may support testing in the field in a large population because NCTS was able to detect exposure to nicotine from all sources (e.g., nicotine replacement therapy, chewing tobacco, cigar, and second- hand smoke; SHS), not just from cigarettes. This study demonstrated that the use of salivary cotinine levels measured by NCTS were well correlated with farmers who working with dry tobacco production. Salivary cotinine levels were also significantly correlated with the prevalence of GTS among the tobacco farmers group at any time to testing across the crop season. This study was different from previous studies that showed that GTS and salivary cotinine levels were correlated in workers who worked in humid conditions because the nicotine penetrates through the skin of the hands of workers who cultivate and harvest tobacco. Finally, although the short-term effects of this exposure may be symptoms of nicotine poisoning as defined of GTS, the long-term effects of such exposure should be investigated and health education programs with health risk exposure for increased awareness of farmers is recommended.

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Conflict of interest

The authors declare no conflict of interest.

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