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Investigation of Salivary Cotinine Levels among Tobacco Farmers in Thailand

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Abstract

Tobacco poisoning has previously been recognized as having adverse effects on health. This study investigated the salivary cotinine levels different between growing season period and to identify factors that can predict changes in salivary cotinine levels. This cross-sectional study included 62 male tobacco farmers. Data on tobacco exposure were collected using a questionnaire, and salivary cotinine levels. A univariable logistic regression analysis revealed that alcohol consumption was significantly associated with salivary cotinine levels. The salivary cotinine levels of male tobacco farmers at the end of the harvest season were significantly higher than those at the beginning of the harvest season (p-value < 0.001). The findings indicate that Thai tobacco farmers are at risk of nicotine exposure. Interventions and measures to raise awareness and reduce the risk of nicotine exposure among tobacco-farmers are required.

Keywords: Nicotine exposure; Exposome; Agriculture; Risk exposure; Health risk

1. Introduction

According to the World Health Organization (WHO) report, over 3.5million hectares of land are harvested for tobacco globally, a figure that continues to rise. In Thailand, traditional methods of tobacco cultivation expose farmers consistently to nicotine present in tobacco leaves, which potentially subjects them to health risks associated with this alkaloid.

The global impact is underscored by the annual deaths worldwide linked to the use of tobacco products. This raises concerns about serious health risks, especially among tobacco farmers who are highly susceptible to nicotine exposure and its harmful effects on various human systems, including the cardiovascular system, reproductive system, respiratory system, kidneys, immune system, and others (Mishra *et al.,* 2015; Pugh *et al.,* 2004; Hansson *et al.,* 1994). A previous study highlighted the significant public health risks posed by nicotine concentrations in tobacco workplaces, with tobacco poisoning recognized as one of the foremost occupational hazards. The global impact is underscored by numerous deaths worldwide each year linked to the use of tobacco products (Yoo *et al.,* 2014; WHO, 2008). Tobacco remains a popular cash-crop choice for many farmers, especially in low- and middle- income countries where the vast majority of tobacco farming takes place (Lecours *et al.,* 2012).

According to the most areas in the north part of Thailand, such as Nan, Chiangmai, Chiang Rai, Payao, and Sukhothai are growing tobacco areas. The northeast part, such as Roi Et, Loei, Nong Khai and Chaiyaphum are also included. The top 3 most tobacco growing area are Phetchabun, Sukhothai and Roi Et. They are also top producers quantitatively (9,279,300 kilograms, 6,398,017 kilograms and 3,414,470 kilograms, respectively).

Sukhothai Province serves as a representative region for the cultivation of traditional Thai tobacco plants, particularly the air-cured Burley tobacco variety. This type of tobacco exhibits distinct properties, such as a transparent structure and a high absorption rate for aromatic, flavored water, alongside a notably high nicotine content ranging from 2.5% to 4.0%. With a provincial production of approximately 6.4 million kilograms from 60,740 acres, Sukhothai Province captures the intricacies and scale of traditional Thai tobacco cultivation. The comprehensive process of traditional Thai tobacco production involves seeding, transplanting, maintaining plants with fertilizer and pesticide applications, and multi-stage harvesting and curing of tobacco leaves. Throughout each stage, tobacco farmers are consistently exposed to tobacco products, highlighting the need for an exploration into potential effects on reproductive health. Cotinine is a substance that has a very long half-life than nicotine, half-life of cotinine is 16-18 hours and having concentration levels in the bloodstream very high 10-15 times compare with nicotine. Therefore, so in research studies about commonly used to measure the amount of cotinine in the saliva to confirm nicotine in the body (Vearrier & Greenberg, 2011). Cotinine can be widely used in further compared to other diagnostic tools because of its higher sensitivity, specificity, long half-life as well as it is the best indicator for distinguishing the tobacco users from non-users. (Raja *et al.,* 2016). Thus cotinine measurement in saliva becomes a non-invasive, easy and well tolerated collection procedure when multiple samples are required over a limited period (Avila-Tang *et al.,* 2013). Another study was shown that cotinine, as a nicotine metabolite, can be a reliable biomarker assessing nicotine exposure in tobacco farmers (Fristiyanwati *et al.,* 2022).

This study aimed to examine whether the salivary cotinine levels of tobacco farmers at the beginning of the harvest season differ from salivary cotinine levels at the end of the harvest season and to identify factors that can predict the changes in salivary cotinine levels. This investigation contributes valuable insights into the occupational health risks within the unique context of tobacco farming in Sukhothai Province, Thailand.

2. Methodology

2.1 Design, setting, and subjects

This cross-sectional study was conducted on tobacco farmers in Thap Phueng Subdistrict, Si Samrong District, Sukhothai Province, Northern Thailand. The area of Si Samrong District was purposively selected because the significance in tobacco cultivation. Participants for the study were chosen using convenience sampling. Tobacco farmers aged 20 to 40 who worked as a tobacco farmer were eligible for inclusion. The sample size for estimating a population proportion (Künzle *et al.,* 2003) was determined by defining the estimated proportion at 17.5%, the confidence level at 95%, the level of precision at 0.05%, and the sample size at 56. Adding 10% of the participants to prevent loss of data yielded a sample size of 62. The final number of participants for data analysis was 62.

2.2 Data collection

Data were collected between March and May 2022. Data were collected two times during processing of cultivation tobacco growing, it was collected at first time in the first week of March, 2022 which is beginning of the harvest season period and to collect the second time in the first week of May, 2021 which is the end of tobacco cultivation.

In-person interviews were conducted by the researchers. An interviewer-administered questionnaire contained the following sections: (a) demographic characteristics such as age, beverage intake, smoking status and alcohol drinking status; (b) tobacco exposure along with activity frequency, use of personal protective equipment (PPE). Salivary cotinine levels, indicative of nicotine exposure, were assessed using NicAlert (Nicalert CE Saliva PI APR 25 2006) from Jant Phamacal Corporation, California, USA) based on the enzyme-linked immunosorbent assay (ELISA) principle, which is a rapid and cost-effective method for verifying low risk exposure and high risk exposure status group. The test strip has seven levels (0 – 6), representing different cotinine levels, with level 0 equivalent to 0 – 15 ng/mL (low risk exposure) and level 6 indicating > 1000ng/ mL of cotinine (Cooke *et al.,* 2008). Salivary samples, collected in the morning after mouth rinsing with water, utilized strips within 10 minutes of opening. Cotinine, selected for its extended half-life, offered a reliable biomarker for exposure assessment (Xie *et al.,* 1997; Kumar *et al.,* 2019). Using receiver operator characteristic curve analysis, the salivary cotinine of cut-points was 15 ng/ml for adults (sensitivity 96.3%, specificity 97.4%). The participant who had the cotinine range (ng/mL) level less than 15 ng/mL were assigned to low risk group, and those of participants who had the cotinine range (ng/mL) level more than 15 ng/mL were assigned to high risk group (Benowitz *et al.,* 2002).

The validity of the questionnaire was checked by three experts in the field of occupational health and environmental. In-person interviews were conducted by researchers. Stimulated saliva samples were collected by the researcher.

2.3 Data analysis

Descriptive statistics, such as frequency and percentage, were used to analyze the sample variables. Chi-square tests were used to investigate the factors (demographic characteristics and tobacco exposure) that are associated with salivary cotinine levels. Binary logistic regression was then used to determine the strength of the association between factors and salivary cotinine levels. Wilcoxon signed-rank test was used for saliva cotinine levels in male tobacco farmers at two study time points analysis. This study examined a single model in which each farm activity was tested independently. All statistical tests were conducted as two-sided analyses, with a predetermined significance level set at $p < 0.05$. All statistical analyses were performed using SPSS Version 22.0.

2.4 Ethical Considerations

This study received ethical approval from the Ethics Committee of the Institutional Review Board at the College of Public Health Sciences, Chulalongkorn University (Code COA No. 115/2564). Written consent was obtained from all male tobacco farmers before their participation.

3. Results and Discussion

The demographics and tobacco exposure of the 62 tobacco farmers under study are presented in Table 1. The participant who had the cotinine range (ng/mL) level less than 15 ng/mL were assigned to low exposure group. And those of participants who had the cotinine range (ng/mL) level more than 15 ng/mL were assigned to high exposure group.

Salivary cotinine levels were found to be 61.3% for low nicotine exposure. In total, the prevalence of high nicotine exposure salivary cotinine levels was 38.7%. Chi-square tests revealed a significant association between salivary cotinine levels and demographic variables including age ($p = 0.011$), and alcohol drinking status ($p = 0.009$).

The salivary cotinine levels of male tobacco farmers in the end of the harvest season period were significantly higher than those of male tobacco farmers in the beginning of harvest season period (Table 2). This cross-sectional study aimed to examine whether the salivary cotinine levels of tobacco farmers at the beginning of the harvest season differ from those at the end of the harvest season and to identify factors predicting changes in salivary cotinine levels.

The majority of male tobacco farmers worked approximately four to seven days per week. During leaf picking and curing processes, farmers frequently used their hands and arms, directly contacting the juice and sap of the plants. Notably, a significant portion of these farmers did not use chemical-resistant rubber gloves or plastic aprons, and their clothing often became wet during work. Wet clothing may increase nicotine exposure through dermal absorption, as the farmers in this area grow Burley tobacco. Our findings revealed that salivary cotinine levels differed significantly between the beginning and end of the harvest season. Previous studies have also reported high nicotine levels on tobacco farms, indicating a significant exposure risk (Yoo *et al.,* 2014). This finding aligns with Park *et al.* (2018), who observed significantly higher cotinine concentrations during the harvesting season compared to the non-harvesting season. Furthermore, nicotine has been shown to be genotoxic at saliva concentrations similar to those achieved during tobacco chewing (Trivedi *et al.,* 1990). For effective protection, suits and gloves should be lightweight and comfortable, given the hot climate in which tobacco farmers work. Gas chromatography-nitrogen phosphorous detection (GC) is a valid, reliable, and commonly used quantitative method to measure cotinine in human urine or saliva (Feyerabend, 1990). However, GC is a time-consuming and relatively expensive method. An alternative method that was chosen in the present study was the NicAlert^{TM} saliva strips test (NCTS) because the test can detect as little as 10 ng/mL cotinine. Furthermore, it requires minimal training to use reliably, can be used anywhere, and provides result within approximately 30 minutes only. In general, providing a urine sample is often unacceptable to people and it is rather difficult to arrange in some settings, but collecting saliva specimen is likely to be more acceptable (Peralta, 2001). The diagnosis accuracy of NCTS when used with saliva was 99% sensitivity and 96% specificity.

Association between factors and saliva cotinine levels in male tobacco farmers is shown in Table 3. The modeled odds of high exposure saliva cotinine levels increased significantly for alcohol drinking (OR = 4.429, 95% CI: 1.408 - 13.928). After controlling for age and smoking status, the modeled odds of high exposure saliva cotinine levels increased. Noah R. Gubner and colleagues found that chronic alcohol abuse may increase

Table 1. Demographic characteristics and tobacco exposure among tobacco farmers, classified by Salivary Cotinine Levels (n = 62)

Variables	n (%)	Salivary Cotinine Levels		p-value*
		Low exposure (n= 38) n (%)	High Exposure (n= 24) n (%)	
Age				
20 - 29	15 (24.2)	5 (13.2)	10 (41.7)	0.011*
30-40	47 (75.8)	33 (86.8)	14 (58.3)	
Beverage intake				
Did not drink	56 (90.3)	33 (86.8)	23 (95.8)	0.243
Did drink	6 (9.7)	5 (13.2)	1 (4.2)	
Smoking status				
Did not smoke	31 (50.0)	18 (47.4)	13 (54.2)	0.602
Smoke	31 (50.0)	20 (52.6)	11 (45.8)	
Alcohol drinking status				
Did not drink	43 (69.4)	31 (81.6)	12 (50.0)	0.009*
Drank	19 (30.6)	7 (18.4)	12 (50.0)	
Hours spent working				
< 8 hours/day	52 (83.9)	31 (81.6)	21 (87.5)	0.537
≥ 8 hours/day	10 (16.1)	7 (18.4)	3 (12.5)	
Working experience				
< 20 years	22 (35.5)	11 (28.9)	11 (45.8)	0.176
≥ 20 years	40 (64.5)	27 (71.1)	13 (54.2)	
Wearing gloves				
No	27 (43.5)	15 (39.5)	12 (50.0)	0.348
Yes	35 (56.5)	23 (60.5)	12 (50.0)	
Wearing raincoat				
No	22 (35.5)	15 (39.5)	7 (29.2)	0.409
Yes	40 (64.5)	23 (60.5)	17 (70.8)	
Wearing plastic apron				
No	37 (59.7)	21 (55.3)	16 (66.7)	0.373
Yes	25 (40.3)	17 (44.7)	8 (33.3)	

Note: Analyzed with a Chi-square test; *Statistical significance at $p < 0.05$, Saliva cotinine level (Low exposure = cotinine range < 15 ng/mL, High exposure = cotinine range > 15 ng/mL).

nicotine metabolism rates (Gubner *et al.,* 2016). In contrast, a recent study showed no significant correlation between cotinine levels and alcohol consumption. Moreover, we identified a significant association between PPE use during the tobacco drying process and sperm count, suggesting that PPE may mitigate certain reproductive health risks associated with nicotine exposure. During leaf picking and curing, farmers often handle leaves directly, transferring them from agricultural carts to curing areas. Cloth gloves, which are often used, may not provide adequate protection against nicotine exposure, especially in the hot climate that promotes sweating and potential skin absorption. This finding supports a prior study that recommended seamless knitted nylon gloves to prevent dermal nicotine absorption during tobacco cultivation (Gehlbach *et al.,* 1979). Use of gloves was also associated with significantly lower nicotine and cotinine levels in urine (Doctor *et al,* 2004). Another study was found that

Table 2. Saliva cotinine levels in male tobacco farmers at two study time points in Thailand

Study time points	Beginning of the Harvest Season Period	End of Harvest Season Period	Comparison of two season periods[a]
	Median	Median	
Participants (n)	62	62	
Salivary cotinine levels (ng/mL)	10	21.67	<0.001*

Notes. [a] Wilcoxon Signed Rank Test, *Association significant at p-value < 0.05

Table 3. Association between factors and Saliva cotinine levels in male tobacco farmers at two study time points in Thailand

Exposure characteristics	Salivary Cotinine Levels		Crude OR (95% CI)	p-value	Adj OR[a] (95% CI)	p-value
	Low exposure n (%)	High exposure n (%)				
Alcohol drinking status						
Did not drink	18 (47.4)	13 (54.2)	ref	0.011*	ref	0.006*
Drank	20 (52.6)	11 (45.8)	4.429 (1.408-13.928)*		5.930 (1.658-21.212)*	
Hours spent working						
< 8 hours/day	31 (81.6)	21 (87.5)	ref	0.715	ref	0.313
≥ 8 hours/day	7 (18.4)	3 (12.5)	0.633 (0.147-2.728)		0.703 (0.235-2.103)	
Working experience						
< 20 years	11 (28.9)	11 (45.8)	ref	0.349	ref	0.387
≥ 20 years	27 (71.1)	13 (54.2)	0.481 (0.166-1.398)		0.753 (0.249-2.275)	
Wearing gloves						
No	15 (39.5)	12 (50.0)	ref	0.350	ref	0.392
Yes	23 (60.5)	12 (50.0)	0.609 (0.215-1.722)		0.665 (0.221-2.002)	
Wearing raincoat						
No	15 (39.5)	7 (29.2)	ref	0.410	ref	0.390
Yes	23 (60.5)	17 (70.8)	1.584 (0.530-4.732)		0.712 (0.238-2.131)	
Wearing plastic apron						
No	21 (55.3)	16 (66.7)	ref	0.370	ref	0.481
Yes	17 (44.7)	8 (33.3)	0.618 (0.213-1.787)		0.704 (0.236-2.104)	

Note: *Statistical significance at p < 0.05, [a] Adjusted for age and smoking; *p < 0.05, ref = refers to the baseline group.

almost 90% of tobacco workers and 72% of paddy workers think work involved with crop farming and processing had health risks (Ali *et al.,* 2022). Ethel Alderete and colleagues found that tobacco farming was associated with significant increased reporting of serious injury (OR = 1.4; 95%CI 1.1 – 2.0), accidental injury by someone else (OR = 1.5; 95% 1.0 – 2.1), assault (OR = 2.2; 95% CI 1.3 – 3.8), and poisoning by exposure to chemicals (OR = 2.5; 95% CI 1.2 – 5.4) (Alderete *et al.,* 2020).

Notably, this study experienced no dropouts or absences among male tobacco farmer participants. However, limitations include the use of a single research area and a small sample size due to time and resource constraints. Future studies should consider integrating lifestyle factors into intervention programs, acknowledging factors such as exercise, alcohol intake, and PPE use. Tailored interventions should address these lifestyle components in addition to nicotine exposure.

4. Conclusion

This finding indicate that salivary cotinine levels of male tobacco farmers were significantly higher at the end of the harvest season than at the beginning. The lack of PPE use remains a potential risk factor for adverse health effects. It is essential to inform tobacco farmers about the potential health risks of their occupational activities. These findings underscore the importance of understanding occupational hazards and their effects on reproductive health outcomes. Recognizing the broader exposome and lifestyle factors that influence male reproductive health is crucial for developing comprehensive strategies and interventions.

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