Biomonitoring of Nicotine Exposure in Tobacco Farmers with Green Tobacco Sickness Symptoms

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ABSTRACT

Introduction: Indonesia is the fifth tobacco producing country in the world. The climate and weather in Indonesia are very suitable for tobacco plantations. At harvest season, tobacco farmers face the risk of being exposed to nicotine compounds due to skin contact with tobacco leaves. This exposure can cause a syndrome known as Green Tobacco Sickness (GTS) with symptoms of nausea, vomiting, weakness, dizziness, headache, insomnia and loss of appetite. These symptoms are not specific enough to describe GTS, so biological monitoring is required. As one of the main metabolites, cotinine has been used as a biological marker to assess nicotine exposure. This study aims to examine the reliability of nicotine biomonitoring in tobacco farmers with symptoms of GTS. **Methods:** We searched the online electronic databases, namely PubMed, Cochrane, and Scopus for appropriate evidence based material. We then made adjustments using the inclusion and exclusion critertia to then conduct a critical review of the selected articles. **Results:** We obtained four articles, consisting of 3 articles examining urinary cotinine as a biomarker of nicotine exposure, and another 1 article examining cotinine saliva. The articles gave us the similar pattern that in tobacco farmers with GTS symptoms there was an increase in cotinine levels, both measured in urine and saliva. **Conclusion:** Based on the articles obtained, cotinine, as a nicotine metabolite, can be a reliable biomarker assessing nicotine exposure in tobacco farmers with GTS symptoms. However, more research is needed to compare the best selection of biological samples such as urine, blood or saliva.

Keywords: biological monitoring, biomonitoring, cotinine, green tobacco sickness, nicotine

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INTRODUCTION

The strategic role of the plantation sub-sector in improving the national economy is described through its contribution to various aspects, one of which is as a contributor to Gross Domestic Product (GDP). In 2018, the plantation sub-sector contributed the highest GDP at 35 percent. In addition, the plantation sub-sector also contributes to the national economy with a high investment value; contributes to the national agricultural commodity trade balance; becomes a source of foreign exchange from export commodities; increases state revenues from excise, export taxes and exit duty; provides food and raw materials industry; absorbs labor; etc. Directorate General of Plantations, 2019).

Tobacco is one of the plantation sub-sector commodities that have made a real contribution as a source of income for farmers and employers. Besides, tobacco is one of the main commodities used in the cigarette industry where its consumption is the largest excise contributor and one of the biggest state revenues Directorate General of Plantations, 2019). With an output of 196,154 tons in 2016, Indonesia ranked fifth among the world's six largest tobacco producing countries (Wardhono, *et al*., 2019). The quality of local tobacco is highly recognized in the international market. Tobacco remains an important economic commodity both for the government and for farmers (Djajadi, 2015).

Tobacco (Nicotiana tabacum L.) belongs to the Solanacaee family, which is an annual herbaceous plant that grows to a height of 1-3 meters. This plant is native to South America, but is cultivated in many countries in the world (Bonamonte *et al*., 2016). In Indonesia, tobacco plantations are spread in almost all regions of the country, with its domination in

East Java, West Nusa Tenggara (NTB), Central Java, and West Java. In 2018, the total area of Indonesian tobacco covered an area of 204.509 hectares, where 99.96 % of them were managed by the private sector (Directorate General of Plantations, 2019).

Tobacco leaves contain chemical compounds that qualitatively and quantitatively depend on several factors such as plant hybrid conditions, climate and soil, leaf height, harvest time and method, type of drying (air or humidity), plant age, and leaf fermentation method and duration. One of the chemical compounds is nicotine (Bonamonte *et al*., 2016).

Nicotine ($C_{10}H_{14}N_2$) was first isolated from tobacco leaves by two German physicians, Wilhelm Heinrich Posselt and Karl Ludwig Reimann (Mishra *et al*., 2015). As the most toxic compound found in tobacco plants, nicotine concentrations vary from 1% to 10% depending on the type of tobacco (Bonamonte *et al*., 2016). Nicotine is a bioactive alkaloid that is a transparent liquid with distinct odor in its pure form. When exposed to air, it turns brown (Mishra *et al*., 2015). It is water soluble and has significant parasympathomimetic and addictive properties. During planting, cultivation, harvesting, curing and baling processes, tobacco farmers are exposed to nicotine which absorbs through the skin especially during humid conditions because clothes become wet from rain, dew, or sweat (Da Mota E Silva *et al*., 2018; Park *et al*., 2018; Cezar-Vaz and Cargnin, 2019; Alves *et al*., 2020). This put farmers at risk of experiencing health problems known as Green Tobacco Sickness (GTS) (Alkam and Nabeshima, 2019).

GTS was initially recorded as "cropper sickness" among tobacco workers in 1970 in Florida, USA. After being known to be caused by nicotine absorption from wet tobacco plants, it was later reported as GTS. GTS is self-limiting and short in duration (Fotedar and Fotedar, 2017). Symptoms of GTS are dizziness, headache, insomnia, nausea, vomiting, weakness, and loss of appetite (Cezar-Vaz and Cargnin, 2019). Despite the morbidity of GTS in almost a quarter of tobacco workers (Cezar-Vaz and Cargnin, 2019) and global prevalence of between 8.2% to 47% (Fotedar and Fotedar, 2017), these symptoms have gotten less attention due to lack of knowledge about GTS even among medical professionals. Many cases are misinterpreted as pesticide toxicity or heat temperature damage (Fotedar and Fotedar, 2017; Park *et al*., 2018).

Nicotine as the main compound in tobacco leaves can cause various health problems not only for smokers but also for tobacco farmers who are exposed during the work process. Although GTS has not been linked with any substantial mortality, it has a considerable influence on the health of tobacco farmers. A quarter of tobacco workers with GTS who seek medical care require hospitalization, and significant hospital expenses are associated with this condition (Mishra *et al*., 2015; Fotedar and Fotedar, 2017). This will certainly have an impact on the productivity of tobacco farming as a product with high economic value. Support from various sectors is needed, one of which is from the health sector through a biomonitoring program.

Biomonitoring is a method for detecting the occurrence of chemical exposure through examination of chemicals or their metabolites in physiological fluids (urine, blood or saliva), tissues (hair), or exhaled air (Boogaard, Hays and Aylward, 2011) . Chemical compounds can be found around us. Without us realizing it, chemicals might enter the body through 3 absorption pathways, namely ingestion, inhalation, and skin absorption. When the exposure scenarios are unknown, biomonitoring is frequently the most reliable exposure assessment tool for poorly defined exposure scenarios since it determines the integrated exposure regardless of the route of exposure. Biomonitoring is often superior to other exposure methods since it specifies integrated exposures regardless of the route of exposure. In terms of risk characterization, biomonitoring is typically preferable to other assessment methods since it assesses actual exposure by capturing individual differences in behavior (e.g. personal hygiene), physiology, and other aspects (e.g. respiration rate). Biomonitoring can also detect metabolic changes and vulnerability (Boogaard, Hays and Aylward, 2011; Haines *et al*., 2019). This can help policymakers prioritize activities and measures, assess the success of policy initiatives aimed at decreasing hazardous substance exposure, and encourage a more holistic approach to health (Ganzleben *et al*., 2017) .

Based on the evidence-based literature, the aim of this paper is to determine which form of biological testing is best to use in assessing nicotine exposure in tobacco farmers experiencing GTS symptoms by considering facilities and methods that are not only practical, but also accurate.

Table 1. Search Strategy Using Keywords

Database	Keyword	Filter	Hit	Selected
PubMed	((nicotine) AND (exposure)) AND (workers)	All fields	70	3
Cochrane	"Nicotine" AND "Exposure" AND "workers"	All fields	1	0
Google Scholar	Nicotine biomonitoring in tobacco farmers or harvesters	Workers	10	1
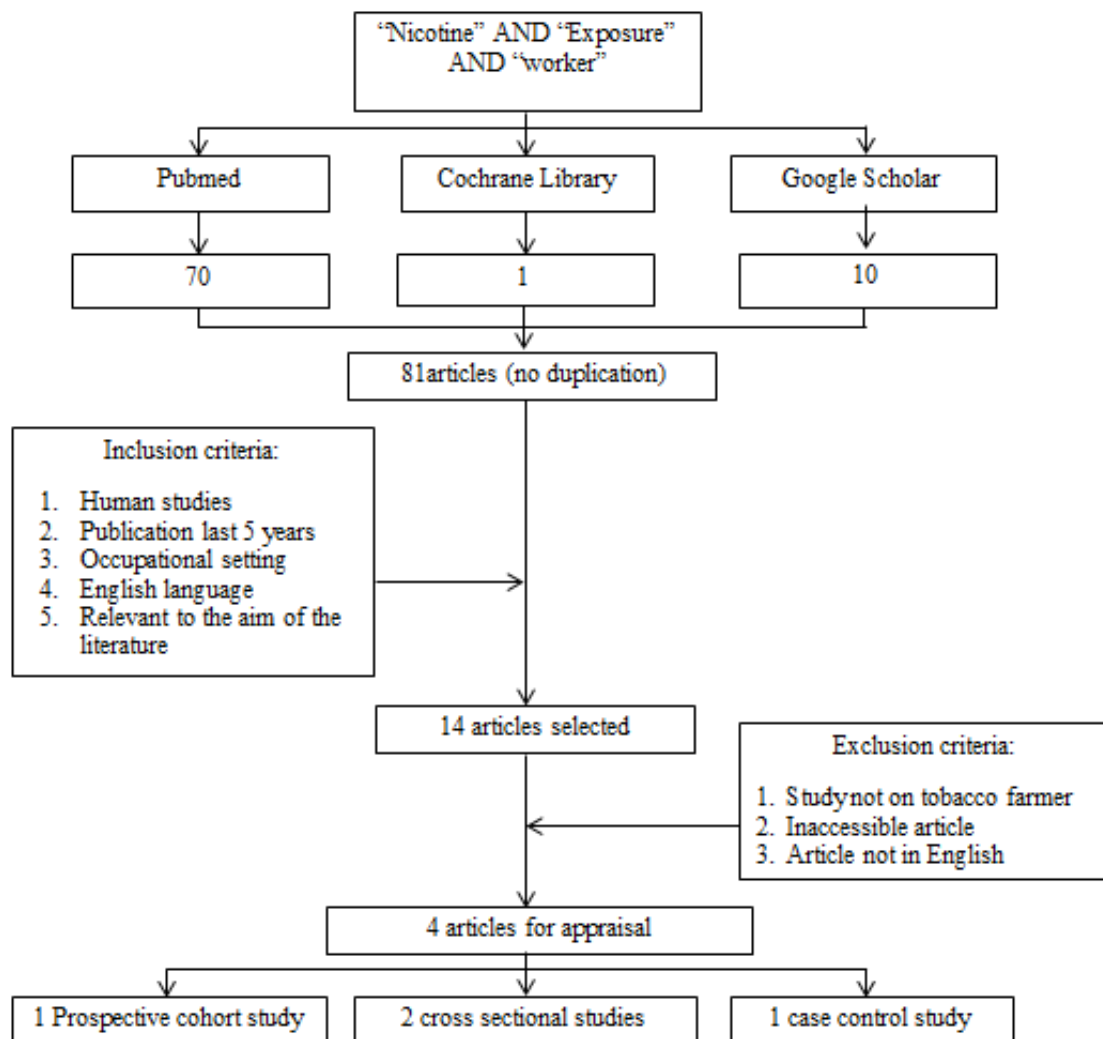
Figure 1. The Process of Article Selection

METHODS

The authors conducted an evidence-based literature search through the PubMed, Cochrane, and Google Scholar electronic databases on December 20, 2020. The keywords used were "nicotine", "exposure", and "workers".

Following the search for publications using the keywords (Table 1), the inclusion and exclusion criteria were used to make the final selection. The inclusion criteria in this searching strategy included human studies, publications within the last 5 years, occupational setting, English language, and articles relevant to the aim of this literature. Meanwhile, studies not on tobacco farmers, inaccessible articles, and articles not in English language were being excluded. The list of articles was reduced using

Table 2. Critical Appraisal Checklist for Diagnostic Study

| Articles | Study Design | No.of Participants | Validity | | | Importance | Applicability | Level of Evidence |
			"The Diagnostic Test were Evaluated in a Representative Spectrum"	"Reference Standard Applied Regardless of the Index Test Result"	"Independent and Blind Comparison"	"Test Characteristics are Presented"	"The Methods for Performing the Test Were Described in Sufficient Detail to Permit Replication"	
Salivary Cotinine Levels As A Biomarker For Green Tobacco Sickness In Dry Tobacco Production Among Thai Traditional Tobacco Farmers (Saleeon *et al.*, 2016)	Pros Cohort	40	Yes	No	Unclear	Yes	Yes	3
Green Tobacco Sickness Among Tobacco Harvesters in a Korean Village (Park *et al.*, 2018)	Cross-sectional	40	Yes	No	Unclear	Yes	Yes	2
Occurrence of green tobacco sickness and associated factors in farmers residing in Dom Feliciano Municipality, Rio Grande do Sul State, Southern Region of Brazil (Campos *et al.*, 2020)	Cross-sectional	354	Yes	No	Unclear	Yes	Yes	2
Use of cotinine biomarker in workers to detect green tobacco sickness (Cezar-Vaz and Cargnin, 2019)	Case Control	111	Yes	No	Unclear	Yes	Yes	4

the inclusion and exclusion criteria, leaving only four articles to be appraised. The article selection strategy can be seen in Figure 1. Finally, 4 articles were selected as they were considered suitable for further assessment using the relevant criteria by "The Oxford Centre for Evidence-Based Medicine for diagnostic study" (Oxford Centre for Evidence Based Medicine, 2020). Table 2 presents the results of the appraisal.

RESULTS

4 articles relevant to the aim of this review were obtained, namely 3 articles from PubMed and 1 article from Google Scholar. Moreover, there were 1 prospective cohort study, 2 cross-sectional studies, and 1 paired case control study. All selected articles examine cotinine as a biomonitoring of nicotine exposure using various methods like NicAlertTM Saliva strip tests (NCTS), High performance liquid chromatography (HPLC), and colorimetry.

Article 1 (Saleeon *et al.*, 2016) is a prospective cohort study that examines the relationship between the incidence of GTS and salivary cotinine levels in dry tobacco processing in Nan Province, Thailand. This study examines nicotine exposure in dry conditions, in contrast to other studies examining GTS in humid conditions. The study was conducted on 40 farmers, 50.0% males and 50.0% females, who were later divided into 2 groups, namely 20 tobacco farmers and 20 non-tobacco farmers. The samples were followed up every 2 weeks for a total of 14 weeks. Salivary cotinine levels were measured using NCTS.

The NCTS test strip used in this study is a semi-quantitative immunoassay technology in the form of a rapid test strip that provides a time-saving alternative compared to other methods (15-30 minutes). The NCTS detects nicotine exposure from all sources. The NCTS samples have a 95% accuracy, a 93% sensitivity, a 95% positive predictive value, and a 93% negative predictive value, making this tool quite reliable in assessing nicotine exposure (Asha and Dhanya, 2015). Although this tool also showed positive results on any nicotine exposure include smoking, it seems that the researchers were trying to reduce the risk of bias by taking samples from farmers who were not smokers - only 1 out of 20 samples of tobacco farmers group was smokers.

Based on seven measurements carried out (T1-T7), it was found that tobacco farmers had higher salivary cotinine levels than non-tobacco farmers at almost all points (p<0.05). For GTS symptoms, headache, vomiting, and dizziness were also associated with increased salivary cotinine, except for nausea. According to this article, the use of PPE by wearing a long sleeved shirt, gloves, and masks is a factor that can reduce the risk of nicotine exposure (Saleeon *et al.*, 2016).

The limitation of this study is that there are no steps to establish the diagnosis of GTS as stated in the title of the article, but instead this study only looks for self-reported symptoms such as headache, dizziness, nausea, and vomiting. The question is whether by finding one of these four symptoms we can immediately diagnose that it is GTS. These symptoms are not specific enough to assess the symptoms of GTS considering that tobacco farmers can also experience this condition due to heat stroke or dehydration considering the tropical conditions in Thailand.

Article 2 Park *et al.* (2018) is a cross sectional study measuring cotinine levels in 40 tobacco harvesters in Korea. This study is interesting because the authors not only assessed the relationship of GTS with increased urinary cotinine levels but also tried to see the best time to show the highest urinary cotinine levels.

Measurements were carried out 5 times, namely 4 times a day during the harvest season from July 20, 2008 to July 30, 2008 (immediately following waking up, following morning work, following afternoon work, and following dinner) and once during the non-harvest season in the following year (2009) using the HPLC with the modified Takeda methods. The results showed that cotinine levels increased significantly in the harvest season compared to the non-harvest season (cotinine levels ranged from 460.63 to 500.71 ng/mg Cr). The highest cotinine concentration was in the morning urine (T1), but there was no significant difference by the time (T1-T4). The concentration in participants were considerably lower during the non-working period (T5) than during the working period (135.40 ng/mg Cr, p<0.01).

In this article, the incidence of GTS was found in 15 out of 40 people (37.5%). The most common GTS symptoms were observed at work, with

symptom onset usually occurring after 3-17 hours of work. Several risk factors that were considered to be associated with the incidence of GTS such as gender, age, and smoking status were also assessed. It was found that GTS occurred more in women than men (55% vs 20%, p <0.05), there was no significant correlation of GTS with age, and the incidence was higher in nonsmokers than in smokers (57% vs 0%, p<0.01).

Article 3 by Campos *et al.* (2020) is a cross sectional study in Dom Feliciano, Rio Grande do Sul, Brazil investigating the factors associated with the occurrence of GTS. Samples were taken from as many as 354 tobacco farmers who were considered to meet the criteria. Urine sampling was carried out in the period October 2011 and March 2012. There were 122 (34.5%) GTS cases among the participants.

Not too different from Article 2 which concluded that GTS symptoms appeared at cotinine levels between 700-1000 ng/mg Cr (Park *et al.*, 2018), in Article 3 the median urine cotinine level for the cases was 755.8ng/mL (632.1 ng/mg of creatinine, p- value 0.01). What was different is that in Article 3 the incidence of GTS was higher in men than in women (61% vs. 39%, p<0.05).

The advantage of Article 3 is that the authors realized that the determination of GTS only through the appearance of symptoms is weak considering that several health conditions show similar complaints. The authors, therefore, included a history of contact tobacco leaves up to 48 hours prior to sampling and an objective assessment of urine cotinine levels >50 ng/mL according to the recommendation of the Brazilian Ministry of Health as parameters in the diagnosis. However, the possibility of pesticide exposure involved in the appearance of GTS symptoms in this study was not ruled out (OR: 3.64, 95% CI: 1.433-9.251, p<0.01).

Article 4 is a paired case control study in the Rio Grande do Sul region of Brazil, assessing urinary cotinine levels between the case group and control group based on smoking status (Everatt *et al.*, 2013). Participants were Burley tobacco farmers, with A total of 111 people consisted of 20 case groups and 91 control groups (ratio 1:4). Urine sampling was carried out during the harvest period, namely December 2016, and additional 4 samples were taken in December 2017 to complete the missing data. The data were then analyzed using the HPLC method, which is more specific and has lower detection limits. The results showed that in the case group, urine cotinine levels were higher than those in the control group (114.9 ng/mL vs 98.5 ng/mL, p<0.092).

The workers began to experience headache (50.0%), skin irritation (40.0%), nausea (35.0%), sickness and general malaise (30.0%), especially in the morning (11; 55.0%). Most of them worked with wet tobacco from the morning dew and in hot weather (70.0%). There was no association between the disease and gender (p= 0.337), age (p= 0.214), ethnicity (p= 1.000), education level (p= 0.713), use (p= 0.385) and frequency of alcoholic beverages (p= 0.584).

The advantage of this study is that the authors only included participants who had never been exposed to pesticides in the last 7 days, so it is almost certain that the symptoms experienced by the case group were not due to pesticide intoxication. Furthermore, as in Article 3, Article 4 defines cases of GTS as the presence of clinical symptoms along with confirmed urinary cotinine levels above the laboratory reference value.

DISCUSSION

Nicotine, 3-(1-methylpyrrolidin-2-yl) pyridine, is well absorbed through inhalation, ingestion, and skin absorption. Nicotine is an addictive central nervous system (CNS) stimulant being mediated via the release of some neurotransmitters, including acetylcholine, beta-endorphins, dopamine, norepinephrine, serotonin, and ACTH (NCBI, 2021). Nicotine intake can result in peripheral vasoconstriction, tachycardia, and increased blood pressure. This substance may potentially cause nausea and vomiting by stimulating the chemoreceptor trigger zone (NCBI, 2021).

Metabolism of nicotine is divided into three steps, namely C-oxidation catalyzed by P450 2A6 (CYP2A6), N-glucuronidation catalyzed by UGT2B10, and N-oxidation catalyzed by FMO3 (Murphy, 2017). Up to 75% nicotine is converted to cotinine largely by the liver enzyme CYP2A6, 15% is processed through other metabolic routes, and a little fraction (10–15%) is eliminated unaltered in urine (Loukola *et al.*, 2015; Benowitz *et al.*, 2016).

Cotinine, the main metabolite of nicotine, is converted exclusively by CYP2A6 to trans-3' hydroxycotinine (3HC); up to 40% is eliminated in urine as 3-hydroxycotinine while 10% is further processed by UGT enzymes into 3-hydro-xycotinine-glucuronide before excretion. UGT enzymes convert

around 15% of cotinine to cotinine-glucuronide, while the rest is metabolized in different ways (Loukola *et al*., 2015). Nicotine and cotinine can be detected in urine, blood and saliva, all of which are organic substances that are easier to use on a regular basis (Cezar-Vaz and Cargnin, 2019).

Cotinine measurement, rather than nicotine testing, has certain benefits and is favoured over nicotine testing. Firstly, since cotinine has a longer half-life than nicotine, it can be used to measure tobacco use over a longer period of time. Nicotine's half-life is estimated to be between 100 and 150 minutes, while cotinine's half-life is estimated to be between 770 and over 1100 minutes. Secondly, nicotine can be measured in a plasma sample, while cotinine can be measured in saliva and urine samples, which are a much less intrusive method of sample collection (Balhara and Sarkar, 2016). As a result, cotinine analysis is now widely used to objectively measure nicotine and tobacco exposure (Balhara and Sarkar, 2016; Murphy, 2017).

Cotinine can be measured in a variety of biological matrices (e.g., blood, saliva, and urine) (Campos *et al*., 2020). Urine samples are preferred and considered suitable for measuring cotinine because urine is easier to collect and has a higher concentration than serum or saliva. Cotinine urine concentrations have been observed to be 10 to 100 times greater than those of serum and saliva. Furthermore, unlike nicotine which is impacted by pH in the kidneys when eliminated in urine, cotinine is unaffected by flow rate or pH; this makes it the best biological exposure index unaffected by diet or other circumstances (Park *et al*., 2018).

Article 1 Saleeon *et al*. (2016) used saliva as a biological sample to measure cotinine levels. Under some conditions when several samples are required over a short period of time, the use of saliva is considered more practical in a tobacco plantation setting because it is simple and well tolerated for the collection approach. In addition, the measurement of cotinine in saliva also becomes a non-invasive method that does not require an expert in sample collection compared to the use of urine or blood samples (Raja, 2016).

Of the four articles above, the use of salivary and urinary cotinine showed a significant relationship with the incidence of GTS. The highest cotinine levels were shown in morning urine as stated in Article 2 (500.71 ng/mg Cr), in accordance with Article 4 which showed that GTS symptoms most often appeared in the morning (55%) (Park *et al*., 2018; Cezar-Vaz and Cargnin, 2019).

However, we still have not been able to determine the cutoff value in determining the diagnosis of GTS. Different studies have suggested different cutoffs for cotinine levels in the urine (Balhara and Sarkar, 2016). According to Article 3, GTS appeared when urinary cotinine levels were 756 ng/mL (632 ng/mg Cr), not too different from Article 2 with 700-1000 ng/mg Cr (Park *et al*., 2018; Campos *et al*., 2020). Article 4 shows a lower level (144 ng/mL), whereas the study was conducted on tobacco farmers specifically the Burley tobacco (Cezar-Vaz and Cargnin, 2019). Non-Virginia tobacco leaves, such as Burley, are known to have about three to four times more nicotine than Virginia tobacco leaves, which were used by the majority of previous research (Cezar-Vaz and Cargnin, 2019). This difference may be caused by several things such as differences in urine collection time, duration of exposure, and laboratory analysis method (Article 1 used NicAlert strip test, Articles 2 and 4 used the HPLC method, while Article 3 used EIA colorimetry). Nicotine metabolism is also influenced by ethnicity-related differences (Murphy, 2017). The action of Enzyme CYP 2A6 (cytochrome P-450, Family 2, Subfamily A, Polypeptide 6), is responsible for the breakdown of nicotine in the liver which is then dispersed in the blood, saliva and urine (Benowitz *et al*., 2016; Park *et al*., 2018; Cezar-Vaz and Cargnin, 2019). The activity of this enzyme shows differences between different races. This may lead to variations in the relative distribution of the excreted metabolites in different populations (Murphy, 2017).

Moreover, cotinine levels are strongly influenced by smoking status. In Articles 2 and 3, smokers showed above average cotinine levels, but the incidence of GTS was lower (Park *et al*., 2018; Campos *et al*., 2020). Compared to smokers, nonsmokers were more likely to develop possible GTS symptoms and might be particularly vulnerable to GTS. The reason is presumably because smokers are tolerant to nicotine and therefore are less likely to have symptoms when exposed to additional nicotine (Saleeon *et al*., 2016). The use of tobacco products also appears to decrease absorption of nicotine and the dermal absorption, and to have a significant inverse relationship to GTS occurrence. Tobacco use causes vascular constriction due to consumption, metabolic adaptation or the tolerance to nicotine's long-term effects (Cezar-Vaz and Cargnin, 2019).

The symptoms of GTS are also not specific enough, making the diagnosis becomes more complicated. Nicotine has physiological and

pathological effects on various organ systems through 3 key mechanisms, namely ganglionic transmission, catecholamines mediated stimulation of nicotinic acetylcholine receptors (nAChRs) on chromaffin cells, and central nervous system (CNS) stimulation on nAChRsGTS (Mishra *et al.*, 2015). However, the precise mechanism by which nicotine causes nausea, vomiting, diarrhea, and increased salivation is yet unknown (Alkam and Nabeshima, 2019). The use of biomarkers helps prove the disease diagnosis and prognosis. As a result, combining clinical presentation with cotinine level assessment allows for more reliable estimations, avoiding other complicating clinical hypotheses, such as work-related intoxications. In Article 3 and Article 4, the assessment of cotinine levels taken from the reference makes the diagnosis of GTS more objective (Cezar-Vaz and Cargnin, 2019; Campos *et al.*, 2020). Sunlight exposure for 7 to 8 hours and greater than 8 hours was found to be positively linked with the occurrence of GTS, indicating a dose-response impact. This result can be explained by nicotine's hydrosoluble and liposoluble properties, as nicotine's skin absorption is aided by humidity and may be exacerbated in warmer conditions due to increased evaporation. Furthermore, heat exhaustion caused by excessive sunshine exposure might cause certain common GTS symptoms (Campos *et al.*, 2020).

The influence of gender as a risk factor for GTS is still questionable. Article 2 shows that GTS was more likely to be experienced by women, while Article 3 shows the opposite (Park *et al.*, 2018; Campos *et al.*, 2020). Some studies attribute this to a subjective reaction, suggesting that women are more likely to report symptoms experienced than men (Campos *et al.*, 2020). Some other studies attribute it to biological variations since women have a larger dermal area (body volume) for nicotine absorption (Cezar-Vaz and Cargnin, 2019)

The use of PPE has been shown to reduce the risk of GTS (Saleeon *et al.*, 2016; Cezar-Vaz and Cargnin, 2019). Article 1 shows that nicotine exposure that occurs during the harvest season is through inhaled tobacco dust in dry condition, while Articles 2, 3, and 4 show nicotine exposure through dermal contact in humid conditions. The high correlation between PPE and cotinine levels is consistent when farmers wear long sleeved shirts, gloves and masks (Saleeon *et al.*, 2016). Appropriate PPE, adjusted to the risk of dermal, oral, or inhalation nicotine entry, contributes greatly to reducing the incidence of GTS. For examples,

cotton gloves offer the least protection (78.5%), but are more comfortable and have a shorter lifespan, whereas rubber gloves provide 93% protection (Cezar-Vaz and Cargnin, 2019). This is a consideration in the selection of PPE; in addition to the effectiveness of protection, it must also consider convenience so that farmers comply with its use (Saleeon *et al.*, 2016)

CONCLUSION

In determining nicotine exposure, cotinine is the strongest biomarker. Since cotinine has a longer half-life in the body, it is superior to measuring nicotine. Urinary cotinine is still considered the best sample for measuring nicotine exposure. Urinary cotinine is a non-invasive test alternative to nicotine exposure. Increased urinary cotinine levels are associated with the occurrence of GTS in tobacco farmers. Salivary cotinine may be more acceptable for assessing exposure to nicotine when urine sample collection may be inconvenient in certain circumstances. In addition to the use of cotinine as a biomarker of nicotine exposure, the most important thing is prevention in the occurrence of GTS itself. The government needs to play a role in educating farmers about the importance of using an appropriate PPE to reduce the risk of contact with tobacco leaves.

ACKNOWLEDGEMENTS

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