Occurrence of green tobacco sickness and associated factors in farmers residing in Dom Feliciano Municipality, Rio Grande do Sul State, Southern Region of Brazil

Ocorrência da doença da folha verde do tabaco e fatores associados entre fumicultores no Município de Dom Feliciano, Rio Grande do Sul, Região Sul do Brasil

Ocurrencia de la enfermedad del tabaco verde y factores asociados en agricultores que residen en el Municipio de Dom Feliciano, Estado de Rio Grande do Sul, Región Sur de Brasil

Élida Campos [1]
Vanessa Indio-do-Brasil da Costa [1]
Sérgio Rabello Alves [2]
Ana Cristina Simões Rosa [2]
Bárbara Rodrigues Geraldino [1]
Beatriz da Cruz Meira [3]
Valéria Cunha [1]
Tânia Maria Cavalcante [4]
Silvana Rubano Turci [2]
Marcia Sarpa [1]
Ubirani Barros Otero [1]

Abstract

This study aimed to evaluate the occurrence of the green tobacco sickness (GTS) and its associated factors in tobacco familiar farmers residing in Dom Feliciano, Rio Grande do Sul State, Brazil. A cross-sectional study was conducted evaluating the sickness in 354 small tobacco farmers, between October 2011 and March 2012. The urinary concentration of cotinine, a biomarker of exposure to nicotine, was determined during the tobacco harvest period. Subjects presenting cotinine urinary levels ≥ 50ng/mL, that had contact with tobacco leaves up to 48 hours before the sample collection and reported at least one disease symptom were deemed as cases. A non-conditional logistic analysis was performed to evaluate the association between GTS and the population characteristics. A total of 122 (34.5%) cases were identified, with 39% of them being smokers and 61% being males. The median cotinine urinary concentrations were 75.6ng/mL (74.1ng/mg of creatinine) for non-cases and 755.8ng/mL (632.1ng/mg of creatinine) for the cases (p-value ≤ 0.01). The multivariate analysis showed a positive association between GTS and sunlight exposure time, exposure to pesticides, worse health status, and inverse association with wood cultivation. This study presented a high GTS prevalence and suggest that the use of urinary cotinine is a significant biomarker to determine GTS cases, influencing in the distribution by sex. Once the tobacco production involves health hazards, is important to implement measures to prevent the harm caused to tobacco farmers, as set in articles 17 and 18 of the World Health Organization Framework Convention on Tobacco Control.

Cotinine; Nicotine; Tobacco Industry

Correspondence
E. Campos
Área Técnica Ambiente, Trabalho e Câncer, Coordenação de Prevenção e Vigilância, Instituto Nacional de Câncer José Alencar Gomes da Silva.
Rua Marquês do Pombal 125, 5º andar, Rio de Janeiro, RJ 20230-240, Brasil.
elidacamp@gmail.com

[1] Coordenação de Prevenção e Vigilância, Instituto Nacional de Câncer José Alencar Gomes da Silva, Rio de Janeiro, Brasil.
[2] Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, Rio de Janeiro, Brasil.
[3] Secretaria da Saúde do Estado do Rio Grande do Sul, Porto Alegre, Brasil.
[4] Secretaria Executiva, Instituto Nacional de Câncer José Alencar Gomes da Silva, Rio de Janeiro, Brasil.

Introduction

The exposure to large quantities of nicotine coming from tobacco farming may cause the green tobacco sickness (GTS), a condition characterized by an acute toxicity caused by the dermal absorption of nicotine [1]. The GTS occurs due to a stimulation or inhibition of receptors in the central nervous system [2], resulting in headache, dizziness, nausea, vomiting, weakness, and abdominal pain [1,2]. The GTS symptoms can be easily confused with ones of other causes, such as toxicity by pesticides [3]. Thus, for a GTS diagnosis, it is necessary that the subject had contact with a green tobacco leaf and presented high nicotine levels in the body [1].

The GTS is a significant cause of sickness among rural workers that cultivate tobacco [4]. Epidemiological studies conducted with tobacco-farming workers in different countries (such as Thailand, India, and the United States) have shown different GTS prevalence, ranging from 8% to 47% [5,6,7]. In Brazil, a study performed in São Lourenço do Sul, Rio Grande Sul State, reported a GTS prevalence of 7% in males and 12% in females [8].

The occurrence of GTS presents a special significance in the national scenario, as Brazil is the main exporter and the second larger producer of tobacco in the world [9]. In such context, the Southern region is responsible for most of the tobacco culture in Brazil, concentrating more than 90% of the production [10]. Thus, considering the importance of tobacco culture in the country, understanding the features of the farmer populations that cultivate tobacco and are associated to this disease is pivotal to implement strategies for preventing and mitigating possible hazards. In such context, it is stressed that the Brazilian government ratified the first public health treaty in the world in 2005: the World Health Organization Framework Convention on Tobacco Control (WHO-FCTC), already in effect in 181 countries. The WHO-FCTC aims to "*protect the present and future generations against the devastating sanitary, social, environmental, and economic consequences generated by the consumption and exposure to tobacco smoke*" and, in its 38 articles, it recommends that measures related to reduction in demand (Part III) and reduction in tobacco offer (Part IV) are adopted. Articles 17 and 18 are highlighted, proposing the adoption of measures to support economically feasible alternate activities and recommending the protection of the environment and the health of persons involved in tobacco planting, respectively [11]. Therefore, in consonance with the WHO-FCTC objectives, this study aims to evaluate the occurrence of GTS and the associated factors in tobacco-producing farmers residing in Dom Feliciano, Rio Grande do Sul State, the thirteenth largest tobacco-producing municipality in the country [12].

Methodology

Study population

This study was conducted from the research entitled *Health and Sustainable Development in the Familiar Agriculture in Tobacco-Producing Regions*, whose objective was to evaluate the health profile in the population residing in Dom Feliciano Municipality and the main risk factors for cancer. Tobacco-producing familiar farmers, residing in Dom Feliciano Municipality, from both sexes, adults (over 18 years old), able to understand and answer to the questionnaires, and speaking Portuguese were selected from such populational inquiry for the present study.

Dom Feliciano has a total of 2,252 tobacco farmers [9], and 354 among them were selected to take part in the survey. Such sample size was calculated considering the prevalence of 0.184 for toxicity by the nicotine present in tobacco leaves [3], with an admissible error of 0.05 and confidence level of 0.95, resulting in a minimum size of 210 subjects.

Data collection

The interviews were structured as medical history and were performed by trained health agents and local supervisors of the team of the Municipal Health Secretariat of Dom Feliciano Municipality. The participants were questioned in two steps: (1) tobacco crop planting, aiming to identify toxicity conditions by pesticides; and (2) tobacco harvest, which is suggested as being the time when

there is the largest exposure to nicotine by requiring larger contact with tobacco leaves. The first step was performed between August and October 2011 and the second step between January and February 2012.

An individual questionnaire and a clinical questionnaire were applied to obtain general information and information on acute toxicity and exposure to pesticides, respectively. Both questionnaires were applied on the first step, and only the clinical questionnaire was applied on the second step. However, in this study, we only use the data from the clinical questionnaire of the second step, as the tobacco harvest step offers larges exposure of the worker to nicotine and smaller contact with pesticides, which toxicity conditions present similarities. In order to determine the food insecurity condition, the Brazilian Scale of Food Insecurity [13] was included in the general information questionnaire. In this study, the variable food insecurity was treated as being dichotomous, with a score equal to zero being classified as food safety and a scorer higher than or equal to one being classified as food insecurity. Alcoholism was determined from the four questions that compose the CAGE questionnaire [14], also included in the general evaluation.

Subjects presenting quantifiable cotinine urinary levels ($\geq$ 50ng/mL), in contact with tobacco leaves in up to 48 hours before the sample collection, and that reported at least one disease symptom (headache, dizziness, nausea, vomiting, weakness, and bellyache) were considered as being GTS cases. The other variables evaluated in this study are described in Tables 1 and 2.

Table 1

Features of the study population. Dom Feliciano Municipality, Rio Grande do Sul State, Brazil (N = 354).

	Total n (%)	Non-cases n (%)	Cases * n (%)
Demographic variables			
Sex			
Male	200 (56.5)	126 (54.3)	74 (60.7)
Female	154 (43.5)	106 (45.7)	48 (39.3)
Age at evaluation (years)			
$\leq$ 25	53 (15.1)	31 (13.4)	22 (18.2)
26 |-| 35	81 (23.0)	48 (20.8)	33 (27.3)
36 |-| 45	105 (29.8)	71 (30.7)	34 (28.1)
46 |-| 55	61 (17.3)	37 (16.0)	24 (19.8)
56 |-| 65	31 (8.8)	25 (10.8)	6 (5.0)
> 65	21 (6.0)	**19 (8.2)**	**2 (1.7)**
Skin color			
Non-white	58 (16.4)	35 (15.1)	23 (19.0)
White	295 (83.6)	197 (84.9)	98 (81.0)
Socioeconomic variables			
Educational level			
High school or higher education	42 (12.5)	25 (11.4)	17 (14.7)
Up to elementary school	293 (87.5)	194 (88.6)	99 (85.3)
Food security			
Yes	271 (78.8)	184 (81.4)	87 (73.7)
No	73 (21.2)	42 (18.6)	31 (26.3)

(continues)

Table 1 (continued)

	Total n (%)	Non-cases n (%)	Cases * n (%)
Health condition variables			
Health status			
Good or very good	147 (41.5)	104 (44.8)	43 (35.2)
Regular	175 (49.4)	106 (45.7)	69 (56.6)
Poor or very poor	32 (9.0)	22 (9.5)	10 (8.2)
Smoking			
Never smoked	122 (34.5)	81 (34.9)	41 (33.6)
Ex-smoker	48 (13.6)	**39 (16.8)**	**9 (7.4)**
Passive smoker	67 (18.9)	42 (18.1)	25 (20.5)
Active smoker	117 (33.1)	70 (30.2)	47 (38.5)
Age when began to smoke (years)			
> 15	85 (54.1)	53 (52.5)	32 (57.1)
≤ 15	72 (45.9)	48 (47.5)	24 (42.9)
Abusive use of alcohol			
No	199 (79.9)	94 (86.2)	50 (78.1)
Yes	50 (20.1)	15 (13.8)	14 (21.9)
Age when began to consume alcohol (years old)			
> 15	161 (59.9)	106 (59.6)	55 (60.4)
≤ 15	108 (40.1)	72 (40.4)	36 (39.6)
BMI			
Light or normal weight (≤ 24.9kg/m^2)	174 (50.1)	118 (53.9)	65 (58.6)
Overweight (from 25.0 to 29.9kg/m^2)	118 (34.0)	74 (33.8)	32 (28.8)
Obesity (≥ 30.0kg/m^2)	55 (15.9)	27 (12.3)	14 (12.6)
Sunlight exposure (times/week)			
≤ 2	7 (2.0)	6 (2.6)	1 (0.8)
3-6	31 (8.8)	21 (9.1)	10 (8.2)
Daily	316 (89.3)	205 (88.4)	111 (91.0)
Time of sunlight exposure (hours)			
≤ 6	96 (27.3)	**77 (33.3)**	**19 (15.7)**
7-8	175 (49.7)	**105 (45.5)**	**70 (57.9)**
> 8	81 (23.0)	49 (21.2)	32 (26.4)
Use of sunscreen or sun protector			
Always	27 (7.6)	16 (6.9)	11 (9.0)
Sometimes	36 (10.2)	24 (10.3)	13 (10.7)
No	7 (2.0)	192 (82.8)	98 (80.3)
Use of hat or cap			
Always	311 (87.9)	204 (87.9)	107 (87.7)
Sometimes	36 (10.2)	22 (9.5)	14 (11.5)
No	7 (2.0)	6 (2.6)	1 (0.8)
Use of long-sleeved shirt			
Always	167 (47.2)	107 (46.1)	60 (49.2)
Sometimes	101 (28.5)	69 (29.7)	32 (26.2)
No	86 (24.3)	56 (24.1)	30 (24.6)
Use of long pants			
Always	192 (54.2)	127 (54.7)	65 (53.3)
Sometimes	106 (29.9)	69 (29.7)	37 (30.3)
No	56 (15.8)	36 (15.5)	20 (16.4)

BMI: body mass index.

* Comparison between cases and non-cases: chi-square or Fisher test (bold: p-value ≤ 0.05).

Table 2

Agricultural practice of the study population. Dom Feliciano Municipality, Rio Grande do Sul State, Brazil (N = 354).

	Total n (%)	Non-cases n (%)	Cases * n (%)
Time of work with tobacco crop (years)			
≤ 10	82 (23.3)	54 (23.5)	28 (23.0)
> 10	270 (76.7)	176 (76.5)	94 (77.0)
Manual cleansing			
No	70 (20.1)	41 (18.1)	29 (24.0)
Yes	278 (79.9)	186 (81.9)	92 (76.0)
Chemical cleansing			
No	233 (67.0)	150 (66.1)	83 (68.6)
Yes	115 (33.0)	77 (33.9)	38 (31.4)
Mechanical cleansing			
No	214 (61.5)	143 (63.0)	71 (58.7)
Yes	134 (38.5)	84 (37.0)	50 (41.3)
Uses manual stove			
No	44 (12.5)	28 (12.2)	16 (13.1)
Yes	308 (87.5)	202 (87.8)	106 (86.9)
Uses electric stove			
No	259 (73.6)	175 (76.1)	84 (68.9)
Yes	93 (26.4)	55 (23.9)	38 (31.1)
Uses manual and electric stove			
No	303 (86.1)	203 (88.3)	100 (82.0)
Yes	49 (13.9)	27 (11.7)	22 (18.0)
Skin injuries at the tobacco harvest			
No	295 (90.8)	183 (90.1)	112 (91.8)
Yes	30 (9.2)	20 (9.9)	10 (8.2)
Tobacco harvest with humid/wet clothes			
No	128 (36.6)	87 (38.2)	41 (33.6)
Yes	222 (63.4)	141 (61.8)	81 (66.4)
Time of harvest of tobacco leaves			
Morning or afternoon	13 (3.7)	9 (4.0)	4 (3.3)
Morning and afternoon	335 (96.3)	217 (96.0)	118 (96.7)
Use of full PPE when working with tobacco **			
Yes	45 (12.7)	29 (12.6)	16 (13.1)
No	308 (87.3)	202 (87.4)	106 (86.9)
Got sick in up to 3 days after the tobacco harvest			
No	235 (67.0)	161 (70.3)	74 (60.7)
Yes	116 (33.0)	68 (29.7)	48 (39.3)
Bean cultivation			
No	38 (10.7)	**31 (13.4)**	**7 (5.4)**
Yes	316 (89.3)	**201 (86.6)**	**115 (94.3)**
Corn cultivation			
No	19 (5.4)	12 (5.2)	7 (5.7)
Yes	335 (94.6)	220 (94.8)	115 (94.3)
Cultivation of vegetables, fruits, and legumes			
No	30 (8.5)	23 (9.9)	7 (5.8)
Yes	323 (91.5)	209 (90.1)	114 (94.2)

(continues)

Table 2 (continued)

	Total n (%)	Non-cases n (%)	Cases * n (%)
Wood cultivation			
No	175 (49.6)	106 (45.9)	69 (56.6)
Yes	178 (50.4)	125 (54.1)	53 (43.4)
Rice cultivation			
No	343 (98.0)	225 (98.3)	118 (97.5)
Yes	7 (2.0)	4 (1.7)	3 (2.5)
Exposure to pesticides			
No	45 (12.7)	29 (12.5)	16 (13.1)
Yes	309 (87.3)	203 (87.5)	106 (86.9)
Age when the contact with pesticides began (years)			
> 15	212 (70.4)	144 (73.5)	68 (64.8)
≤ 15	89 (29.6)	52 (26.5)	37 (35.2)
Got sick after applying/working with pesticides			
No	208 (69.8)	140 (72.2)	68 (65.4)
Yes	90 (30.2)	54 (27.8)	36 (34.6)
Use of full PPE when applying pesticides ***			
No	246 (85.4)	165 (86.4)	81 (83.5)
Yes	42 (14.6)	26 (13.6)	16 (16.5)

PPE: personal protective equipment.

* Comparison between cases and non-cases: chi-square or Fisher test (bold: p-value ≤ 0.05);

** Joint wear of gloves, overall, or long-sleeved shirt and pants, boots, and coat;

*** Joint wear of gloves, overall, or long-sleeved shirt and pants, boots, and mask.

Biological analysis

• Standards, reagents, and quality policy

Negative calibrator, 0ng/mL (6626493/2012-05); negative control, 50ng/mL (6623399/2012-11); cutoff calibrator, 500ng/mL (6623400/2012-11), positive control, 5,000ng/mL (6623401/2012-11); plate (6625998/2012-08); enzyme conjugate (662497/2012-08); substrate reagent (6614535/2013-02); stopping reagent (6625819/2013-05). All supplies for the enzyme immunoassay (EIA) were purchased from OraSure Technologies, Inc. (Bethlehem, USA) using a kit (662498/2012-05).

The colorimetric creatine kit, model K016 with standard, alkaline, and acid reagents and picric acid (0158/2013-11) were purchased from Bioclin Ltda. (Belo Horizonte, Brazil). Due to a laboratory quality policy (accreditation by the Joint International Commission in 2012) and to the initial start procedures for adopting the ABNT NBR ISO/IEC 17025:(2005), all precision instruments (e.g., glassware, automatic pipettes, spectrophotometer, etc.) were previously calibrated and certified.

• Used instruments

Microplate reader able to read at dual wavelength of 450nm and 630nm, model Asys – Expert Plus, purchased from Biochrom Ltd. (London, UK); microplate washer, model Stat Fax – 2600, purchased from Awareness Technology, Inc. (Palm City, USA); UV-Visible spectrophotometer, model UV-1601, purchased from Shimadzu Co (Kyoto, Japan).

- **Analytical method principle and procedure to determine cotinine urinary levels**

The EIA for cotinine is a competitive microplate immunoassay for qualitative and semiquantitative determination of such compound in urine samples. Reagents and samples were brought to room temperature (20ºC±2ºC) before the analysis. Ten microliters of sample, calibrator, or control were added to each well test and properly labeled. One hundred microliters of enzyme conjugate were transferred to each testing well of the plate, and incubation was performed for 30 minutes at room temperature (20ºC±2ºC) in the dark. Each well was washed six times with 300 microliters of distilled water by using a suitable plate washer. One hundred microliters of substrate reagent were added to each well and were incubated at room temperature for 30 minutes in the dark (20ºC±2ºC). One hundred microliters of stopping reagent were transferred to each well. The absorbance was measured at a dual wavelength of 450nm and 630nm within 30 minutes since the reaction was stopped. For the quantification procedure, curve calibrators with different cotinine concentrations (n = 3, in duplicate) were prepared by plotting the negative absorbance values of raw absorbance data of cotinine observed in negative, low, medium and high calibrators against such metabolite analyte concentrations. The positive absorbance values of samples in such standard curve and the final concentrations were compared. The assay sensitivity was 50ng/mL for quantification (LOQ). No specificity was found in cross-reactivity to 16 compounds of toxicological interest at 10,000ng/mL in preserved human urine [15].

- **Assay to determine creatinine urinary level**

Creatinine was measured in urine (250 microliters of sample volume) by final point colorimetry (modified Jaffe method) [16].

Statistical analysis

The descriptive analysis of the features of the study population was performed by frequency distribution and univariate analysis of categorical variables by using the chi-square test. Central trend and dispersion measurements were calculated for continuous variables. The normality of the continuous variables was evaluated by using the Kolmogorov-Smirnov test. Bivariate analyses stratified by gender and smoking were performed, using the non-parametric group comparison Mann-Whitney, Fisher tests, or chi-square, with Bonferroni post-hoc test. Cotinine levels under the quantification limit were considered as being zero.

Bivariate and multivariate non-conditional logistic regression analyses were performed to evaluate the associate factors (independent variables) and GTS occurrence (dependent variable). The associate variables were included in the multivariate model with the outcome in the bivariate analyses and a statistical significance level of p-value ≤ 0.20. According to a forward procedure, variables associated to the dependent variable at a significance level ≤ 0.05 were kept in the final model. The statistical analyses of the collected data were performed using the software SPSS for Windows version 17.0 (https://www.ibm.com/).

This study was approved by the Ethics Research Committe of the José Alencar Gomes da Silva National Cancer Institute (n. 120/09) and all subjects that accepted to participate in the survey signed the Free Informed Consent Term.

Results

The study population was composed by 354 subjects, which 57% being males, 84% having declared to be white, and 68% being up to 45 years old (median = 39 years old; range = 18-87 years old). Among the participants who declared educational level, less than 15% studied in high school or had higher education, 21% did not have food safety, half of the population declared its health status as regular and was identified as overweight or obese. Active smoking was reported by 33% of the population, and 20% presented abusive alcohol consumption. Most population reported daily sunlight exposure of seven or more hours of exposure daily. However, continuous use of sunscreen was reported by

only 8% of the farmers. The frequencies of regular wear of others protection forms, such as hat or cap, long-sleeved shirt, and long pants were of 88%, 47%, and 54%, respectively (Table 1).

Only two farmers produced Burley-type tobacco and more than 90% of the participants reported to perform tasks in all tobacco culture steps (seedbed preparation = 96%; soil preparation = 90%; tobacco planting = 97%; tobacco crop care = 96%; tobacco harvest = 98%; drying of tobacco leaves = 92%; classification of tobacco leaves = 98%; crop cleansing = 98%). Table 2 presents the agricultural characteristics of the population. Only 13% reported being wearing full PPE (personal protective equipment) when working. Over three quarters of the population had cultivated tobacco for over ten years. The cleaning of the crop was done manually by the most farmers, while only between 30-40%, approximately, used chemical and mechanical techniques. The use of manual and electric stove was reported by 88% and 26% of the farmers, respectively. More than half of the participants wore humid clothes by the time of the harvest, which mostly occurred in the morning and afternoon times (96%). Skin injuries by the time of the tobacco harvest were reported by 9% of the farmers and one third of them reported to having gotten sick in up to three days after the tobacco harvest. Cultivation of bean (89%), corn (95%), vegetables, fruits, and legumes (92%), wood (50%), and rice (2%) were present besides tobacco. Referring to pesticides, 87% reported to use such chemical compounds, with 30% of them reporting having started their use as early as the age of 15. Only 15% of the pesticide users reported to having using full PPE, and 30% got sick after the application of such compounds.

Among the 354 participants of the study, 122 were identified as being GTS cases and, among these latter, 60.7% were males and 61.5% were non-smokers. The median cotinine urinary level in the population was 299ng/mg of creatinine (336ng/mL), and the GTS cases had a median concentration of 632ng/mg of creatinine (756ng/mL) (Table 3). When stratified by smoking and sex, higher cotinine levels were seen in smokers when compared to non-smokers (cases: 1,343 vs. 316ng/mg of creatinine, p-value ≤ 0.05) and in males than in females (cases: 655 vs. 609ng/mg of creatinine, p-value = 0.86), although the difference was not statistically significant between sexes. The observed prevalence of GTS was 34.5%. Cotinine levels above 50ng/mL and recent contact with tobacco leaves were observed in 69% and 65% of the participants, respectively. The most frequent characteristic GTS symptom was headache (52), followed by dizziness (31), weakness (27), nausea (21), bellyache (20), and vomiting (10) (Table 4).

A difference between cases and non-cases was observed for the variables age, smoking, time of sunlight exposure, and bean cultivation (Tables 1 and 2). Higher educational level, as well as higher frequencies of former smokers, manual crop cleansing, and wood cultivation, were seen for males in the stratified bivariate analysis by sex (data not presented in a table). Furthermore, getting sick during or up to three days after the harvest presented an association to the disease among males. Referring to females, the cases were younger and presented higher time of sunlight exposure. An association between GTS and bean cultivation, skin contact with tobacco leaves, and exposure to pesticides was also seen for females. Considering smoking, both smoking and non-smoking subjects, the non-cases were older. Other GTS associated variables among non-smokers were time of sunlight exposure, bean cultivation, and exposure to pesticides. Lower educational level and higher frequency of subjects who presented abusive alcohol use were seen among the cases in smokers.

Table 5 shows the result of the final multivariate model, simultaneously adjusted for time of sunlight exposure, current exposure to pesticides, health status and wood cultivation. The analysis showed a statistically significant positive association between GTS and sunlight exposure (7-8 hours: OR = 2.26; 95%CI: 1.16-4.40 / > 8 hours: OR = 2.39; 95%CI: 1.14-5.03). Subjects with GTS presented a chance of exposure to pesticides almost 4 times higher (95%CI: 1.43-9.25), as well as a chance of reporting their health status as being regular twice higher (95%CI: 1.17-3.38), when compared to non-cases. However, a lower chance of wood cultivation was seen among the cases (OR = 0.60; 95%CI: 0.37-1.00) when compared to GTS non-cases (Table 5).

Table 3

Cotinine urinary levels in the study population. Dom Feliciano Municipality, Rio Grande do Sul State, Brazil.

	n *	Mean (SD)	Minimum	P25	P50	P75	Maximum
Total							
ng/mL	354	726 (954)	< LOQ	< LOQ	336	1,215	5,849
ng/mg of creatinine		961 (2,337)	< LOQ	< LOQ	299	1,114	31,560
**Non-cases **							
Total population	232						
ng/mL		533 (800)	< LOQ	< LOQ	76	1,027	5,375
ng/mg of creatinine		816 (2,433)	< LOQ	< LOQ	74	938	31,560
Males **	126						
ng/mL		685 (817)	< LOQ	< LOQ	417	1,246	5,375
ng/mg of creatinine		868 (1,476)	< LOQ	< LOQ	325	1,133	9,963
Females	106						
ng/mL		353 (744)	< LOQ	< LOQ	< LOQ	292	3,931
ng/mg of creatinine		754 (3,229)	< LOQ	< LOQ	< LOQ	296	31,560
Non-smokers **	162						
ng/mL		189 (435)	< LOQ	< LOQ	< LOQ	161	2,619
ng/mg of creatinine		164 (345)	< LOQ	< LOQ	< LOQ	169	1,846
Smokers	70						
ng/mL		1,330 (882)	< LOQ	809	1,315	1,747	5,375
ng/mg of creatinine		2,325 (4,029)	< LOQ	710	1,358	2,399	31,560
Cases							
Total population	122						
ng/mL		1,093 (1,109)	50	279	756	1,655	5,849
ng/mg of creatinine		1,236 (2,125)	47	246	632	1,345	19,048
Males **	74						
ng/mL		1,017 (845)	90	298	888	1,659	4,086
ng/mg of creatinine		1,079 (1,402)	47	228	655	1,311	8,939
Females	48						
ng/mL		1,211 (1,425)	50	243	646	1,618	5,849
ng/mg of creatinine		1,477 (2,911)	64	282	609	1,509	19,048
Non-smokers **	75						
ng/mL		557 (509)	50	175	420	771	2,310
ng/mg of creatinine		604 (772)	47	163	316	750	4,768
Smokers	47						
ng/mL		1,949 (1,264)	96	1,180	1,692	2,021	5,849
ng/mg of creatinine		2,244 (3,038)	171	731	1,343	2,645	19,048

LOQ: quantification limit; P: percentile; SD: standard deviation.

* Percentile above the quantification limit: total population = 68.9%; non-cases – total population = 52.3%; males = 65.9%; females = 36.8%; non-smokers = 34%; smokers = 95.7%;

** Mann-Whitney test statistically significant (p-value ≤ 0.05): non-cases vs. cases (total population); males vs. females (non-cases); non-smokers vs. smokers (non-cases and cases).

Table 4

Traits related to green tobacco sickness (GTS) in the study population. Dom Feliciano Municipality, Rio Grande do Sul State, Brazil.

	Total	Males		Females		Non-smokers		Smokers	
		Non-cases	Cases	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Cotinine urinary levels (ng/mL)									
≤ 50	110 (31.1)	43 (34.1)	-	67 (63.2)	-	107 (66.0)	-	3 (4.3)	-
> 50	244 (68.9)	83 (65.9)	74 (100.0)	39 (36.8)	48 (100.0)	55 (34.0)	75 (100.0)	67 (95.7)	47 (100.0)
Contact with tobacco leaves in the last 24/48 hours									
No	123 (34.7)	54 (42.9)	-	69 (65.1)	-	85 (52.5)	-	38 (54.3)	-
Yes	231 (65.3)	72 (57.1)	74 (100.0)	37 (34.9)	48 (100.0)	77 (47.5)	75 (100.0)	32 (45.7)	47 (100.0)
GTS symptoms									
Headache									
No	170 (48.0)	91 (72.2)	20 (27.0)	51 (48.1)	8 (16.7)	94 (58.0)	17 (22.7)	48 (68.6)	11 (23.4)
Yes	184 (52.0)	35 (27.8)	54 (73.0)	55 (51.9)	40 (83.3)	68 (42.0)	58 (77.3)	22 (31.4)	36 (76.6)
Dizziness									
No	244 (68.9)	103 (81.7)	46 (62.2)	70 (66.0)	25 (52.1)	114 (70.4)	47 (62.7)	59 (84.3)	24 (51.1)
Yes	110 (31.1)	23 (18.3)	28 (37.8)	36 (34.0)	23 (47.9)	48 (29.6)	28 (37.3)	11 (15.7)	23 (48.9)
Nausea									
No	280 (79.1)	116 (92.1)	50 (67.6)	80 (75.5)	34 (70.8)	132 (81.5)	47 (62.7)	64 (91.4)	37 (78.7)
Yes	74 (20.9)	10 (7.9)	24 (32.4)	26 (24.5)	14 (29.2)	30 (18.5)	28 (37.3)	6 (8.6)	10 (21.3)
Vomiting									
No	318 (89.8)	120 (95.2)	57 (77.0)	96 (90.6)	45 (93.8)	149 (92.0)	65 (86.7)	67 (95.7)	37 (78.7)
Yes	36 (10.2)	6 (4.8)	17 (23.0)	10 (9.4)	3 (6.3)	13 (8.0)	10 (13.3)	3 (4.3)	10 (21.3)
Weakness									
No	260 (73.4)	105 (83.3)	53 (71.6)	73 (68.9)	29 (60.4)	120 (74.1)	51 (68.0)	58 (82.9)	31 (66.0)
Yes	94 (26.6)	21 (16.7)	21 (28.4)	33 (31.1)	19 (39.6)	42 (25.9)	24 (32.0)	12 (17.1)	16 (34.0)
Bellyache									
No	282 (79.7)	116 (92.1)	58 (78.4)	72 (67.9)	36 (75.0)	128 (79.0)	57 (76.0)	60 (85.7)	37 (78.7)
Yes	72 (20.3)	10 (7.9)	16 (21.6)	34 (32.1)	12 (25.0)	34 (21.0)	18 (24.0)	10 (14.3)	10 (21.3)

Table 5

Multivariate analysis between green tobacco sickness and associated factors in the study population. Dom Feliciano Municipality, Rio Grande do Sul State, Brazil (n = 301).

	OR [Ref.: no]	95%CI	p-value
Time of sunlight exposure (hours) [Ref.: ≤ 6 hours]			
7-8	2.26	1.159-4.404	**0.02**
> 8	2.39	1.136-5.028	**0.02**
Current exposure to pesticides [Ref.: no]	3.64	1.433-9.251	**0.01**
Health status [Ref.: good or very good]			
Regular	1.99	1.173-3.376	**0.01**
Poor or very poor	1.06	0.394-2.847	0.91
Wood cultivation [Ref.: no]	0.60	0.366-0.998	**0.05**
Constant	0.08		0.00

95%CI: 95% confidence interval; OR: odds ratio; Ref.: reference category.
Notes: model simultaneously adjusted by time of sunlight exposure, current exposure to pesticides, health status and wood cultivation; bold: p-value ≤ 0.05.

Discussion

GTS occurrence was observed in the current study in 34.5% of the population, with a median cotinine equal to 756ng/mL and 76ng/mL (632ng/mg and 74ng/mg of creatinine) in cases and non-cases, respectively. Sunlight exposure, use of pesticides, as well as regular health status were positively associated to GTS, while wood cultivation presented a negative association to the disease occurrence.

Studies conducted in several countries have reported different GTS occurrence frequencies, which varied higher or lower when compared to the ones seen in the present study. A sectional study conducted in Thailand, where only the presence of symptoms was considered to determine GTS, verified a case prevalence of 23%, being 18% in males and 26% in females [7]. In India, a study conducted with tobacco farmers and non-tobacco farmers reported a GTS prevalence of 47% in the farmers who cultivated tobacco [6]. When stratified by sex, a higher disease frequency was seen in females (56%) than in males (43%) [6]. Considering the toxicity symptoms and the time of work with tobacco, American studies reported GTS occurrence frequencies from 2% to 18%, varying due to the year period [3,5,17]. A disease prevalence from up to 10% and 16% in males and females, respectively, was identified in a Brazilian study that considered subjects who manifested tobacco toxicity symptoms in up to two days after the tobacco harvest as being GTS cases [8]. The observed range of GTS frequencies could be attributed to the different methodologies used by such studies, as well as to the different traits of the tobacco productive processes in the different locations, according to the type of growing tobacco [3,5,6,7,8,17].

Contrarily to the results found in other studies [6,7,8], a slightly higher GTS prevalence in Dom Feliciano was seen in males (37%) instead of females (31%). However, the results seen in the present study are similar to the ones in the other two national studies that measured the cotinine urinary levels [10,18]. These results suggest that not using such parameter to define GTS cases may bias the results, as females could have more facility in reporting the symptoms more precisely when compared to males [8], even with lower exposure [18].

The cotinine can be determined in different biological matrices (e.g., blood, saliva, and urine) [19]. Though the Brazilian Ministry of Health sets cotinine levels as one of the parameters to define GTS cases, many studies do not analyze such nicotine metabolite, only considering the symptoms and the time of exposure to tobacco leaves to determine the disease cases [3,5,6,7,8,17]. A cross-sectional study with tobacco farmers in Rio Grande do Sul found no difference in urinary cotinine levels between GTS symptomatic and asymptomatic subjects, suggesting that cotinine is not an indicator of nicotine poisoning [20]. However, is important to consider the cotinine level since the symptoms of GTS are nonspecific [3] and that this nicotine metabolite is one of the parameters of the case definition of GTS [1]. The population of Dom Feliciano presented high cotinine levels (cases = 1,093ng/mL; non-cases = 533ng/mL) when compared to other studies that evaluated such metabolite in urine [10,18]. A case-control study conducted in Rio Grande do Sul reported a mean cotinine urinary concentration of 432ng/mL and 353ng/mL for GTS cases and controls, respectively [10]. Stratifying by smoking, cotinine levels of 742ng/mL in cases and 821ng/mL in controls smokers, and 255ng/mL and 68ng/mL for cases and controls non-smokers, respectively [10], were identified. Other Brazilian study conducted in Arapiraca, Alagoas State, identified median cotinine urinary levels of 811ng/mL in GTS cases and 1,293ng/mL in controls in smokers, while levels of 288ng/mL and 156ng/mL were detected in cases and controls, respectively, for non-smokers [18]. Considering the significance of the kidney function in the cotinine urinary concentration [19], the levels of such metabolite were presented as adjusted and non-adjusted by creatinine in the present study. However, the studies do not perform this correction in general, which makes the comparison of the found results impossible.

A sunlight exposure for a period from 7 to 8 hours and longer than 8 hours were positively associated to GTS occurrence, being suggestive of dose-response effect. This result can be explained by the hydrosoluble and liposoluble features of nicotine [3,21], as the dermal absorption of nicotine is facilitated by humidity [18] and is possibly aggravated in hot environments due to higher transpiration. Furthermore, some typical GTS symptoms can be also caused by exhaustion by heat cause by an exaggerated sunlight exposure [4]. Likewise, an acute toxicity by pesticides, substances widely used by tobacco farmers (87%), has shown strong association to the GTS occurrence. A recent study by the same research group [22], it was verified that the tobacco farmers in Dom Feliciano used insecticides

(pyrethroids, organophosphates), herbicides (dinitroaniline, isoxazolidinone, substituted glycine), and fungicides (dicarboximide) that can present clinical manifestations similar to the GTS ones [23], which could justify the association found for such environmental contaminant [4]. In such aspect, it is important to highlight the association verified in the bivariate analysis between bean cultivation and GTS. Bean cultivation presents a released use of 134 pesticides from 57 different chemical groups, with pyrethroids, triazoles, and organophosphates being highlighted among such groups [24], and which muscarinic manifestations (such as colic and vomiting) are similar to the GTS symptoms [25].

The perception of the health condition of a subject as being regular also presented a positive association to GTS, while the health status reported as being poor or very poor did not present any statistically significant association. Understanding the health perception from physical and subjective dimensions and the capacity for working composes a significant factor to refer the health status [26]. Thus, the observed results could be explained by the higher susceptibility of those who present debilitated health, but are able to work, as subjects that perceive their health as poor or very poor could perform the field activities less intensively, being less exposed to the tobacco leaves. Referring to the inverse association verified for wood cultivation, this could result from a smaller farmer's dependence on the tobacco crop due to the production of wood, which would protect the farmer against the harmful effects of the exposure to tobacco leaves. Similar results were seen by Fassa et al. [8] for dairy production in a study conducted in farmers in São Lourenço do Sul, Rio Grande do Sul State. Thus, it is highlighted that WHO-FCTC prescribed a diversification of crops in tobacco-producing areas, aiming a gradual substitution of the smoke cultivation [11].

The memory bias from the data obtainment by a reminding report is a possible limitation in such study. However, the use of cotinine levels to set GTS cases is strongly highlighted, according to a recommendation from the Brazilian Ministry of Health [1], as well as the presentation of such metabolite levels adjusted by creatinine, a significant indicator of kidney function [19].

Conclusion

The tobacco-producing farming population in Dom Feliciano Municipality, in the Southern region of Brazil presented high GTS prevalence. The results suggest that the use of urinary cotinine is a significant biological marker to determine GTS cases, influencing in the disease prevalence distribution by sex. The study indicated an association between GTS and sunlight exposure, use of pesticides, health perception, and wood cultivation.

Brazil's position regarding tobacco production and export and the health hazards from the exposure to nicotine, as well as the intensive use of pesticides and other risk factors typical of tobacco cultivation (such as exhaustion and dependence on tobacco industry), evidence the significance of this theme for public health and the urge for implementing measures to prevent the harm caused to such workers, as set in Articles 17 and 18 of WHO-FCTC, which set alternatives for such crop in order to protect the health and the environment against its negative effects.

Contributors

E. Campos participated in the statistical analysis, interpretation of data, writing of the manuscript and approval of the final version. V. I.-B. Costa, V. Cunha, T. M. Cavalcante and M. Sarpa contributed in the critical review and approval of the final version. S. R. Alves, A. C. S. Rosa and B. C. Meira collaborated in the laboratory analysis, critical review and approval of the final version. B. R. Geraldino contributed in the interpretation of data, critical review and approval of the final version. S. R. Turci and U. B. Otero participated in the research conception, critical review and approval of the final version.

Additional informations

ORCID: Élida Campos (0000-0003-1604-8845); Vanessa Indio-do-Brasil da Costa (0000-0002-0360-143X); Sérgio Rabello Alves (0000-0002-4181-1163); Ana Cristina Simões Rosa (0000-0003-0547-1138); Bárbara Rodrigues Geraldino (0000-0002-7061-1574); Beatriz da Cruz Meira (0000-0001-7107-1242); Valéria Cunha (0000-0003-0944-3337); Tânia Maria Cavalcante (0000-0003-0866-4672); Silvana Rubano Turci (0000-0003-0811-9252); Marcia Sarpa (0000-0001-8976-4653); Ubirani Barros Otero (0000-0003-1464-2410).

Acknowledgments

References

1. Ministério da Saúde. Doença da folha verde do tabaco. http://bvsms.saude.gov.br/bvs/dicas/204_doenca_folha_verde.html (accessed on 27/Oct/2014).

2. Riquinho DL, Hennington EA. Cultivo do tabaco no sul do Brasil: doença da folha verde e outros agravos à saúde. Ciênc Saúde Colet 2014; 19:4797-808.

3. Arcury TA, Vallejos QM, Schulz MR, Feldman SR, Fleischer AB, Verma A, et al. Green tobacco sickness and skin integrity among migrant Latino farmworkers. Am J Ind Med 2008; 51:195-203.

4. Riquinho DL, Hennington EA. Health, environment and working conditions in tobacco cultivation: a review of the literature. Ciênc Saúde Colet 2012; 17:1587-600.

5. Arcury T, Quandt S, Preisser J. Predictors of incidence and prevalence of green tobacco sickness among Latino farmworkers in North Carolina, USA. J Epidemiol Community Health 2001; 55:818-24.

6. Parikh JR, Gokani VN, Doctor PB, Kulkarni PK, Shah AR, Saiyed HN. Acute and chronic health effects due to green tobacco exposure in agricultural workers. Am J Ind Med 2005; 47:494-9.

7. 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.

8. Fassa AG, Faria NMX, Meucci RD, Fiori NS, Miranda VI, Facchini LA. Green tobacco sickness among tobacco farmers in southern Brazil. Am J Ind Med 2014; 57:726-35.

9. Sindicato da Indústria do Tabaco. Brasil é líder mundial em exportação de tabaco. http://sinditabaco.com.br/sobre-o-setor/exportacoes/ (accessed on 24/Feb/2015).

10. Bartholomay P, Iser BPM, Oliveira PPV, Santos TEHH, Malta DC, Sobel J, et al. 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. Instituto Nacional de Câncer José Alencar Gomes da Silva; Organização Mundial da Saúde. Convenção-quadro para o controle do tabaco. Rio de Janeiro: Instituto Nacional de Câncer José Alencar Gomes da Silva; 2011.

12. Onde mais se produz tabaco no Sul Brasil? Portal do Tabaco 2016; 25 jan. http://portaldotabaco.com.br/onde-mais-se-produz-tabaco-no-sul-do-brasil/.

13. Segall-Corrêa AM, Escamilla RP, Maranha LK, Sampaio MFA. (In)segurança alimentar no Brasil: validação de metodologia para acompanhamento e avaliação. Relatório técnico. Campinas: Departamento de Medicina Preventiva e Social, Faculdade de Ciências Médicas, Universidade Estadual de Campinas; 2003.

14. Bernadt MW, Mumford J, Taylor C, Smith B, Murray RM. Comparison of questionnaire and laboratory tests in the detection of excessive drinking and alcoholism. Lancet 1982; 1:325-8.

15. Dhar P. Measuring tobacco smoke exposure: quantifying nicotine/cotinine concentration in biological samples by colorimetry, chromatography and immunoassay methods. J Pharm Biomed Anal 2004; 35:155-68.

16. Junge W, Wilke B, Halabi A, Klein G. Determination of reference intervals for serum creatinine, creatinine excretion and creatinine clearance with an enzymatic and a modified Jaffé method. Clin Chim Acta 2004; 344:137-48.

17. 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.

18. Oliveira PPV, Sihler CB, Moura L, Malta DC, Torres MCA, Lima SMCP, et al. First reported outbreak of green tobacco sickness in Brazil. Cad Saúde Pública 2010; 26:2263-9.

19. Malafatti L, Martins I. Aspectos analíticos da determinação de cotinina em matrizes biológicas. Rev Bras Toxicol 2009; 22:9-20.

20. Fassa AG, Meucci RD, Fiori NS, Carrett MLV, Faria NMX. Urinary cotinine in tobacco farmers in Southern Brazil. Rev Saúde Pública 2018; 52:70.

21. Arcury TA, Quandt SA. Health and social impacts of tobacco production. J Agromedicine 2006; 11:71-81.

22. Campos E, Silva VSP, Mello MSC, Otero UB. Exposure to pesticides and mental disorders in a rural population of Southern Brazil. Neurotoxicology 2016; 56:7-16.

23. Costa LG. Toxic effects of pesticides. In: Klaassen CD, Watkins III JB, editors. Casarett & Doull's essentials of toxicology. 2nd Ed. New York: McGraw-Hill; 2010. p. 309-22.

24. Ministério da Agricultura, Pecuária e Abastecimento. AGROFIT – Sistema de Agrotóxicos Fitossanitários. http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed on 20/Nov/2017).

25. King AM, Aaron CK. Organophosphate and carbamate poisoning. Emerg Med Clin North Am 2015; 33:133-51.

26. Riquinho DL, Gerhardt TE. A transitoriedade nos estados de saúde e doença: construção do cotidiano individual e coletivo em uma comunidade rural. Trab Educ Saúde 2010; 8:419-37.

Resumo

O estudo teve como objetivo avaliar a ocorrência de doença da folha verde (DFV) e fatores associados entre fumicultores familiares no Município de Dom Feliciano, Estado do Rio Grande do Sul, Brasil. Foi realizado um estudo transversal sobre DFV em 354 pequenos produtores de tabaco, entre outubro de 2011 e março de 2012. Foi medida a concentração urinária da cotinina, um biomarcador da exposição à nicotina, durante a temporada de colheita do tabaco. Os casos de DFV foram definidos como indivíduos com níveis urinários de cotinina ≥ 50ng/mL, que tiveram contato com folhas de tabaco em até 48 horas antes da coleta da amostra sanguínea e que relatavam pelo menos um sintoma da doença. Foi realizada uma análise logística não condicional para avaliar a associação entre DFV e as características da população. Foram identificados um total de 122 (34,5%) casos da doença, entre os quais 39% eram fumantes e 61% eram do sexo masculino. As concentrações urinárias medianas de cotinina foram 75,6ng/mL (74,1ng/mg de creatinina) para não-casos e 755,8ng/mL (632,1ng/mg de creatinina) para os casos (valor de p ≤ 0,01). A análise multivariada mostrou uma associação positiva entre DFV e tempo de exposição à luz solar, exposição a pesticidas e pior estado de saúde geral, e uma associação inversa com cultivo de lenha. O estudo identificou alta prevalência de DFV e sugere o uso da cotinina como biomarcador significativo para identificar os casos de DFV, influenciando a distribuição por sexo. Uma vez que a fumicultura envolve danos à saúde, é importante implementar medidas para prevenir os impactos para os fumicultores, de acordo com os artigos 17 e 18 da Convenção-Quadro para o Controle do Tabaco da Organização Mundial da Saúde.

Cotinina; Nicotina; Indústria do Tabaco

Resumen

Este estudio visó evaluar la ocurrencia de la enfermedad del tabaco verde (GTS por sus siglas en inglés) y sus factores asociados en pequeños agricultores de tabaco, que residen en Dom Feliciano, Rio Grande do Sul, Brasil. Se realizó un estudio transversal, evaluando la enfermedad del tabaco verde, en 354 pequeños agricultores de tabaco, entre octubre de 2011 y marzo 2012. La concentración urinaria de cotinina, un biomarcador de la exposición a la nicotina, se determinó durante el periodo de cosecha del tabaco. Los individuos que presentan niveles de cotinina urinarios ≥ 50ng/mL, que tuvieron contacto con hojas de tabaco hasta 48 horas antes de la recogida de la muestra, e informaron de al menos un síntoma de la enfermedad, fueron considerados casos. Se realizó un análisis logístico no condicional para evaluar la asociación entre GTS y las características de la población. Se identificaron un total de 122 (34,5%) casos, siendo un 39% de ellos fumadores y un 61% hombres. Las concentraciones medias de cotinina urinaria fueron 75,6ng/mL (74,1ng/mg de creatinina) para los no casos y 755,8ng/mL (632,1ng/mg de creatinina) para los casos (valor de p ≤ 0,01). El análisis multivariado mostró una asociación positiva entre el GTS y las horas de exposición a la luz solar, exposición a los pesticidas, un estatus peor de salud, y la asociación inversa con la cultura maderera. Este estudio presentó una alta prevalencia de GTS y sugiere que el uso de la cotinina urinaria es un biomarcador significativo para identificar casos de GTS, influenciando en la distribución por sexo. Debido a que la producción de tabaco implica perjuicios para la salud, es importante implementar medidas para prevenirlos en los agricultores del tabaco, como está estipulado en los artículos 17 y 18 del Convenio Marco para el Control del Tabaco de la Organización Mundial de la Salud.

Cotinina; Nicotina; Industria del Tabaco