Brief Report

Impact of eggplant consumption on urine cotinine examination results

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Abstract

Background and Objective: A urinary cotinine test is performed to assess the smoking status. Cotinine is a nicotinic metabolite. However, foodstuffs from the plant family *Solanaceae*, such as eggplant, contain trace amounts of nicotine, which may have affected the cotinine test results. This study investigated the impact of eggplant consumption on urine cotinine concentration tests.

Methods: Six young women who were healthy and non-smokers consumed meals devoid of *Solanaceous* ingredients for three days before consuming 300 grams of eggplant. After eating eggplants, the participants did not consume *Solanaceous* food throughout the study. During the study, participants' urine cotinine concentrations were sampled periodically using a urine nicotine and cotinine test kit.

Results: Four participants' urine tested positive for cotinine, whereas that of two participants tested negative. The time when a positive result was recorded after consuming eggplants varied among the individuals. Participants' urine cotinine concentrations differed as well.

Conclusions: Urine cotinine concentrations may increase after consuming *Solanaceous* foods, even in non-smokers. When assessing the smoking status, the influence of *Solanaceous* foods should be considered when measuring urine cotinine levels.

Keywords: Nicotine, Cotinine, *Solanaceae* foods, Urine, Smoking status

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I. Introduction

Cigarette smoking causes various respiratory and cardiovascular diseases[1,2]. Cigarette nicotine is one of three harmful substances, along with carbon monoxide(CO) and tar, and it is more addictive than alcohol or amphetamine[3]. Nicotine is metabolized to cotinine, mainly in the liver. Cotinine can be found in the blood, urine, and saliva[4]. Therefore, measuring urine cotinine concentration is useful for assessing smoking status.

Measurement of exhaled CO is conducted in smoking cessation clinics in Japan to confirm the smokers' smoking status. However, heat-not-burn tobacco, the use of which has spread rapidly in recent years, produces low amounts of CO because tobacco leaves are not burned. Therefore, the measurement of cotinine concentration in urine is highlighted as a novel method for assessing heat-not-burn smoking status.

Meanwhile, foodstuffs in the *Solanaceae* plant family, such as eggplants, tomatoes, and potatoes, contain nicotine[5,6], and their consumption can affect the measurement of cotinine concentrations in urine tests to assess smoking status. This study investigated the impact of eggplant consumption on urine cotinine concentration tests.

II. Participants and methods

1. Participants

We recruited six healthy, non-smoking female students aged 21–22 years from a women's university in Kyoto, Japan. Self-reported height and weight were recorded. Using a questionnaire (Figure 1)[7], we confirmed that the participants had not been exposed to second-hand smoke. Written informed consent was obtained from all participants.

2. Methods

Figure 2 shows the study design. From days 1 to 7, the participants consumed three designated meals (breakfast at 7 a.m., lunch at noon, and dinner at 6 p.m.) that did not contain *Solanaceous* ingredients (Tokatsu Foods Corporation, Japan), except for breakfast on day 1 and breakfast on day 4. On day 4, participants consumed 300 grams of eggplant at 9 a.m. Wrapped eggplant was heated in a microwave oven. A 300-gram serving of eggplant is considered to contain approximately 30 µg of nicotine, equivalent to the amount of nicotine when exposed to passive smoking[5].

The participants were also asked not to consume any snacks or

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	Questionnaire	Answer
Q1	Age	(　　　)
Q2	Sex	1. Male　2. Female 3. Other　4. Don't want to answer
Q3	Height, Weight	(　　　) cm, (　　　) kg
Q4	Do you smoke? (include heat-not-burn tobacco)	1. Yes →answer Q 5,6,7~ 2. No →answer Q 8~
Q5	If you answered the above question with yes, how long have you been smoking?	For (　　　) years
Q6	Which do you smoke?	1. Tobacco 2. Heat-not-burn tobacco 3. Both
Q7	How many cigarettes do you smoke?	Tobacco: (　　　) Heat-not-burn tobacco: (　　　)
Q8	How many people are in your family?	(　　　)
Q9	Do you have any disease in your liver and kidney?	Yes / No
Q10	Did you feel exposed to passive smoking in these three days?	Yes / No
Q11	Were smokers around you in these three days?	Yes Friend Family or roommate Coworker Other No
Q12	Did someone smoke in the following locations around you in these three days?	Yes Bathroom / Bedroom In a car Living or dining No
Q13	Did you go to a place with a lot of tobacco smoking in these three days?	Yes Pachinko parlor Japanese style pub Restaurant No
Q14	How many hours or days per week on average do you spend in places that smell like smoke?	(　　　) hours/day on average

Figure 1. Questionnaire about second-hand smoking

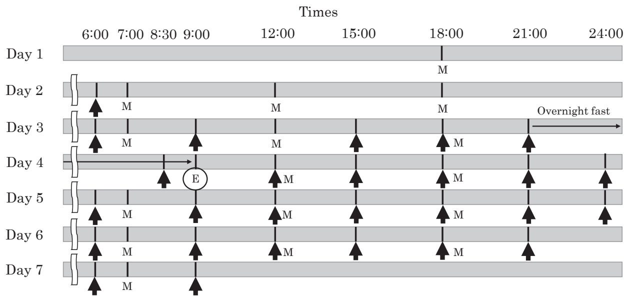

Figure 2. Study design
M: Meal (without *Solanaceous* ingredients), Ⓔ: intake of 300 grams of eggplant,
▲: Urine sampling and intake of 300 mL water

drinks containing *Solanaceous* foods during the study. Their urine was sampled from days 2 to 7. The participants consumed 300 mL of water after urine collection. Urine samples were stored in sample collection containers at 2–8°C and tested within 48 hours of collection.

We used urinary nicotine and cotinine test kits (Nic Check®, SCETI K.K., Japan)[8] to measure the urine cotinine levels. The Nic Check® test strip contained four different chemicals spotted at definite intervals along its length[9]. When urine diffused up the test strip, potassium thiocyanate mixed with chloramine-T on the strip, releasing cyanogen chloride. Cyanogen chloride then reacts with nicotine and its metabolites in urine. Diethylthiobarbituric acid reacts with the resulting glutaraldehyde to produce a pale pink to dark pink color along the length of the test strip as well as in the liquid remaining at the bottom of the tube. This kit uses a dedicated color chart to measure cotinine and nicotine levels both qualitatively and quantitatively. Nic Check® has detection thresholds of 5 μg/mL and 2.5 μg/mL for nicotine and cotinine, respectively. The examination of urinary cotinine proceeds as follows.

(1) Place about 0.5 to 1.0 mL of collected urine in a test tube.
(2) Place the Nic Check® test strips in a test tube.
(3) After 15 min, a color chart was used to check the color change indicated by numbers.

If the test strip turned light or dark pink, the sample was considered positive for nicotine or cotinine. The degree of color change was scored as shown in Figure 3. Three researchers checked the color change of each cotinine test strip. The average

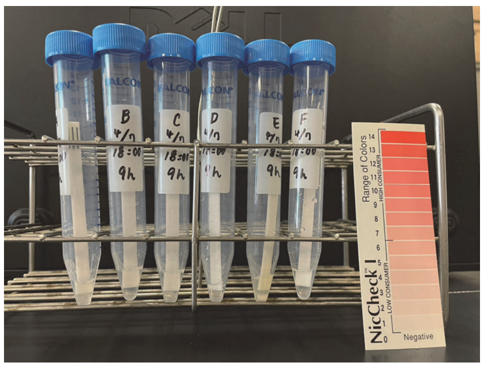

Figure 3. Color chart and changes in test strips

of the scores given by the three researchers was determined as the color number of each sample. Color changes of 1 or higher were considered positive.

3. Ethical statement

This study was approved by the Research Ethical Committee of Kyoto Women's University (approval number 2021-41) and performed in accordance with the guidelines of the Declaration of Helsinki.

III. Results

Table 1 presents the characteristics of the participants. The participants were not exposed to passive smoking for three days before the start of the experiment. Figures 4 and 5 show the results of cotinine examination after eggplant consumption. Four participants' urine samples tested positive for cotinine (score of 1 or 2, Figure 4), whereas two participants' urine samples tested negative (Figure 5). The time between eggplant intake and cotinine detection varied individually (minimum, three hours and maximum, 51 hours). The urine cotinine score also differed among the participants. In participants C and D, cotinine was detected in the urine before the consumption of eggplant.

Table 1. Characteristics of the participants

ID	Height (cm)	Weight (kg)	BMI (kg/m^2)	Passive smoking from 3 days before test
A	151	43	18.9	no
B	159	62	24.5	no
C	154	46	19.4	no
D	160	59	19.5	no
E	160	64	25.0	no
F	157	54	21.9	no

IV. Discussion

In this study, four of the six participants' urine samples tested positive for cotinine after consuming eggplant; they were not exposed to smoking three days before consuming eggplant. We observed individual differences in the time elapsed between eggplant consumption, cotinine detection, and cotinine

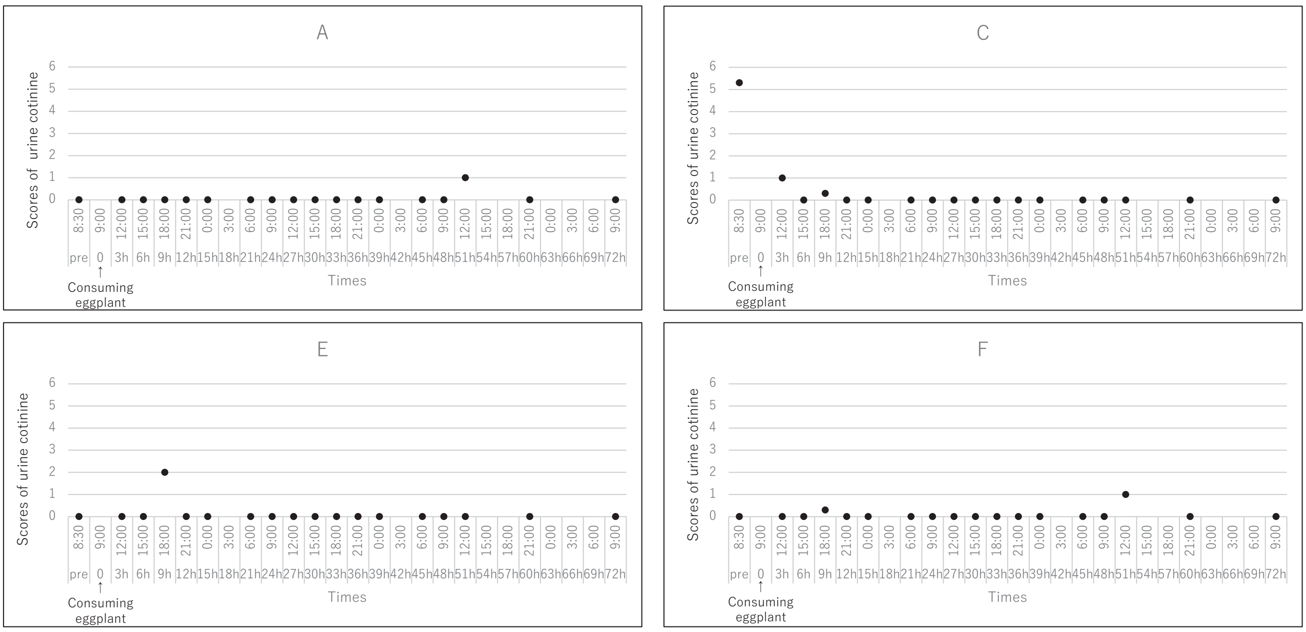

Figure 4. Positive urine cotinine test results after eating eggplant (n=4)

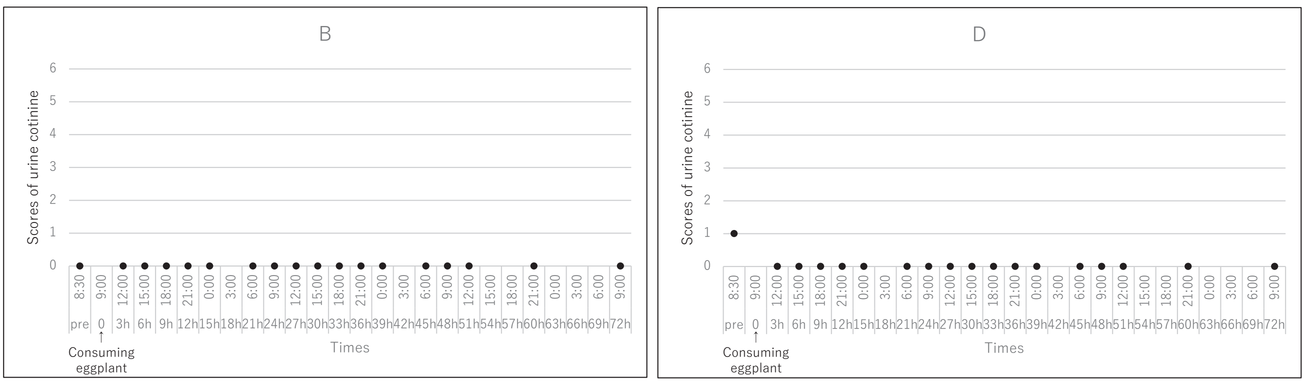

Figure 5. Negative urine cotinine test results after eating eggplant (n=2)

concentrations. This study is the first to investigate the effect of eggplant consumption on urine cotinine levels using a urine nicotine and cotinine test kit.

Nicotine is metabolized primarily by the liver enzymes cytochrome P450 (CYP) 2A6, UDP-glucuronosyltransferase (UGT), and flavin-containing monooxygenase (FMO)[4], although 10% of the nicotine dose is excreted unchanged in urine[10]. The major metabolic pathways of nicotine to form cotinine and trans-3'-hydroxycotinine are catalyzed by CYP2A6[11], and cotinine is excreted in the urine. Nicotine and cotinine are also converted to *N*-glucuronides mainly by UGT2B10, and trans-3'-hydroxycotinine is converted to *O*-glucuronide primarily by UGT2B17[10]. Nicotine has a half-life of three hours, and cotinine has a half-life of 20 hours[12]. However, the time required for nicotine metabolism varies among people, which is attributed to the genetic polymorphisms of CYP2A6, UGT 2B10, and UGT2B17 genes[10]. For example, Japanese individuals show lower cotinine formation and a higher frequency of CYP2A6 defective alleles than Koreans, Caucasians, and African Americans[10]. In addition to genetic factors, diet, age, sex, medications (e.g., estrogen-containing hormone preparations), physical conditions (e.g., pregnancy and kidney disease), and smoking influence nicotine metabolism[4]. Furthermore, the excretion of cotinine in urine may depend on the pH of the urine[13]. These factors may explain the individual differences in cotinine concentrations and the time required for metabolizing nicotine.

Two participants' samples tested positive for cotinine before consuming eggplant (participants C and D), although the participants consumed meals that did not contain *Solanaceous* foods three days before consuming eggplant. It may be because, for some people, nicotine metabolism takes more than three days, and these participants may have consumed *Solanaceous* foods before the start of this study. Another reason may be that the cotinine test kit used in this study reacts with niacin (nicotinic acid), and high concentrations of niacin in the meals we served might have affected the results.

This study had some limitations. First, there was no control group. Second, all participants were young and non-smoking women. Therefore, the results cannot be generalized.

V. Conclusions

The urine cotinine concentration–nicotine metabolite–may increase even in non-smokers after consuming *Solanaceous* foods like eggplant. When assessing smoking status, the consumption of *Solanaceous* foods should be considered before measuring urine cotinine levels.

Acknowledgments

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