การประเมินระดับก๊าซคาร์บอนมอนอกไซด์ในลมหายใจ เพื่อบ่งชี้การสูบบุหรี่ของกลุ่มนักศึกษาระดับอุดมศึกษา ในเขตกรุงเทพมหานครและปริมณฑล

ชมพูนุท สินธุพิบูลยกิจ* และ ทินกร เพิ่มพงศ์ไพบูลย์

กลุ่มวิชาเคมีคลินิก คณะเทคนิคการแพทย์ มหาวิทยาลัยหัวเฉียวเฉลิมพระเกียรติ จังหวัดสมุทรปราการ

บทคัดย่อ

การตรวจระดับก๊าซคาร์บอนมอนอกไซด์ในลมหายใจเป็นวิธีที่ใช้ประเมินการสูบบุหรี่ซึ่งมีค่าตัดสิน (cut-off) แตกต่างกันตามลักษณะของกลุ่มตัวอย่างที่ศึกษา การเริ่มสูบบุหรี่เมื่ออายุน้อยกว่า 20 ปี มีความสัมพันธ์กับจำนวนมวนบุหรี่ที่สูบมากขึ้น ระยะเวลาในการสูบและระดับการติดนิโคตินที่มากขึ้น ซึ่งส่งผลต่อความสำเร็จในการเลิกบุหรี่ที่ลดลง การศึกษานี้จึงมีวัตถุประสงค์เพื่อใช้การตรวจระดับก๊าซคาร์บอนมอนอกไซด์ในลมหายใจในการรณรงค์การลด ละ เลิกบุหรี่ ในกลุ่มตัวอย่างนักศึกษาและหาค่าตัดสินที่เหมาะสมสำหรับประเมินการสูบบุหรี่ โดยวัดระดับก๊าซคาร์บอนมอนอกไซด์ในลมหายใจและสัมภาษณ์นักศึกษาที่อาศัยในเขตกรุงเทพมหานครและปริมณฑลจำนวน 389 คน พบว่ากลุ่มนักศึกษาที่สูบบุหรี่ (200 คน) มีระดับก๊าซคาร์บอนมอนอกไซด์ในลมหายใจสูงกว่ากลุ่มที่ไม่สูบบุหรี่ (138 คน) และกลุ่มที่ได้รับควันบุหรี่มือสอง (51 คน) ตามลำดับ [median (95% CI); 9.00 (8.00, 11.00) ppm vs 3.00 (3.00, 3.00) ppm vs 2.00 (2.00, 3.00) ppm, $p < 0.001$] มีค่าตัดสินที่เหมาะสมที่ความเข้มข้น ≥ 6 ส่วนในล้านส่วน (ppm) ซึ่งมีความไวและความจำเพาะของวิธีเท่ากับร้อยละ 76.50 และ 96.38 ตามลำดับ โดยความไวของวิธีมีค่าเพิ่มขึ้นเป็นร้อยละ 84.12 เมื่อทดสอบในกลุ่มที่สูบบุหรี่มวนสุดท้ายภายใน 6 ชั่วโมง กลุ่มนักศึกษาที่ตั้งใจจะเลิกสูบบุหรี่ (123 คน) มีคะแนนความตระหนักต่อพิษภัยของการสูบบุหรี่สูงกว่ากลุ่มที่ไม่ต้องการเลิกสูบบุหรี่ (4.02 ± 0.95 vs 3.14 ± 1.31, $p < 0.001$) งานวิจัยนี้แสดงให้เห็นว่าการตรวจระดับก๊าซคาร์บอนมอนอกไซด์ในลมหายใจสามารถนำมาใช้เพื่อสร้างความตระหนักต่อพิษภัยของการสูบบุหรี่ในกลุ่มนักศึกษาได้โดยมีค่าตัดสินที่เหมาะสมที่ความเข้มข้น ≥ 6 ส่วนในล้านส่วน

คำสำคัญ: ก๊าซคาร์บอนมอนอกไซด์ในลมหายใจ การสูบบุหรี่ นักศึกษา

***ผู้รับผิดชอบบทความ E-mail address:** chompunoot.sint@gmail.com

รับบทความ: 4 พฤษภาคม 2561 แก้ไขบทความ: 11 กรกฎาคม 2561 รับตีพิมพ์บทความ: 16 สิงหาคม 2561

Exhaled Carbon Monoxide Level as an Indicator of Smoking among Undergraduate Students in Bangkok Metropolis and Bangkok Metropolitan

Chompunoot Sinthupibulyakit* and Thinnakorn Permpongpaiboon

Faculty of Medical Technology, Huachiew Chalermprakiet University, Samut Prakan Province, Thailand

Abstract

Measurement of exhaled carbon monoxide (eCO) has been used to evaluate tobacco smoking with a range of eCO cut-off point depending on specific populations. Early initiation of smoking before 20 years of age has been associated with greater consumption, longer duration of smoking, and increased nicotine dependence, consequently, influencing smoking cessation. This study aimed to apply eCO test as a tool for smoke free environment campaign by examining baseline eCO levels, the sensitivity and specificity of eCO test, and optimal cut-off value for smoking assessment in samples of the undergraduate students. Total of 389 undergraduate students living in Bangkok Metropolis and Bangkok Metropolitan underwent a measurement of eCO levels and completed questionnaire-based interview seeking demographic information and details of exposure to tobacco smoke, smoking habits and smoking-related knowledge. Active smokers (n = 200) had significantly higher eCO levels than non-smokers (n = 138) and passive smokers (n = 51) [median (95% CI); 9.00 (8.00, 11.00) ppm vs 3.00 (3.00, 3.00) ppm vs 2.00 (2.00, 3.00) ppm, $p < 0.001$], respectively. The eCO level at ⩾ 6 ppm was optimal cut-off value to classify smokers, with sensitivity of 76.50% and specificity of 96.38%. When excluding data of smokers with > 6-hour since last cigarette, sensitivity increased to 84.12%. Obviously, active smokers who desired to quit smoking (n = 123) had a significantly higher awareness scores than those who did not (n = 73) (4.02 ± 0.95 vs 3.14 ± 1.31, $p < 0.001$). In conclusion, eCO test with optimal cut-off at ⩾ 6 ppm is an effective tool to validate smoking status among undergraduate students and raise the student's awareness on adverse effect of smoking. The reliability of test increased if an individual smoked with ⩽ 6-hour prior to test.

Keywords: Exhaled carbon monoxide, Tobacco smoking, Undergraduate students

---

***Corresponding author E-mail address:** chompunoot.sint@gmail.com
Received: May 4, 2018 Revised: July 11, 2018 Accepted: August 16, 2018

Introduction

Smoking is responsible for considerable morbidity and mortality worldwide. It is usually associated with noncommunicable diseases (NCDs), such as lung cancers, heart and respiratory diseases, and a major factor in deaths from communicable diseases such as tuberculosis, and lower respiratory infection.(1) Smoking increases the risk of dying from cancer and other diseases in cancer patients and survivors. It can induce insulin resistance, causes diabetes and general adverse effects on the body including inflammation and impaired immune function.(2) In addition to smoking, exposure to secondhand tobacco smoke has been causally linked to cancer, respiratory and cardiovascular diseases, and to adverse effects on the health of infants and children.(2-6) In 2016, the prevalence of current tobacco smoking in Thai adult were 20.7%, of which 18.2% were daily smokers.(7) Smokers mostly started their first cigarette smoking at the age of 17.8 years and continuing smoke habit at the age of undergraduate,19.5-years-old.(8) Carbon monoxide (CO) is a poisonous, colorless and odorless gas found in tobacco smoke. It is 200 times faster at binding with hemoglobin in red blood cell than oxygen molecule, resulting in reduction of oxygen transport, causing hypoxia. The symptoms of CO poisoning may vary depends on the amount and duration of exposure ranging from headache, dizziness, nausea, increased heart rate to death.(9) The measurement of exhaled carbon monoxide (eCO) is a method to evaluate smoking status as CO is rapidly absorbed into the bloodstream when lit cigarettes are inhaled. It has been shown in several studies to be an effective and easy tool for smoking assessment as it is non-invasive with high sensitivity and specificity.(10-12) Particularly, the available measurement of eCO level with portable hand-held eCO analyzer, which can immediately share the results to the participants especially smoker and thus can depict the harmful effects of smoking. This may affect smoker's subsequent smoking behavior. Thereby, eCO measurement can be used as part of the smoking prevention campaign and intervention as the numerous evidences show that eCO test was suitable for both clinical and community-based studies.(13-16) However, previous studies revealed that the optimal eCO cut-off values for evaluating smoking status were varied in different populations depending on the characteristics of studied populations and the intended use of the eCO test.(17-22) In this study, we aimed to apply eCO test as a tool for smoke free environment campaign in undergraduate students by estimating baseline exhaled carbon monoxide levels, examining the sensitivity and specificity of the eCO test and defining the optimal cut-off value for recent smoking assessment in samples of the undergraduate students living in Bangkok metropolis and Bangkok metropolitan.

Materials and methods

The study was approved by the ethics review committee at Huachiew Chalermprakiet University. Total of 389 undergraduate students living in Bangkok Metropolis and Bangkok Metropolitan were recruited in the study; 200 active smokers, 51 passive smokers and 138 non-smokers. Participants were classified as active smokers if they had smoked a cigarette within the last 24 hours. Passive smokers were those who do not smoke but considered themselves to be exposed to tobacco smoke from intimate friends or relatives at least once per week during the past week. Non-smokers were defined as participants who do not smoke or refrained from smoking for more than 6 months and considered themselves had not been exposed to smoke exhaled by smokers. Informed consents were obtained from all participants. Background information about their smoking-related knowledge, awareness of tobacco smoking effects, smoking habits and exposure to passive smoke was obtained using questionnaire-based interview. Active smokers were asked to complete the Fagerstrom Test of Nicotine Dependence (FTND). All participants underwent a measurement of exhaled carbon monoxide (eCO) levels by using electrochemical sensor-based piCO$^+$ Smokerlyzer® (Bedfont Scientific Ltd, England) and the results were informed immediately with short advices. The eCO levels in all groups were reported as part per million (ppm). The eCO levels between different groups were compared using Kruskal—Wallis H test and Mann—Whitney U test. The receiver operating characteristic (ROC) curve, sensitivity, specificity, positive and negative predictive values were calculated to evaluate validity of eCO test for assessment of smoking status among participants. Spearman's Rho correlation coefficients were calculated to assess the relationships between eCO levels and smoking behaviors and nicotine dependence. The awareness score about adverse effect of tobacco smoking was calculated using linear scale from no awareness (score = 0) to maximum awareness (score = 5). Kruskal—Wallis H test and Mann—Whitney U test were used to compare parameters between different groups.

Results

The demographic characteristics of 389 participants comprising 255 male and 134 female undergraduate students are shown in Table 1. The median (95% confidence interval) of eCO level in active smokers was 9.00 (8.00, 11.00) ppm (range 1-41); non-smokers was 3.00 (3.00, 3.00) ppm (range 0-8); passive smokers was 2.00 (2.00, 3.00) ppm (range 1-6). As expected, the eCO levels were significantly higher in active smokers compared with passive smokers and non-smokers ($p < 0.001$). In non-smokers, male had a higher eCO level than female [4.00 (3.00, 4.00) ppm vs 3.00 (3.00, 3.00) ppm, $p < 0.01$]. In passive smokers, there was no significant correlation between eCO levels and frequency of passive

Table 1 Demographic characteristics and exhaled carbon monoxide (eCO) levels of study participants

Characteristics	Tobacco smoking		
	Non-smokers (n = 138)	Passive smokers (n = 51)	Active smokers (n = 200)
Age, year (mean ± SD)	21.58 ± 2.80	21.18 ± 1.20	21.70 ± 1.66
Gender, n (%)			
Male	40 (29.0%)	25 (49.0%)	190 (95.0%)
Female	98 (71.0%)	26 (51.0%)	10 (5.0%)
eCO level, n			
0 ppm	4		
1 ppm	10	14	1
2 ppm	27	17	8
3 ppm	46	16	9
4 ppm	28	3	11
5 ppm	18		18
6 ppm	2	1	12
7 ppm	2		18
8 ppm	1		13
9 ppm			11
10 ppm			9
11-15 ppm			35
16-20 ppm			36
21-25 ppm			11
26-30 ppm			5
≥ 31 ppm			3
eCO level (ppm), median (95% confidence interval)	3.00 (3.00, 3.00)	2.00 (2.00, 3.00)[a]	9.00 (8.00, 11.00)[a, b]

[a] significant difference in eCO level at $p < 0.001$, compared with non-smokers group.

[b] significant difference in eCO level at $p < 0.001$, compared with passive smokers group.

smoke exposure ($p > 0.05$). Active smokers had smoked for 4.88 ± 2.82 year (range 1-17) and had average FTND score of 2.53 ± 2.18 (range 0-8). There were significantly positive correlations between eCO level of active smokers and duration of smoking habit (r = 0.181, $p < 0.05$), daily cigarettes consumption (r = 0.375, $p < 0.001$), number of days smoked in a week (r = 0.433, $p < 0.001$), and FTND scores (r = 0.528, $p < 0.001$) (Table 2). The significant increases of the eCO levels were observed in smokers with increasing number of cigarettes smoked per day ($p < 0.001$), increasing smoking frequency ($p < 0.001$), and the increase of FTND scores ($p < 0.001$) as shown in Table 3. There were negative correlations between the age of smoking initiation and the duration of smoking habit (r = -0.848, $p < 0.001$), daily cigarettes consumption (r = -0.473, $p < 0.001$), number of days smoked in a week (r = -0.426, $p < 0.001$), and FTND scores (r = -0.284, $p < 0.001$) (Table 2).

Table 2 Correlations between exhaled carbon monoxide (eCO) level, age of smoking initiation and smoking characteristics

Correlations	r	*p*-value
eCO level and		
Duration of smoking habit	0.181	0.010[a]
Daily cigarette consumption	0.375	0.000[b]
Frequency of smoking (days per week)	0.433	0.000[b]
Fagerstrom Test of Nicotine Dependence (FTND) score	0.528	0.000[b]
Age of smoking initiation *and*		
Duration of smoking habit	-0.848	0.000[b]
Daily cigarette consumption	-0.473	0.000[b]
Frequency of smoking (days per week)	-0.426	0.000[b]
Fagerstrom Test of Nicotine Dependence (FTND) score	-0.284	0.000[b]

[a] significant correlation at $p < 0.05$.

[b] significant correlation at $p < 0.001$.

Table 3 Exhaled carbon monoxide (eCO) levels in active smokers

Characteristics	n	eCO level (ppm), median (95% confidence interval)
Number of cigarettes smoked per day		
1-5	88	7.00 (6.00, 8.00)
6-10	79	13.00 (9.00, 15.00)[a]
11-15	14	12.00 (8.03, 18.00)[a]
16-20	15	13.00 (8.50, 17.00)[a]
≥ 21	4	13.00 (8.00, 29.00)
Frequency of smoking (days per week)		
1-2	16	5.00 (3.00, 6.00)
3-4	27	6.00 (4.00, 7.00)
5-6	29	10.00 (7.00, 15.00)[b,c]
7	128	11.50 (10.00, 14.00)[b,c]
Fagerstrom Test of Nicotine Dependence (FTND) score		
Very low (0-2)	113	7.00 (6.00, 8.00)
Low (3-4)	46	12.00 (9.00, 16.00)[d]
Moderate (5)	11	15.00 (11.00, 20.00)[d]
High (6-7)	28	16.00 (10.00, 19.00)[d]
Very high (8-10)	2	24.00 (18.00, 30.00)[d]

[a] significant difference in eCO level at $p < 0.05$, compared with 1-5 cigarettes smoked per day.

[b] significant difference in eCO level at $p < 0.001$, compared with 1-2 days per week.

[c] significant difference in eCO level at $p < 0.01$, compared with 3-4 days per week.

[d] significant difference in eCO level at $p < 0.01$, compared with very low FTND score.

The eCO level was inversely related to the lapsed time since last cigarette smoked as its level decreased over time (Table 4). High eCO levels were observed within individuals who smoked within the last 6 hours. Consistently, the eCO results of smokers with ⩽ 6 hours since last cigarette smoked were statistically significantly higher, compared to smokers with > 6 hours since last cigarette smoked ($p < 0.001$).

When active smokers and nonsmokers were examined as a whole, a cut-off indicating the optimal equilibrium between sensitivity and specificity was ⩾ 6 ppm. However, when using the data of smokers with ⩽ 6 hours since last cigarette smoked, the optimal cut-off point was

Table 4 Exhaled carbon monoxide (eCO) levels in active smokers with various length of time since the last cigarette

	Length of time since the last cigarette (h)					
	1 (n = 123)	2 (n = 36)	3 (n = 7)	4-6 (n = 4)	7-12 (n = 4)	13-24 (n = 26)
eCO (ppm)	12.00 (10.00,14.00)	11.00 (8.00, 14.00)	9.00 (7.00, 12.00)	6.50 (2.00, 8.00)[a,b]	5.00 (5.00, 7.00)[a,b,c]	4.00 (4.00, 6.00)[a,b,c]
	≤ 6 h since the last cigarette (n = 170)			> 6 h since the last cigarette (n = 30)		
eCO (ppm)	11.00 (10.00, 13.00)			5.00 (4.00, 5.99)[d]		

NOTE: data shown as median (95% confidence interval).

[a]significant difference in eCO level at $p < 0.05$, compared with 1 hour since the last cigarette.

[b]significant difference in eCO level at $p < 0.05$, compared with 2 hours since the last cigarette.

[c]significant difference in eCO level at $p < 0.05$, compared with 3 hours since the last cigarette.

[d]significant difference in eCO level at $p < 0.001$, compared with length of time since the last cigarette ≤ 6 h group.

⩾ 6 ppm with increased sensitivity. At cut-off value of ⩾ 6 ppm, the positive predictive value (PPV) and negative predictive value (NPV) were 96.84% and 73.89%, respectively. The exclusion of smokers with > 6 hours since last cigarette smoked data increased the NPV (83.13%). As shown in Fig. 1A, the significant contribution to the area under the curve (AUC) with 95% confidence interval was 0.912 (0.881, 0.943) at the eCO cut-off of ⩾ 6 ppm ($p < 0.001$), suggesting a good diagnostic accuracy to predict smoking status. With the data for smokers who had last cigarette over 6 hours prior to eCO testing excluded from the ROC analysis (Fig. 1B), the area under the curve at eCO cut-off of ⩾ 6 ppm increased marginally to AUC with 95% confidence interval of 0.936 (0.906, 0.965), $p < 0.001$.

As depicted in Table 5, the background knowledge about tobacco smoking of 3 study groups by questionnaire-based interview showed that most of the students knew the definition of second-hand smoke (76.3%) but not third-hand smoke (33.2%). The health risks of tobacco smoking participants mostly knew were lung cancer (96.7%), emphysema (83.8%), laryngeal cancer (75.1%), asthma (63.5%), cardiovascular disease (58.6%), hypertension (46.8%) and diabetes (23.7%). The average score on the awareness of the adverse effect of tobacco smoking due to eCO testing was 3.86 ± 1.13 (total score = 5) (Table 6). Active smokers had significantly lower awareness scores compared to passive smokers and non-smokers (3.70 ± 1.17 vs 3.92 ± 1.23 vs 4.07 ± 0.99, $p < 0.01$). Obviously, smokers

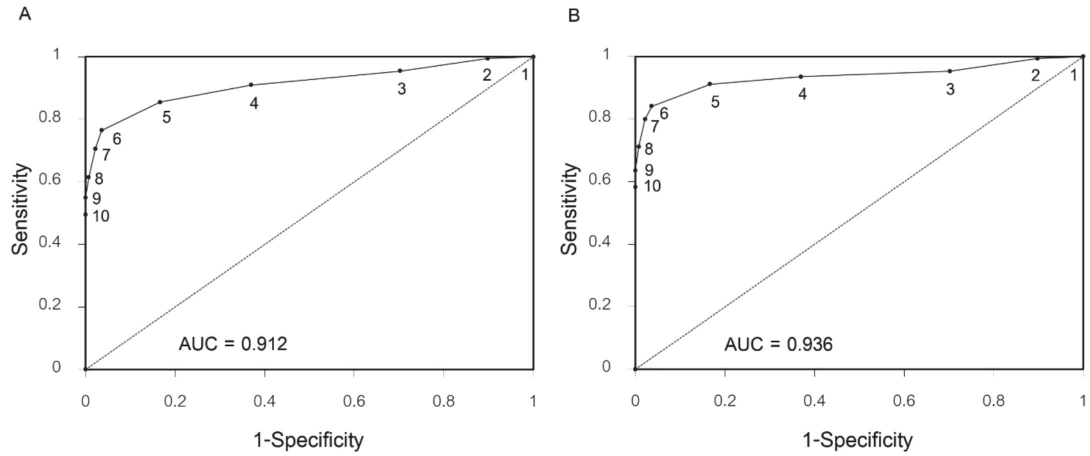

Fig. 1 Receiver operating characteristic (ROC) curve. 1-specificity (x-axis) was plotted against sensitivity at exhaled carbon monoxide (eCO) cut-off levels from 1 ppm to 10 ppm. The numbers placed along the ROC curve indicate eCO cut-off levels. (A) Data analysis using non-smokers and active smokers. (B) Data analysis using non-smokers and active smokers with less than 6-hour last cigarette smoked. Area under curve (AUC) was calculated.

Table 5 Background knowledge on tobacco smoking in active smokers, passive smokers, and non-smokers from questionnaire-based interview

Background knowledge	Tobacco smoking		
	Non-smokers (n = 138)	**Passive smokers (n = 51)**	**Active smokers (n = 200)**
Types of tobacco smoke (n, %)			
Second-hand smoke	115 (83.3%)	25 (49.0%)	157 (78.5%)
Third-hand smoke	60 (43.5%)	8 (15.7%)	61 (30.5%)
The health risks of tobacco smoking (n, %)			
Lung cancer	138 (100.0%)	50 (98.0%)	188 (94.0%)
Emphysema	130 (94.2%)	40 (78.4%)	156 (78.0%)
Laryngeal cancer	98 (71.0%)	37 (72.5%)	157 (78.5%)
Asthma	98 (71.0%)	36 (70.6%)	113 (56.5%)
Cardiovascular disease	87 (63.0%)	28 (54.9%)	113 (56.5%)
Hypertension	65 (47.1%)	29 (56.9%)	88 (44.0%)
Diabetes	38 (27.5%)	11 (21.6%)	43 (21.5%)

Table 6 Awareness scores of adverse effect of tobacco smoking due to eCO measurement from questionnaire-based interview

Awareness score, n	Tobacco smoking			
	Non-smokers (n = 138)	**Passive smokers (n = 51)**	**Active smokers (n = 200)**	**Total (n = 389)**
No awareness (0)	4	1	8	13
Very low (1)	0	2	3	5
Low (2)	1	1	6	8
Moderate (3)	20	15	59	94
High (4)	65	9	72	146
Very high (5)	48	23	52	123
Average scores (mean ± SD)	4.07 ± 0.99	3.92 ± 1.23	3.70 ± 1.17[a]	3.86 ± 1.13

Awareness score, n	Smokers who desired to quit smoking (n = 123)	Smokers who did not desire to quit smoking (n = 73)
No awareness (0)	2	6
Very low (1)	0	3
Low (2)	2	4
Moderate (3)	28	30
High (4)	49	22
Very high (5)	42	8
Average scores (mean ± SD)	4.02 ± 0.95	3.14 ± 1.31[b]

[a] significant difference in awareness scores at $p < 0.001$, compared with non-smokers group.
[b] significant difference in awareness scores at $p < 0.001$, compared with smokers who desired to quit smoking group.

who desired to quit smoking had a significantly higher awareness scores than smokers who did not want to quit smoking (4.02 ± 0.95 vs 3.14 ± 1.31, $p < 0.001$). Out of 196 smokers, 123 smokers (62.8%) decided to quit smoking, of which 66 smokers (53.7%) want to stop smoking by themselves, 33 smokers (26.8%) need guidance from counseling (The Thai National Quitline center; The Quitline 1600) and 24 smokers (19.5%) decided to try 0.5% sodium nitrate mouthwash to stop smoking.

Discussion

The eCO measurement has been shown to be an immediate, non-invasive, simple and effective test for confirming smoking status.[10, 14, 18] These findings indicate that eCO can be effectively used to validate smoking status in undergraduate student population. In this study, we demonstrated the significantly high level of eCO in active smokers, compared to non-smokers and passive smokers, consistently with the previous studies.(11, 23, 24) The eCO levels of passive smokers are about the same levels as non-smokers and did not correlate to the frequency of passive smoke exposure, although other studies have demonstrated increased eCO levels with passive smoking in non-smokers.(23, 25) These dissimilarities may depend on inclusion criteria of passive smokers in each study including the frequency and duration of exposure and other factors such as the amount of CO exposed, the exhale rate of subjects and the lapsed times between exposure and eCO testing as the decline rate of eCO was about 2.1 to 7.5 ppm per hour, depending on the initial eCO level.(25, 26) In non-smokers, male students had higher eCO levels than females, correlating to the study by Zhang *et al.*(15) Our results were consistent with other studies that the higher eCO levels in smokers were associated with the longer duration of smoking, higher daily cigarette consumption, increase of smoking frequency and a higher FTND score.(10, 22, 23, 27) We found that the early initiation of cigarette smoking is significantly related to greater consumption (frequency and number of cigarettes smoked), longer duration of smoking, and increased nicotine dependence.

The eCO levels significantly decreased inversely to the time since last cigarette smoked. This significantly negative correlation between the time of last cigarette smoked and eCO levels could be explained based on the half-life of CO being 5 to 6 hours.(28, 29) Our results support this as the significantly higher eCO levels in smokers with ⩽ 6 hours last cigarette smoked, relative to smokers who smoked last cigarette more than 6 hours.

The eCO cut-off level that has been used to validate smoking status in different population groups can be in a range of 3 to 10 ppm, which depends on the specific characteristics of population such as ethnics, age and sociocultural patterns of smoking.(12, 14, 19, 22, 30) In this study, the ROC analysis revealed that the eCO level at ⩾ 6 ppm was the optimal cut-off value to classify undergraduates who had smoked from non-smokers, with sensitivity of 76.50% and specificity of 96.38%. Furthermore, the results demonstrated that the sensitivity of the eCO test increased from 76.50% to 84.12% and the negative predictive value (NPV) increased from 73.89% to 83.13%, when excluding the data of smokers with more than 6-hour since last cigarette, suggesting that lapsed time since last cigarette smoked is the factor which should be considered when using eCO test to assess smoking status. Hence, this cut-off level may be useful in detecting smoking status in individual students who have smoked within the last six hours.

As the results, forty-seven (23.5%) active smokers had eCO values below the ⩾ 6 ppm cut-off. Twenty of these (42.6%) had the last cigarette more than 6 hours prior to the eCO test. These could be due to the short half-life of eCO as seen in our study that the eCO level decreased over the time since the last cigarette. Nonetheless, smokers who smoke only few cigarettes per day can also have normal eCO level.(10, 14, 19, 26) On the other hand, there were five (3.6%) non-smokers with eCO $\geqslant$ 6 ppm. These discrepancies may imply false self-report or exposure to other sources such as public transportation.

The questionnaire-based interview revealed the background knowledge about tobacco smoking of undergraduate students. About 66.8% of all participants did not know about third-hand smoke. Fewer than 50% of the students knew that cigarette smoking causes some health risks such as hypertension and diabetes, similar to study by Phanucharas and Chalongsuk.(31) Thus, the less knowledge about tobacco smoke adverse effects may result in the less concern regarding tobacco smoking. However, we demonstrated the effectiveness of using eCO test in smoke free environment campaign as it can raise the awareness about the adverse effect of tobacco smoking, especially in smokers who determined to stop smoking, compared with smokers who did not want to quit smoking. These were probably due to the immediate results reflecting participant's smoking status that could denote the harmful effects of tobacco smoking. The data also showed that the average age of smoking initiation in smokers was 16.81 ± 2.56 years (range 7-25), similar to the results (17.1 and 17.8 years) reported by Phanucharas and Chalongsuk, and Tobacco Control Research and Knowledge Management Center, respectively.(8, 31) It has been described that initiation of cigarette smoking before 20 years of age has been associated with greater consumption, longer duration of smoking, and increased nicotine dependence, consequently, influencing smoking cessation.(32) For these reasons, the use of eCO test for smoke free environment campaign among undergraduate students may potentially raise smoking student's concern and lower the number of young smokers in the long run.

Conclusion

The use of eCO test which provides a quick, simple, non-invasive and inexpensive method for smoking status assessment was an effective tool to raise the undergraduate student's awareness on smoking effect, especially in smokers. The immediate eCO result reports demonstrated instantly the adverse effects of smoking. Our results suggested that eCO at $\geqslant$ 6 ppm is an optimal cut-off to verify smokers with high sensitivity and specificity. The lapsed time since last cigarette was the factor affecting eCO results in students who smoked, increased sensitivity was observed in smokers within less than 6-hour after the last cigarette.

Acknowledgements

This work was financially supported by The Medical Technology Professional Alliance against Tobacco.

References

1. World Health Organization. Effects of tobacco on health. [homepage on the Internet]. [cited 2018 Apr 3]. Available from: http://www.euro.who.int/en/health-topics/disease-prevention/tobacco/data-and-statistics/effects-of-tobacco-on-health
2. U.S. Department of Health and Human Services. The Health Consequences of Smoking—50 Years of Progress: A Report of the Surgeon General. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health, 2014.
3. Leung GM, Ho LM, Lam TH. Secondhand smoke exposure, smoking hygiene, and hospitalization in the first 18 months of life. Arch Pediatr Adolesc Med 2004; 158: 687-93.
4. Papathanasiou G, Mamali A, Papafloratos S, Zerva E. Effects of Smoking on Cardiovascular Function: The Role of Nicotine and Carbon Monoxide. Health science journal 2014; 8: 274-90.
5. Hori M, Tanaka H, Wakai K, Sasazuki S, Katanoda K. Secondhand smoke exposure and risk of lung cancer in Japan: a systematic review and meta-analysis of epidemiologic studies. Jpn J Clin Oncol 2016; 46: 942-51.
6. Leung LT, Ho SY, Wang MP, Lam TH. Secondhand smoke from multiple sources, Thirdhand Smoke and Respiratory Symptoms in Hong Kong Adolescents. Nicotine Tob Res 2018; 20:192-8.
7. World Health Organization. WHO REPORT ON THE GLOBAL TOBACCO EPIDEMIC, 2017: Monitoring tobacco use and prevention policies. Geneva: World Health Organization; 2017: 156-7.
8. Tobacco Control Research and Knowledge Management Center, Mahidol University. Tobacco consumption control situation in Thailand, 2016. 1st ed. Bangkok: Mahidol University, 2016; 20-47. (in Thai)
9. Goldstein M. Carbon monoxide poisoning. J Emerg Nurs 2008; 34: 538-42.
10. Middleton ET, Morice AH. Breath carbon monoxide as an indication of smoking habit. Chest 2000; 117: 758-63.
11. Marrone GF, Paulpillai M, Evans RJ, Singleton EG, Heishman SJ. Breath carbon monoxide and semiquantitative saliva cotinine as biomarkers for smoking. Hum Psychopharmacol 2010; 25: 80-3.

12. Chatrchaiwiwatana S, Ratanasiri A. Exhaled carbon monoxide levels among tobacco smokers by age. Southeast Asian J Trop Med Public Health 2017; 48: 429-37.
13. Hung J, Lin CH, Wang JD, Chan CC. Exhaled carbon monoxide level as an indicator of cigarette consumption in a workplace cessation program in Taiwan. J Formos Med Assoc 2006; 105: 210-3.
14. MacLaren DJ, Conigrave KM, Robertson JA, Ivers RG, Eades S, Clough AR. Using breath carbon monoxide to validate self-reported tobacco smoking in remote Australian Indigenous communities. Popul Health Metr 2010; 8: 2.
15. Zhang Q, Li L, Smith M, *et al.* Exhaled carbon monoxide and its associations with smoking, indoor household air pollution and chronic respiratory diseases among 512 000 Chinese adults. Int J Epidemiol 2013; 42: 1464-75.
16. Campbell KA, Cooper S, Fahy SJ, *et al.* 'Opt-out' referrals after identifying pregnant smokers using exhaled air carbon monoxide: impact on engagement with smoking cessation support. Tob Control 2017; 26: 300-6.
17. SRNT Subcommittee on Biochemical Verification. Biochemical verification of tobacco use and cessation. Nicotine Tob Res 2002; 4: 149-59.
18. Low EC, Ong MC, Tan M. Breath carbon monoxide as an indication of smoking habit in the military setting. Singapore Med J 2004; 45: 578-82.
19. Pearce MS, Hayes L. Self-reported smoking status and exhaled carbon monoxide: results from two population-based epidemiologic studies in the North of England. Chest 2005; 128: 1233-8.
20. Sandberg A, Sköld CM, Grunewald J, Eklund A, Wheelock ÅM. Assessing recent smoking status by measuring exhaled carbon monoxide levels. PLoS One 2011; 6: e28864.
21. Doruk S, Demirtas I, Aksit H, Erkorkmaz U, Seyfikli Z. Assessment of exposure to tobacco smoke: measurement of exhaled carbon monoxide and hair nicotine. Turk J Med Sci 2012; 42: 739-45.
22. Guan NC, Ann AY. Exhaled carbon monoxide levels among Malaysian male smokers with nicotine dependence. Southeast Asian J Trop Med Public Health 2012; 43: 212-8.
23. Deveci SE, Deveci F, Açik Y, Ozan AT. The measurement of exhaled carbon monoxide in healthy smokers and non-smokers. Respir Med 2004; 98: 551-6.
24. Chatrchaiwiwatana S, Ratanasiri A. Exhaled carbon monoxide level and smoking status in urban Khon Kaen adults. J Med Assoc Thai 2008; 91: 1669-76.

25. Kumar R, Mahakud GC, Nagar JK, *et al.* Breath carbon monoxide level of non-smokers exposed to environmental tobacco smoke. Indian J Chest Dis Allied Sci 2011; 53: 215-20.
26. Leitch DN, Harkawat R, Askew J, Masel P, Hendrick DJ. Relation of expired carbon monoxide to smoking history, lapsed time, TLCO measurement and passive smoking. Respir Med 2005; 99: 32-8.
27. Cunnington AJ1, Hormbrey P. Breath analysis to detect recent exposure to carbon monoxide. Postgrad Med J 2002; 78: 233-7.
28. Peterson JE, Stewart RD. Absorption and elimination of carbon monoxide by inactive young men. Arch Environ Health 1970; 21: 165-71.
29. Jo WK, Oh JW. Evaluation of CO exposure in active smokers while smoking using breath analysis technique. Chemosphere 2003; 53: 207-16.
30. Cropsey KL, Eldridge GD, Weaver MF, Villalobos GC, Stitzer ML. Expired carbon monoxide levels in self-reported smokers and nonsmokers in prison. Nicotine Tob Res 2006; 8: 653-9.
31. Phanucharas D, Chalongsuk R. Smoking behavior and smoking-related knowledge of students at Silpakorn University, Thailand. Silpakorn U Science & Tech J 2009; 3: 34-43.
32. Caponnetto P, Polosa R. Common predictors of smoking cessation in clinical practice. Respir Med 2008; 102: 1182-92.