Original Article

Carbon Monoxide in the Expired Air and Urinary Cotinine Levels of e-Cigarette Users

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Abstract

OBJECTIVES: The aim of this descriptive study was to determine the sociodemographic characteristics of electronic (e)-cigarette users for clarifying the causes of e-cigarette smoking and to identify the carbon monoxide (CO) and urinary cotinine levels of the volunteers.

MATERIALS AND METHODS: Twenty volunteers who smoked e-cigarettes completed a questionnaire, and their exhaled CO and urinary cotinine levels were measured. An enzyme-linked immunosorbent assay kit was used for cotinine analysis.

RESULTS: Overall, 85% of the participants were males, 60% were married, and 75% were college/university graduates. The median age of participants was 38.5 years. The participants' main reasons for starting to smoke were peer influence and curiosity. The participants' main reasons for smoking e-cigarettes were to quit and reduce smoking the conventional cigarettes and cost effectiveness. Only three people knew that smoking was harmful to health. The participants' CO levels were measured as a median of 3, lowest of 1, and highest of 22. Cotinine levels were "positive" in all samples. A moderate and statistically significant correlation was found between the amount of fluids used by the participants in 1 day (mL) and cotinine levels in urine specimens (Pearson correlation test, r=0.511, p=0.025).

CONCLUSION: The study is an important proof of the country's scientific work on e-cigarettes. Preventive strategies should be very strictly implemented for any tobacco products, including e-cigarettes, as they harm individuals and the community.

KEYWORDS: Carbon monoxide, electronic cigarette, urinary cotinine

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INTRODUCTION

The electronic (e)-cigarette, which is marketed as a "new" product by the tobacco industry, is highly dependent on health and is addictive due to its nicotine content. E-cigarettes, which are known to be the most common type of Electronic Nicotine Delivery System (ENDS), vaporize a solution, and the users inhale the solution. The content is majorly nicotine with other substances, such as propylene glycol that can be present with or without glycerol, and other flavoring agents. Other toxicants like formaldehyde and acrolein are also reported as contents of e-cigarettes [1].

The present evidence-based science accepts e-cigarette as a tobacco product, and major preventive strategies are strongly recommended by the World Health Organization (WHO) [2]. Unfortunately, the use of ENDS, including e-cigarette use, is increasing, and many new brands are entering the market. The use among youth is alarming, and data from North America and other developed countries highlight the sharp increase in the prevalence rates [3]. ENDS products are available worldwide, and anyone opting for it can easily procure and be addicted. Many ecigarette users are adolescents who do not use conventional cigarettes, and this challenging detail makes the situation more complex. The users probably do not know about the nicotine content in e-cigarettes [4], which is very dangerous and deceptive. Scientists warn the public on protecting people from e-cigarettes [5]. A Harvard group recently confirmed "no safe dose" in smoking and declared the only safe cigarette as to be "never smoker." The ethical perspective of smokingrelated issues also comments on limiting the discussions with the (nicotine) addiction characteristics of smoking [6].

Due to all these harmful effects, informing and raising awareness is extremely valuable to protect the identified risks of e-cigarette in the society. The correction of the deceptive "harm reduction" perception of the community is also the social responsibility of the scientists [7]. In this regard, scientists should design e-cigarette researches to compare the effects of users with non-smokers, which is also ethically acceptable.

With this background, the aim of this study was to determine the sociodemographic characteristics of e-cigarette users for clarifying the causes of e-cigarette smoking and estimating the exhaled carbon monoxide (CO) and urine cotinine levels of

the users. Using our results, we also aimed to produce simple and understandable information for the community to protect them from dangers of any type of nicotine exposure.

MATERIALS AND METHODS

Participants
In this descriptive study, twenty volunteers who smoked e-cigarettes completed a questionnaire. The median age of the participants was 38.5 years. The youngest individual was aged of 22 years and the oldest was 69 years. Banners and e mails were used to invite participants. Since e-cigarette smokers were not interested in participating, the "call" was repeated several times.

Inclusion criteria were declaring to be an e-cigarette smoker and to be volunteer for participation in the study.

Questionnaire
A questionnaire was prepared by the researchers to obtain the sociodemographic characteristics, define smoking details, and understand the dynamics of e-cigarette use. The questionnaire was completed before the measurement of exhaled CO and collection of urine cotinine samples.

Participants did not answer few of the questions in the questionnaire. Hence, some of the items given in the tables were presented over 19 participants.

Exhaled CO Measurement
Exhaled CO levels of the participants were measured after the completion of the questionnaire and submission of urine samples for cotinine. CO™ Smokerlyzer (Bedfont Scientific, Kent, England) was used to measure exhaled CO levels. The results were given in part per million (ppm), and the sensitivity of the sensor was reported as 1 ppm by the manufacturer. The halflife of CO is between 5-6 hours in the body [8] and is probably restored to normal after 24 and 48 hours if one is not exposed to smoking [9].

Urinary Cotinine Assay
Urinary cotinine is a widely used biomarker for active tobacco use and/or passive smoking [9,10]. Urine samples were collected from the volunteers and directed immediately on ice to the toxicology laboratory and stored at -80°C in until further use. The Cotinine Direct (Serum/Urine) ELISA Kit (EIA-5496, Marburg, Germany) was used to measure cotinine levels in urine samples according to the manufacturer instructions. Each sample was assayed in duplicates. The sensitivity of the kit was reported as 1 ng/mL by the manufacturer.

Ethical Issues
The ethical permission was obtained from Hacettepe University Non-interventional Clinical Researches Ethics Board (26.08.2015, GO 15/540-17). Informed consent of the participants for all steps of the study were also obtained prior to initiation of the study. When data collection was completed, the participants were informed about the hazards of e-cigarette. A "quit" program is among the plans of the study.

Statistical Analysis
Descriptive statistics was performed to define the sociodemographic and cigarette use for the participants. The IBM Statistical Package for Social Sciences (IBM SPSS Statistics Corp.; Armonk, NY, USA) 23.0 program was used for statistical analysis. Pearson and Spearman tests were used for correlation analysis to understand the relationship between ecigarette use and other characteristics.

RESULTS

The sociodemographic characteristics of the participants are presented in Table 1. Twenty people participated in the study. The median age of the participants was 38.5 years. A majority of the participants were males (n=17) and married (n=12). Eight participants reported a disease (asthma, diabetes mellitus, hepatitis, hypertension, coronary heart disease, etc.). Seven individuals were on regular medication.

The median value of e-cigarette use duration was 5.5 months (minimum=0.3, and maximum 25 months). The median value of monthly expenses was 60 TL. The most frequently stated reasons for e-cigarette use was to quit (n=11) and to reduce (n=8) the conventional cigarette. Cost effectiveness, curiosity, and influence of the comprehensive smoke-free implementation were other three items that were declared by the participants.

The participants were also asked about their reasons for smoking. They reported that peer influence, addiction, and

Table 1. Sociodemographic characteristics of the participants

Characteristics	n	%
Sex		
Male	17	85
Female	3	15
Marital status		
Married	12	60
Not married	5	25
Widowed	3	15
Educational status		
Secondary	1	5
High	4	20
University	15	75
Occupational status		
No	4	20
Yes	16	80
Economic status		
Good	7	35
Fair	13	65
Disease		
No	12	60
Yes	8	40
Use medicine		
No	13	65
Yes	7	35
Total	20	100

curiosity were among the reasons for smoking, which was consistent with literature [11].

In Table 2, e-cigarette smoking characteristics of the participants are presented. Eighteen participants used e-cigarette daily. E-cigarette was provided most frequently from the Internet (35%) and friends/relatives (30%). All participants reported that the e-cigarettes used included nicotine (n=20). Fifteen out of 19 e-cigarette users used e-cigarettes, which did not mimic conventional cigarettes. Eleven participants never smoked a conventional cigarette using an e-cigarette.

Only nine participants stated the addictive characteristics of the e-cigarettes, and three participants highlighted the hazards/risks for health.

Four out of 19 participants thought to quit e-cigarette, and only one participant had attempted to quit.

Table 2. E-cigarette smoking characteristics of the participants

Characteristics	n	%
Previous e-cigarette use (n=20)		
No	14	70
Yes	6	30
Frequency of e-cigarette use (n=19)		
Everyday	18	90
Frequently	1	10
Provided from (n=20)		
Internet	7	35
Friend/relative	6	30
Shop	3	15
All	2	10
Other	2	10
Type of e-cigarette (n=19)		
Not mimicking conventional cigarette	15	79
Other	4	21
Nicotine content (n=20)		
Yes	20	100
No	-	-
Conventional cigarette use wit e-cigarette (n=20)		
No	11	55
Yes	9	45
Change in conventional cigarette smoke (n=20)		
No smoking	11	55
Reduced	9	45
Knowledge on e-cigarette hazards on health (n=20)		
No hazards on health	3	15
A little bit but not as much as conventional cigarette	13	65
Yes	3	15
No idea	1	5
Knowledge about addictive property of e-cigarette		
No	7	35
Yes	9	45
No idea	4	20
Thought to quit e-cigarette smoking (n=19)		
No	15	79
Yes	4	21
Attempted to quit e-cigarette smoking (n=19)		
No	18	95
Yes	1	5

Table 3. Exhaled CO levels of the e-cigarette users

CO level (ppm)	n	%
1	4	20
2	5	25
3	3	15
4	1	5
5	1	5
6	1	5
10	2	10
12	1	5
20	1	5
22	1	5

Table 4. Urine cotinine levels of the participants

Sample number	Cotinine	Level of cotinine (ng/mL)
1	Positive	633.28±34.06
2	Positive	654.54±16.81
3	Positive	351.78±15.73
4	Positive	638.20±48.58
5	Positive	818.24±17.28
6	Positive	739.13±34.90
7	Positive	778.33±85.57
8	Positive	318.63±134.85
9	Positive	604.64±179.46
10	Positive	710.24±70.54
11	Positive	709.45±7.06
12	Positive	740.55±64.89
13	Positive	825.30±49.50
14	Positive	785.07±35.29
15	Positive	745.69±10.10
16	Positive	641.39±18.37
17	Positive	577.58±56.20
18	Positive	490.33±73.59
19	Positive	779.26±32.24
20	Positive	788.63±24.60

Values were given as mean ± standard deviation for each paired sample.

Table 5. Correlation between the e-liquid reserve amount (mL) per day and urine cotinine levels

	Mean	SD	min	max	r	p
All participants (n=20)						
Reserve e-liquid per day (mL)	3.5	2.5	0.3	8.0	0.511*	0.025
Cotinine	666.5	143.0	318.6	825.3		
Conventional cigarette smokers using e-cigarette (n=9)						
Reserve e-liquid per day (mL)	3.4	2.8	0.3	8.0	0.108**	0.799
Cotinine	643.1	147.1	351.8	818.2		
Only e-cigarette users (n=11)						
Reserve e-liquid per day (mL)	3.7	2.3	0.3	6.0	0.511**	0.109
Cotinine	685.7	143.4	318.6	825.3		

*Pearson correlation test; **Spearman correlation test

Exhaled CO Levels

In Table 3, exhaled breath CO levels of the participants are presented. The values changed from 1 ppm to 22 ppm.

The urine cotinine levels of all participants were found positive and are presented in Table 4. Due to the specifications of the kit used, actual cotinine levels of urine samples were expressed as positive. However, using a standard curve, which was prepared according to kit procedure, cotinine levels were also calculated and given as numbers. The levels were in the range of 318.8-825.3 ng/mL. The mean cotinine level determined was 709.9 ng/mL.

The correlation between the e-liquid reserve amount (mL) per day and urine cotinine levels are presented in Table 5.

A normal distribution was found between e-liquid reserve amount used per day and urine cotinine levels of all participants (One-Sample Kolmogorov–Smirnov Test, p=0.444 and p=0.625). The correlation was suggested as a medium strength correlation (Pearson correlation test, r=0.511, p=0.025). A low strength correlation was found among e-cigarette and conventional cigarette smokers (Spearman correlation test, r=0.108, p=0,799), and a medium strength correlation was found among only the e-cigarette smokers (Spearman correlation test, r=0.511, p=0.109).

DISCUSSION

The results of our research have contributed to the disclosure of "Tobacco: deadly in any form or disguise," which was declared by WHO in 2006 [12]. The conclusion is that the nicotine metabolite cotinine is detected in urine samples of all e-cigarette smokers, which is very valuable in this context and is important for the accumulation of evidence. When compared with the non-smokers' expected values ("0"), the detected levels in the smokers are thought to be alarming. Thus, the tobacco industries' attempts end with new products of tobacco, such as e-cigarette, which deceive the users, as they lack information on the "new" agenda. The World Medical Association has entrusted the responsibility to medical staff to grapple with the industry in its statement on health hazards of tobacco and tobacco-derived products [13]. The outline was drawn very clearly in the Framework Convention on Tobacco Control, which was implemented in February 2005 [14].

Participants were found to be unaware of their level of knowledge and perception about ecigarettes. For example, certain individuals believe that e-cigarettes are not harmful to health (Table 2). This can be interpreted as a finding that the tobacco industry's misleading perception of different tobacco products is reflected in the society. Therefore, the need for correct information regarding e-cigarette threats to spread rapidly in the community is emerging. It is seen that the correct information about e-cigarette addiction is not known to all participants (Table 2).

Remarkably, most of the e-cigarette smokers do not consider quitting smoking of e-cigarettes (Table 2). As there is no "safe dose" of tobacco use, e-cigarette users should be informed in detail about the health risks of tobacco use. With the increase in the marketing tactics of the tobacco industry on e-cigarettes [15], the need for the systematic tobacco control campaigns are recommended to be met with.

A few limitations can be noted in our study. Firstly, the number of the participants in our descriptive study did not allow the generalization of the results for the community. Nevertheless, the number of the participants in our study was higher than that of other published studies on e-cigarettes, which was systematically reviewed by Marsot and Simon [16]. Secondly, cotinine levels measured using the enzymelinked immunosorbent assay kit was not confirmed using another method, such as high-performance liquid chromatography or gas chromatography-mass spectroscopy. Thirdly, we had difficulty contacting the e-cigarette users, and we did not predict this difficulty at the beginning of the study. Majority of the ecigarette users were "happy" with their e-cigarette smoking behavior, and it was not easy for us to convince them to participate in our study. Lastly, we would like to stress the CO level measurement(s). The fact that the time of the last e-cigarette use was not recorded was a limitation, and this limited our critics on the issues. In contrast, the exhaled CO levels of the participants varied from 1 ppm to 22 ppm (Table 3), and most of the assessed values were over 5-6 ppm [8]. Exhaled CO is a well-known biomarker of a recent smoking history [9].

In conclusion, this study wherein nicotine exposure, CO, and urine cotinine levels are found to increase due to e-cigarette

use and/or nicotine exposure, contributed to the scientific evidence about the e-cigarette use indicators at both the national and the international levels. Further research targeting the limitations of this study is the most important expectation. Evidencebased quitting programs for e-cigarette users could also be planned and implemented, as the dynamics of e-cigarette use may be different from conventional cigarette smoking.

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