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Short communication

A longitudinal study of cotinine in long-term daily users of e-cigarettes

Jean-François Etter*

Institute of Global Health, Faculty of Medicine, University of Geneva, Switzerland

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ABSTRACT

Background: It is not clear whether, in established vapers, cotinine levels remain stable or change over time.
Methods: We enrolled 98 exclusive users of e-cigarettes on websites and forums dedicated to smoking cessation and to e-cigarettes. We collected saliva vials by mail in 2013–2014 (baseline), and collected a second saliva vial eight months later (follow-up) in the same participants. Participants had not used any tobacco or nicotine medications in the previous five days. Cotinine in saliva was analyzed with liquid chromatography–mass spectrometry. Use of e-cigarettes, tobacco and nicotine medications was self-reported.
Results: All participants were former smokers, and 99% were using e-cigarettes daily. They had already been using e-cigarettes for nine months on average at baseline. The median cotinine level was 252 ng/mL at baseline (quartiles: 124–421 ng/mL) and 307 ng/mL at follow-up (114–466 ng/mL, $W = 0.9$, $p = 0.4$ for change over time). The median concentration of nicotine in refill liquids was 11 mg/mL at baseline (quartiles: 6–15 mg/mL) and 6 mg/mL at follow-up (5–12 mg/mL) (Wilcoxon signed rank test: $W = 5.2$, $p < 0.001$ for change over time). The median volume of e-liquid used per month was 80 mL at baseline (quartiles: 50–130 mL) and 100 mL at follow-up (60–157 mL, $W = 3.3$, $p = 0.001$ for change over time).
Conclusion: In experienced e-cigarette users enrolled online, cotinine levels were similar to levels usually observed in cigarette smokers. Over time, these users decreased the concentration of nicotine in their e-liquids, but increased their consumption of e-liquid in order to maintain their cotinine levels constant.

1. Introduction

Experienced vapers (e-cigarette users) can obtain substantial amounts of nicotine from these devices (Etter and Bullen, 2011; Vansickel and Eissenberg, 2013), and some may even obtain amounts of nicotine similar to the amounts typically observed in smokers (Etter, 2014; Etter and Bullen, 2011; Vansickel and Eissenberg, 2013). The amount of nicotine obtained from e-cigarettes is a crucial point, because it may determine the effects of e-cigarettes on nicotine withdrawal symptoms, their ability to replace combustible cigarettes, their efficacy for smoking cessation and relapse prevention, and their addictiveness (Le Houezec, 2003). In experimental studies, levels of cotinine (a metabolite of nicotine) either remained constant (Pacifici et al., 2015) or decreased (Adriaens et al., 2014; McRobbie et al., 2015; van Staden et al., 2013) after smokers switched from tobacco cigarettes to e-cigarettes. In an observational study, no change in cotinine levels was observed two months after smokers switched to e-cigarettes (Berg et al., 2014). One element that has not yet been documented in longitudinal studies is whether, in established vapers who have already quit smoking for some time, levels of nicotine and cotinine remain stable over time. Thus, the objective of this study was to assess change over time in saliva cotinine levels in experienced vapers.

2. Methods

We posted a registration form in French and English on the smoking cessation website *Stop-Tabac.ch* in 2013–2014 (Wang and Etter, 2004). Participation was limited to current e-cigarette users who lived in Switzerland, France, or the US. We asked websites informing about e-cigarettes or selling them and specialized discussion forums to publish links to the registration form (the survey was briefly advertised as an academic survey for current vapers). This online form covered current and past use of e-cigarettes and tobacco, the number of cigarettes per day that former smokers smoked before quitting, postal address, household income (below or above the average household income in the respondent's country), age and sex. Participants were >18 years and were not compensated. The study was approved by the ethics committee of the Geneva University Hospitals.

We sent to current vapers a plastic vial (Salivette, Sarstedt), a consent form, a questionnaire and a stamped return envelope (Etter et al., 1998). Eight months after the first assessment, we posted a second vial and questionnaire to participants who

* Corresponding author at: Institute of Global Health, University of Geneva, CMU, Case Postale, CH-1211 Geneva 4, Switzerland. Fax: +41 22 379 04 52.
E-mail address: Jean-Francois.Etter@unige.ch

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had already returned one saliva vial. This questionnaire covered e-cigarette use, brand and model (free text fields), whether their e-cigarettes contained nicotine, nicotine concentration in e-liquids, puffs/day, number of refills per day, quit date (in ex-smokers), and use in the past five days of any tobacco (smoked or smokeless) and nicotine medications.

Participants were instructed to collect the saliva samples no less than 30 min after eating or drinking. Upon receipt, vials were stored at −4 °C then shipped by express mail to ABS Laboratories (Herts, UK) for cotinine analysis by liquid chromatography–mass spectrometry (Etter, 2014; Etter and Bullen, 2011; Etter et al., 2005).

2.1. Analyses

We classified e-cigarettes in three categories according to answers to the free text fields on brand and model names and to answers to a question on use of pre-filled cartridges vs. refillable tanks: (1) first generation: disposable models that resemble a cigarette (usually discarded after 200 puffs), pre-filled cartridges and cartomizers, (2) second generation: larger models with refillable tanks (e.g., Ego), with fix or variable voltage, and (3) third generation: advanced personal vaporizers (electric output regulated electronically or mechanically, temperature control devices, tube or box batteries, rebuildable atomizers, replaceable coils, large format tanks, mods). To assess change over time, we used Wilcoxon Signed Rank Tests for medians, paired-samples *t*-tests for means, and McNemar tests for dichotomous variables.

3. Results

We posted vials to 442 vapers and received 255 vials back (a 58% response rate) between October, 2013 and May, 2014. For the second assessment, we received 127 vials back (50% of 255, 29% of 442), between October and December, 2014. The median interval between the first and second assessments was 8.1 months.

Of the 127 people who participated in both assessments, 98 used exclusively e-cigarettes at both assessments (and no tobacco or nicotine medications in the past 5 days), 21 were dual users of both e-cigarettes and tobacco or nicotine medications at either assessment, and eight either did not use e-cigarettes or could not be classified. All further analyses included only the 98 participants who were using only e-cigarettes at both time points, the other groups were not analysed because of their small size.

3.1. Characteristics of the sample at the first assessment

The median age of these 98 participants was 46 years, most were men (67%), most (68%) had obtained a diploma that gives access to University, and household income tended to be above average (below average = 25%, about average = 30%, above average = 37%). Participants lived in France (48%), Switzerland (29%) and the US (23%). All participants were former smokers, they had quit smoking nine months (280 days) before the first assessment and had been smoking 25 cigarettes per day before they quit (medians).

At the first assessment, almost all participants (99%) were using e-cigarettes daily, and they had already been using e-cigarettes for nine months (270 days) on average (Table 1). Participants refilled their tanks twice a day on average. At the first assessment, participants used mainly third generation (53%) and second generation models (44%), and few participants used first generation models (1%).

3.2. Changes between the first and second assessments

Cotinine concentrations in saliva remained stable between the first and second assessments (Table 1). The concentration of nicotine in refill liquids decreased but the volume of e-liquid used per month increased between the first and second assessments. The number of puffs per day on e-cigarettes remained unchanged. The change over time in the proportions of participants who used second generation vs. third generation models was not statistically significant (Table 1).

In participants who used third generation models at both assessments, nicotine concentrations in e-liquids decreased from 11 mg/ml at the first assessment to 6 mg/mL at follow-up (Wilcoxon Signed Rank Test: $W = 4.1$, $p < 0.001$), the corresponding figures for participants who used second generation models at both assessments were 11 and 9.5 mg/mL respectively ($W = 3.3$, $p = 0.001$).

4. Discussion

Between the 9th and the 17th month after they started vaping (between 2013 and 2014), experienced users of second and third generation e-cigarettes enrolled online decreased the concentration of nicotine in their e-liquids, but they increased their consumption of e-liquid and maintained their saliva cotinine levels constant.

This suggests that participants compensated for the decreased nicotine strength of their liquids by using more liquid. As a consequence of using more liquid, they probably inhaled more vapor and therefore possibly increased their exposure to inhaled substances other than nicotine. This is an undesirable outcome, because e-cigarette aerosols, although they are less toxic than smoke, may not be innocuous, particularly when, as in this study, vaping is intensive (250 puffs/day) and prolonged (Farsalinos and Polosa, 2014). These vapers would probably be better off if they inhaled fewer puffs of an aerosol containing more nicotine, which would also enable them to keep their cotinine levels constant while decreasing their exposure to the other substances in the aerosol.

There are various reasons why these vapers decreased the nicotine concentration in their liquids. First, during 2013–2014, new models appeared, and even though the use of third generation models did not increase significantly in this sample, the characteristics of these models may nevertheless have changed. New models include electronical or mechanical regulation of the temperature, the voltage and the wattage, high wattage devices, sub-Ohm coils (that operate at <1 Ω), rebuildable atomizers, replaceable coils, large format tanks, etc. These newer devices deliver more power, more cloud density, more intense flavors and a better 'throat hit' than older models (Etter, 2015). These new models cannot be used with liquids that have high nicotine concentrations, because the taste of the vapor would be too harsh and the "throat hit" too strong. These new models are used with low-nicotine liquids (3–6 mg/mL) but require using more liquid to get satisfactory blood nicotine levels (Dave, 2016).

It is also possible that newer e-cigarette models and newer e-liquids are more efficient at transferring nicotine from the liquid to the aerosol or from the aerosol to the blood, or that the chemical composition of the aerosol changed over time (e.g., change in the ratio of free-base nicotine to protonated nicotine; El-Hellani et al., 2015). However, this would not explain why users increased their consumption of liquid.

The fact that nicotine concentrations in e-liquids also decreased slightly in users of second generation models suggests that, independently from the technological evolutions that apply mostly to third generation models, some participants reduced the strength of their liquids either as an intermediate step before stopping nicotine use, or because of fears about nicotine (Silla et al., 2014). These fears may have been triggered by media reports (Yates, 2015) and by public health experts who misperceive or misreport the relative risks of combustion, smokeless tobacco and nicotine (McNeill et al., 2014).

It is also possible that, independently from technological evolutions, some vapers increased their consumption of liquid because they changed their puffing and inhalation patterns. This would occur if they increasingly liked the act of inhaling and exhaling vapor, the flavours, or the gestures of vaping. It is also possible