Nicotine & Tobacco Research, 2020, 1114–1122
doi:10.1093/ntr/ntz185
Original investigation
Received April 5, 2019; Editorial Decision September 14, 2019; Accepted September 26, 2019

Original investigation

Real-World Evidence of Differences in Biomarkers of Exposure to Select Harmful and Potentially Harmful Constituents and Biomarkers of Potential Harm Between Adult E-Vapor Users and Adult Cigarette Smokers

Douglas Oliveri PhD, Qiwei Liang PhD, and Mohamadi Sarkar, MPharm., PhD, FCP

Altria Client Services LLC, Richmond, VA

Corresponding Author: Mohamadi Sarkar, MPharm, PhD, FCP, Altria Client Services LLC, Center for Research and Technology, 601 East Jackson Street, Richmond, VA 23219, USA. Telephone: 804-335-2537; E-mail: mohamadi.sarkar@altria.com

Abstract

Introduction: Real-world evidence regarding likely long-term health effects of e-vapor products (EVP) under actual use conditions relative to cigarette smoking is not well studied.

Methods: In this cross-sectional, observational study, biomarkers of exposure (BOE) to select harmful and potentially harmful constituents and biomarkers of potential harm (BOPH) relevant to smoking-related diseases were measured in exclusive adult EVP users (AEVP, $n = 144$) and exclusive adult cigarette smokers (AS, $n = 73$). AEVP used their own brand of EVP for 6+ months following 10+ years of cigarette smoking and AS smoked own brand of cigarettes for 10+ years. Subject recruitment and informed consent were obtained online and urine/blood samples were collected at local clinical laboratories, representing a new paradigm for collecting real-world evidence.

Results: The levels of total NNAL (NNK metabolite), 3-hydroxypropyl mercapturic acid (acrolein metabolite), and carboxyhemoglobin (carbon monoxide measure) were 46% to 86% lower in AEVP compared with AS ($p \leq .0001$) as was nicotine equivalents (nicotine and its five metabolites; 36%, $p < .01$). The levels of some BOPH were significantly lower in AEVP compared with AS for 11-dehydrothromboxane-B2 (29%, $p = .04$; platelet activation), 8-epi-prostaglandin F2α (23%, $p = .02$; oxidative stress) and soluble intercellular adhesion molecule-1 (16%, $p = .02$; endothelial function).

Conclusions: This study demonstrates the feasibility of a new approach for collecting real-world evidence. Substantially lower levels of BOEs (NNK, nicotine, acrolein, carbon monoxide) and favorable differences in BOPHs (platelet activation, oxidative stress, endothelial function) suggest EVP users may have lower health risks than cigarette smokers.

Implications: Cigarette smoking causes serious diseases. Switching from a combustible tobacco product to a noncombustible product is a potential harm reduction pathway for adult smokers unable or unwilling to quit. Real-world evidence regarding the relative risk of EVP use compared with cigarettes is not well established. This study provides data specific to BOE to tobacco smoke constituents and biomarkers of potential harm collected under actual use conditions in a real-world setting. The totality of evidence suggests that exclusive EVP use may present lower health risk compared with smoking cigarettes.

Introduction

There is overwhelming scientific consensus that cigarette smoking is addictive and causes lung cancer, heart disease, COPD (chronic obstructive pulmonary disease), and other serious diseases in smokers.[1] Quitting tobacco use is the most effective means of reducing the risk of tobacco-related disease. For those unable or unwilling to quit tobacco, completely switching from cigarettes to noncombustible products has the potential to reduce the risk of smoking-related diseases. There is evidence that many adult smokers are interested in reduced risk alternatives to cigarettes. According to an internal analysis of Wave 1 data (2013–2014) from the Population Assessment of Tobacco and Health (PATH) study, 55% of adult smokers are seeking reduced risk alternatives. A growing body of evidence indicates that noncombustible products like heat-not-burn tobacco products, e-vapor products, snus and traditional smokeless tobacco products have the potential to reduce risk from smoking-related diseases.[2–6] Many in public health[7–9] have recognized the existence of a continuum of risk among tobacco products, with conventional, combustible cigarettes at the highest end of that spectrum and noncombustible products on the lower end. FDA has also acknowledged this continuum of risk and proposed that the potential for innovation can lead to less harmful products.[10]

E-vapor products (EVPs) are a category of noncombustible products, with the ability to deliver nicotine without burning tobacco. As a result, many of the combustion-related chemicals are either absent or present at extremely low levels.[11]

There is an ongoing public health debate regarding the long-term health benefit of switching from cigarettes to EVPs.[12–15] Nevertheless, there is a growing body of evidence that suggests EVPs may present lower risks than cigarettes, including chemical analyses of harmful and potentially harmful constituents (HPHCs)[16,17] and randomized clinical trials. Notably, many randomized clinical trials[18–21] involve "forced-switching" from smoking to EVPs, which suffer from the limitation of adherence to the study EVP, particularly since adult smokers may not immediately switch if the EVP does not provide as satisfactory an experience as cigarette smoking. Adherence to the study EVP in a "forced-switching" setting is further complicated by the inability of many adult smokers to exclusively use the study EVP uninterrupted by occasional cigarettes throughout the study duration typically required to assess measurable changes in health effects (ranging from 3 to 12 months). Additionally, participants in an RCT are unable to select their preferred product type of flavor, therefore risking noncompliance and reversal back to cigarette smoking.

Therefore, observational studies assessing the impact of EVPs under real-world conditions offer a unique opportunity as smokers have self-selected to switch to EVPs of their own volition. There have been few studies collecting real-world evidence on EVP users and they report biomarkers of exposure (BOE)[11,22,23] or pulmonary function[24] but do not include biomarkers of inflammation or oxidative stress. Furthermore, no reports exist in the published literature that differentiates between cartridge and tank EVP users. Here, we present a systematic assessment of biomarker data among matched EVP users and smokers (by age, gender, body mass index [BMI], and smoking history) under real-world conditions.

This study compares BOE to tobacco smoke constituents and biomarkers of potential harm (BOPH) in a cross section of adult former cigarette smokers who are current users of EVPs relative to current smokers of conventional cigarettes. This is the first study to report real-world evidence of differences in BOE and BOPH following a long-term product switch (6+ months of self-reported exclusive use) from conventional cigarettes to EVPs.

Methods

Study Design

We hypothesize that adult smokers currently using EVP for at least 6 months after smoking 10 cigarettes per day for 10 years will have lower levels of BOEs and exhibit favorable levels of BOPHs compared with a matched control group of adult smokers. We utilized a cross-sectional study design because we wanted to gather observations under actual use conditions in the real-world from participants who had made switching decisions on their own volition and were using their own products. This cross-sectional, observational study represents a new approach for collecting real-world evidence—a "virtual" study where study participation is supported via an online portal and a call center, and the biological specimens are collected at local laboratories. The study was overseen by Covance Inc. between January 2017 and June 2017. The study was conducted in accordance with Good Clinical Practice (GCP) based on the International Conference on Harmonization guidelines, and the corresponding US Code of Federal Regulations (CFR) governing the Protection of Human Subjects (21 CFR 50), IRBs (21 CFR 56), and the Basic Principles of the Declaration of Helsinki. Prior to the start of the study, the study documents were approved by an independent Institutional Review Board (Chesapeake IRB, Columbia, MD—currently known as Advarra).

Participant recruitment (additional details provided in Supplementary Appendix A: Participant Recruitment and Prescreening) focused on each of the four regions defined by the US Census Bureau to provide a geographically diverse study population. Interested participants were prescreened by phone based on the inclusion and exclusion criteria, and qualified participants were provided study details including the responsibilities for the online enrollment process and laboratory visit. These prescreened participants were directed to an online portal for completion of enrollment, including rescreening, creating an account on the portal, ID verification, e-consent with a call center agent on the phone to answer questions, scheduling of the laboratory visit with the call center agent, and completion of online questionnaires. The entire enrollment process, including the phone call with the call center agent and the completion of the online questionnaires, lasted approximately 30 min. A laboratory kit with instructions was shipped to the participants after completion of the online questionnaires.

On the morning of the laboratory visit, the participants collected a first-void morning urine sample (at least 4 hours of retention in the bladder overnight) and stored at ambient temperature. Previous research has demonstrated that the first-morning void is the optimum spot urine collection time for estimating exposure to cigarette smoke constituents.[25] Anthropometric measurements and blood specimens (8-h fasting) were drawn at the local clinical laboratory site and the biological specimens were shipped to a central lab for processing and analysis on the day of the visit.

The study was conducted in two groups for which participants self-identified: exclusive adult EVP users (AEVP) and exclusive adult cigarette smokers (AS). Exclusive AEVP identified themselves as former smokers (minimum of 10 cigarettes per day for at least 10 years) and were exclusively using EVPs for at least 6 months and no other tobacco- or nicotine-containing products during that period. Exclusive AS were individuals who currently smoked 10 or

more conventional cigarettes and have smoked 10 or more cigarettes per day for at least 10 years and did not use other tobacco or nicotine-containing products (including EVPs) in the past 30 days. Six months of EVP use was considered as sufficient for smokers to acclimatize use behavior and manifest a measurable change in BOPH based on a previous switching study with a reduced exposure product.[26]

AEVP were subdivided into two equal groups, tank-based and cartridge-based EVPs. Each participant was assigned to a respective age–gender–BMI category. The distribution of BMI within each age–gender quota in the AEVP group was used to define the age–gender–BMI quotas for the AS group as a means to generate appropriately matched controls. Recruitment of AS was initiated once the target quotas for AEVPs were filled.

Participants

Participants included males or females, 30–65 years of age, who self-identified as AEVP or AS. Participants were required to be in good health (self-reported) and have a BMI between 18.5 and 39.9 kg/m^2. Participants were required to reside within 30 miles of a local laboratory network site (LabCorp Patient Service Centers) and have access to the internet, an active e-mail address and phone number. Participants who did not meet the smoking history, age, or BMI requirements and/or who were pregnant or planning to become pregnant were excluded.

Products and Product Use

No study product was provided by the sponsor or study investigator. Participants were instructed to use their own brand of EVP or conventional cigarettes ad libitum throughout study duration (approximately 30 days from electronic consent and online questionnaire completion to biological specimen collection).

Study Measures

BOE to specific HPHCs assessed as part of the study included: a biomarker of nicotine-derived nitrosamine ketone (NNK) exposure—4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides, NNAL-*O*-glucuronide, and NNAL-*N*-glucuronide (total NNAL); a biomarker of nicotine exposure—nicotine equivalents or the molar sum of nicotine, nicotine glucuronide, cotinine, cotinine glucuronide, *trans*-3′-hydroxy cotinine, and *trans*-3′-hydroxy cotinine glucuronide[23]; a biomarker of acrolein exposure—3-hydroxypropyl mercapturic acid (3-HPMA); and a biomarker of carbon monoxide exposure—blood carboxyhemoglobin (COHb). Total NNAL, nicotine equivalents, and 3-HPMA were measured from urine and COHb was measured from blood samples.

Biomarkers of potential harm related to mechanisms involved in smoking-related diseases evaluated as part of the study included: a biomarker related to inflammation—white blood cell (WBC) count; a biomarker related to cardiovascular risk—high-density lipoprotein cholesterol (HDL-C); a biomarker related to platelet activation 11-dehydrothromboxane B2; a biomarker related to oxidative stress 8-epi-prostaglandin F2α; and a biomarker related to endothelial function—soluble intercellular adhesion molecule-1 (sICAM-1). White blood cell count, HDL-C, and sICAM-1 were measured from serum and 11-dehydrothromboxane B2 and 8-epi-prostaglandin F2α were measured from urine.

Urine creatinine was measured in the spot urine collection and was used to adjust the concentration values of nicotine equivalents, total NNAL, 3-HMPA, 11-dehydrothromboxane B2, and 8-epi-prostaglandin F2α. The hematology panel provided details regarding complete and differential blood counts.

Biomarker analysis was performed by Celerion (Lincoln, Nebraska), NMS Labs (Willow Grove, Pennsylvania), and Covance (Indianapolis, Indiana and Harrogate, England).[27] The urinary BOEs, 8-iso-prostaglandin-F2α and 11-dehydrothromobane B2, were measured by LC-MS/MS, COHb by GC-MS/MS, creatinine and HDL-C were measured by colorimetric assays, and sICAM was measured by immunoassay. All the methods were validated using best practices, e.g., 21 CFR Part 58, Guidance for Industry – Bioanalytical Method Validation. Urinary nicotine equivalents were calculated as the molar sum of total nicotine, total cotinine, and total *trans*-3′-hydroxycotinine excreted in the spot urine sample. The values of individual components reported as below the limit of quantitation were set to one-half of the limit of quantitation.

Statistical Analysis

The sample size was based on detecting a difference in total NNAL between AEVP and AS because this biomarker is tobacco-specific,[27] correlates with overall cigarette smoke exposure,[28] and has a relatively long half-life.[29] Assuming a two-sided *t*-test and 5% type I error rate, a sample size of 150 participants for the AEVP group and 75 participants for the AS group provided at least 80% power to detect a difference.

We used an analysis of variance (ANOVA) model, to detect differences in total NNAL between AEVP and AS, with total NNAL level as the response variable and classification variables study group, age group (30 to <45, ≥45), gender, BMI (<25, ≥25), race (white, non-white), study group × age group, and study group × BMI group as model terms. We selected those variables that were most likely to influence the biomarker levels as confounders based on previous publications.[30] The ANOVA model was also used to examine the difference in all other BOE and biomarkers of potential harm between AEVP and AS as well as between tank and cartridge AEVPs. For biomarkers not normally distributed, a natural log-transformation was applied, and geometric mean was used in such cases. Data were analyzed using SAS v9.3 or above (SAS Institute Inc., Cary, NC).

Results

Following the initial telephone screening, 417 participants were rescreened for the study within the online portal; of which, 200 participants were categorized as screen failures. The most common reason for screen failure was an inability to verify identification at the online portal (n = 100), followed by smoking <10 cigarettes per day (n = 32), and unwillingness or inability to provide informed consent (n = 22). Of the 217 participants who were enrolled in the study, 197 participants completed. Among those who completed, 194 participants were included in the biomarker analysis set; 3 participants were excluded due to major protocol violations related to pregnancy and age.

A summary of demographics is provided in Table 1 and product use history is provided in Supplementary Table S1. The mean age was statistically significantly higher for AS than AEVP (47 vs. 44 years of age, p = .0399); however, the distribution of participants within the age groups was similar (p = .4672). Nevertheless, any potential impact of age on the biomarker outcomes was taken into consideration in the statistical model. For the AEVP subgroups, there were 35 males

and 35 females in the tank-based subgroup and 36 males and 26 females for the cartridge-based subgroup; the latter not as equally distributed due to relaxing the quota requirement to recruit the necessary number of participants. The majority of AS and AEVP were Caucasian (77.4% and 54.5%, respectively) with a higher proportion of African Americans for AEVP (30.3%) compared to AS (17.7%). The differences in demographics related to race (p = .0056) and ethnicity (p = .0342) were statistically significant. The statistical differences related to race was based on five specific categories. As some categories were comprised of a small number of participants, they were combined into two broader categories (white vs. nonwhite) in the analysis of variance model for race. In the model used to assess BOE and BOPH, the difference between AEVP and AS was adjusted for race and age, among other factors, and therefore the differences in the distribution observed at baseline did not impact the group inferences.

Tobacco Use History

A summary of tobacco use history for AS and AEVP is provided in Table 1. Overall, AS reported smoking cigarettes nearly every day during the past 30 days (mean, 29.3 days). On the days on which AS smoked cigarettes, the majority (74.2%) reported smoking 11–30+ cigarettes per day. Similarly, AEVP reported the use of EVP most days during the past 30 days (mean, 24.1 days). Participants in the tank-based subgroup reported use of tank EVP for 20.3 days (mean) but also used cartridge EVP for 10.8 days (mean). Participants in the cartridge-based EVP subgroup reported the use of cartridge EVP for 17.2 days (mean) but also used tank EVP for 7.0 days (mean) during the past 30 days. Participants in the tank-based EVP subgroup tended to use more e-liquid on days they used EVP compared with users of cartridge-based EVP. Participants in the cartridge-based EVP subgroup tended to use EVP cartridges with a higher concentration of nicotine compared with users of tank-based EVP. The extensive poly-use of different types of EVP (e.g., tank-based, cartridge-based) among AEVP diminished the ability to evaluate differences in tobacco use history between tank and cartridge-based EVP. Therefore, the sub-groups should be referred to as predominant tank and predominant cartridge-based EVP users.

Exposure to Select HPHCs

The descriptive statistics for the BOE and BOPH are shown in Tables 2 and 3 describes the least squares mean (LS Mean) values based on the statistical model. Overall, AEVP had statistically significantly lower levels of BOE to specific HPHCs compared with AS (Table 3 and Figure 1). This corresponded to lower levels in EVP users compared with AS by 86% for total NNAL (LS geometric mean, p < .0001), 36% for urinary nicotine equivalents (LS mean, p = .0035), 46% for level 3-HPMA (LS geometric mean, p = .0001), and 47% reduction for COHb (LS geometric mean, p < .0001).

The predominant tank and cartridge-based AEVP had lower levels of BOE when compared with AS. No specific trends were evident between the AEVP subgroups as shown in Table 2.

Biomarkers of Potential Harm

As shown in Table 3 and Figure 1, AEVP had statistically significantly lower levels of BOPH compared with AS, with the exception of WBC count and HDL-C. This corresponded to 9% lower levels for WBC count (LS geometric mean, p = .0588), 29% lower levels

Table 1. Demographics and Tobacco Use Summary

			AEVP			
Demographics	Statistic	AS (N = 62)	All AEVP (*N* = 132)	Tank-based (*N* = 70)	Cartridge-based (*N* = 62)	*p*-Value[a] AS vs. all AEVP
Age (years)						
	Mean	47.1	44.4	44.0	44.9	0.0399
	SD	8.48	8.31	8.6	7.95	
	Median	46.5	44.0	44.0	45.5	
Age group						
30 to <45 years	*n* (%)	28 (45.2)	67 (50.8)	37 (52.9)	30 (48.4)	0.4672[b]
45 to ≤65 years	*n* (%)	34 (54.8)	65 (49.2)	33 (47.1)	32 (51.6)	
Gender						
Male	*n* (%)	30 (48.4)	71 (53.8)	35 (50.0)	36 (58.1)	0.4826
Female	*n* (%)	32 (51.6)	61 (46.2)	35 (50.0)	26 (41.9)	
Race						
White	*n* (%)	48 (77.4)	72 (54.5)	43 (61.4)	29 (46.8)	0.0056
Black/African American	*n* (%)	11 (17.7)	40 (30.3)	16 (22.9)	24 (38.7)	
Asian	*n* (%)	1 (1.6)	2 (1.5)	2 (2.9)	0 (0.0)	
Multiracial	*n* (%)	0 (0.0)	12 (9.1)	6 (8.6)	6 (9.7)	
Other	*n* (%)	1 (1.6)	5 (3.8)	3 (4.3)	2 (3.2)	
Prefer not to answer	*n* (%)	1 (1.6)	1 (0.8)	0 (0.0)	1 (1.6)	
Ethnicity						
Hispanic or Latino	*n* (%)	3 (4.8)	21 (15.9)	12 (17.1)	9 (14.5)	0.0342
Not Hispanic or Latino	*n* (%)	58 (93.5)	109 (82.6)	57 (81.4)	52 (83.9)	
Prefer not to answer	*n* (%)	1 (1.6)	2 (1.5)	1 (1.4)	1 (1.6)	
BMI						
<25 kg/m^2	*n* (%)	24 (38.7)	34 (25.8)	17 (24.3)	17 (27.4)	0.0661
≥25 kg/m^2	*n* (%)	38 (61.3)	98 (74.2)	53 (75.7)	45 (72.6)	

AEVP = adult e-vapor product users; AS = adult smokers; N = number of participants; SD = standard deviation.

[a]Based on the Chi-square test or Fisher exact test (where the expected frequency is five or less) for categorical data and t-test for continuous data.

Table 2. Descriptive Statistics for Biomarkers of Exposure to Specific Tobacco Constituents and Biomarkers of Potential Harm in Adult Smokers and Adult Exclusive Users of EVP

	N[a]	*n* missing	Mean	SD	%CV	Min, max
Biomarkers of exposure						
Total NNAL (ng/g creatinine)						
AS	57	5	332.7	331.6	99.7	4, 1407
AEVP	126	6	144.4	219.7	152.1	1, 1054
AEVP Tank based	*67*	*3*	*130.5*	*194.7*	*149.2*	*1, 773*
AEVP Cartridge based	*59*	*3*	*160.3*	*245.7*	*153.2*	*1, 1054*
Nicotine equivalents (mg/g creatinine)						
AS	57	5	10.1	6.3	63.0	0, 29
AEVP	127	5	6.3	7.3	116.8	0, 29
AEVP Tank based	*68*	*2*	*7.0*	*7.7*	*110.3*	*0, 29*
AEVP Cartridge based	*59*	*3*	*5.5*	*6.8*	*125.5*	*0, 24*
3-Hydroxypropylmercapturic acid (µg/g creatinine)						
AS	54	8	1878.2	1728.3	92.0	145, 8962
AEVP	124	8	876.8	835.5	95.3	49, 4768
AEVP Tank based	*65*	*5*	*899.0*	*929.9*	*103.4*	*96, 4768*
AEVP Cartridge based	*59*	*3*	*852.3*	*724.6*	*85.0*	*49, 3879*
Carboxyhemoglobin (% saturation)						
AS	62	0	4.9	2.6	52.5	1, 12
AEVP	131	1	2.9	2.2	78.0	1, 12
AEVP Tank based	*70*	*0*	*2.8*	*2.2*	*80.8*	*1, 12*
AEVP Cartridge based	*61*	*1*	*3.0*	*2.2*	*75.4*	*1, 10*
Biomarkers of potential harm						
White blood cells (×10^3/µL)						
AS	62	0	7.3	2.1	29.4	3.2, 11.2
AEVP	128	4	6.6	2.1	31.1	3.3, 16.3
AEVP Tank based	*68*	*2*	*6.4*	*1.9*	*29.8*	*3.3, 13.6*
AEVP Cartridge based	*60*	*2*	*6.8*	*2.1*	*32.3*	*3.6, 16.3*
High-density lipoprotein cholesterol (mg/dL)						
AS	60	2	56.0	17.6	31.5	22, 101
AEVP	124	8	55.4	14.4	25.9	21, 106
AEVP Tank based	*65*	*5*	*56.0*	*14.9*	*26.6*	*21, 89*
AEVP Cartridge based	*59*	*3*	*54.6*	*13.8*	*25.2*	*34, 106*
11-dehydrothromboxane B2 (ng/g creatinine)						
AS	57	5	952.6	825.2	86.6	23, 4368
AEVP	125	7	844.2	1496.2	177.2	27, 13077
AEVP Tank based	*66*	*4*	*978.9*	*1981.2*	*202.4*	*27, 13077*
AEVP Cartridge based	*59*	*3*	*693.4*	*586.1*	*84.5*	*72, 3006*
8-Epi-prostaglandin F2α (ng/g creatinine)						
AS	57	5	480.9	435.3	90.5	36, 3047
AEVP	127	5	342.7	275.2	80.3	52, 1897
AEVP Tank based	*68*	*2*	*334.1*	*281.8*	*84.4*	*52, 1897*
AEVP Cartridge based	*59*	*3*	*352.7*	*269.5*	*76.4*	*57, 1633*
Soluble intercellular adhesion molecule-1 (ng/mL)						
AS	55	7	266.89	101.4	38.0	21.1, 538.0
AEVP	117	15	217.92	103.6	47.5	21.1, 642.2
AEVP Tank based	*66*	*4*	*227.58*	*120.2*	*52.8*	*21.1, 642.2*
AEVP Cartridge based	*51*	*11*	*205.42*	*76.4*	*37.2*	*21.1, 416.3*

AEVP = adult e-vapor product users; AS = adult smokers; CI = confidence interval; CV = coefficient of variation; *N* = number of participants; SD = standard deviation.

The observations for the AEVP subgroups (Tank based and Cartridge based) are shown in italics. Since many of the participants in these sub-groups reported dual use of tank and cartridge based products, caution should be used when drawing any specific inferences regarding observations for the different types of EVPs.

for 11-dehydrothromboxane B2 (LS geometric mean, p = .0433), 23% lower levels for 8-epi-prostaglandin F2α (LS geometric mean, p = 0.0194), and 16% lower levels for sICAM-1 (LS mean, p = .0165). The levels of HDL-C (LS mean, p > .05) were observed to be 2% higher in AEVP users relative to AS (Table 3). In general, similar trends were observed for BOPH levels for the predominant tank and cartridge-based AEVP compared with AS is shown in Table 2.

Discussion

We report here real-world evidence regarding BOE and BOPH in adult-exclusive EVP users and adult cigarette smokers using their own products under actual use conditions. Additionally, we provide the first report of BOE and BOPH in EVP users. The study adds to the growing body of evidence regarding the potential health effects of EVPs relative to cigarette smoking. Overall, statistically significantly

Table 3. Adjusted Model for Biomarkers of Exposure to Specific Tobacco Constituents and Biomarkers of Potential Harm in Adult Smokers and Adult-Exclusive Users of EVP

Group	N[a]	LS mean [95% CI]	LS mean ratio or difference [95% CI] (Group vs. AS)	*p*-Value[b]
Biomarkers of exposure				
Total NNAL (ng/g creatinine)[c]				
AS	57	230.1 [130.2, 406.7]		
AEVP	126	31.6 [20.7, 48.2]	0.14 [0.07, 0.26]	<0.0001
AEVP Tank based	*67*	*35.0 [19.1, 64.0]*	*0.15 [0.07, 0.33]*	*<0.0001*
AEVP Cartridge based	*59*	*28.6 [16.3, 50.1]*	*0.12 [0.06, 0.27]*	*<0.0001*
Nicotine equivalents (mg/g creatinine)				
AS	57	10.0 [7.9, 12.0]		
AEVP	127	6.4 [4.9, 7.9]	-3.5 [-5.9, -1.2]	0.0035
AEVP Tank based	*68*	*6.4 [4.9, 7.9]*	*-3.5 [-5.9, -1.2]*	*0.0035*
AEVP Cartridge based	*59*	*7.1 [4.9, 9.3]*	*-2.9 [-5.6, -0.2]*	*0.0385*
3-Hydroxypropylmercapturic acid (μg/g creatinine)[c]				
AS	54	1232.4 [942.9, 1610.8]		
AEVP	124	666.0 [545.1, 813.7]	0.54 [0.40, 0.74]	0.0001
AEVP Tank based	*65*	*677.1 [504.6, 908.5]*	*0.55 [0.38, 0.79]*	*0.0016*
AEVP Cartridge based	*59*	*655.1 [505.2, 849.6]*	*0.53 [0.37, 0.77]*	*0.0008*
Carboxyhemoglobin (% saturation)				
AS	62	4.1 [3.5, 4.9]		
AEVP	131	2.2 [1.9, 2.5]	0.53 [0.43, 0.66]	<0.0001
AEVP Tank based	*70*	*2.2 [1.8, 2.7]*	*0.54 [0.42, 0.69]*	*<0.0001*
AEVP Cartridge based	*61*	*2.2 [1.8, 2.6]*	*0.53 [0.41, 0.68]*	*<0.0001*
Biomarkers of potential harm				
White blood cells ($\times 10^3/\mu L$)				
AS	62	6.9 [6.4, 7.5]		
AEVP	128	6.3 [5.9, 6.7]	0.91 [0.82, 1.00]	0.0588
AEVP Tank based	*68*	*6.1 [5.6, 6.7]*	*0.89 [0.79, 0.99]*	*0.0404*
AEVP Cartridge based	*60*	*6.4 [5.9, 7.0]*	*0.93 [0.83, 1.05]*	*0.2404*
High-density lipoprotein cholesterol (mg/dL)				
AS	60	56.5 [52.9, 60.0]		
AEVP	124	57.9 [55.1, 60.6]	1.4 [-3.1, 5.9]	0.5382
AEVP Tank based	*65*	*58.8 [54.9, 62.7]*	*2.4 [-2.9, 7.6]*	*0.3768*
AEVP Cartridge based	*59*	*56.9 [53.0, 60.8]*	*0.4 [-4.8, 5.7]*	*0.8692*
11-Dehydrothromboxane B2 (ng/g creatinine)[c]				
AS	57	664.8 [499.3, 885.2]		
AEVP	125	471.4 [379.5, 585.6]	0.71 [0.51, 0.99]	0.0433
AEVP Tank based	*66*	*461.7 [336.0, 634.4]*	*0.69 [0.47, 1.03]*	*0.0696*
AEVP Cartridge based	*59*	*481.3 [362.7, 638.6]*	*0.72 [0.49, 1.07]*	*0.1056*
8-Epi-prostaglandin F2α (ng/g creatinine)[c]				
AS	57	374.1 [309.9, 451.5]		
AEVP	127	288.6 [251.1, 331.7]	0.77 [0.62, 0.96]	0.0194
AEVP Tank based	*68*	*283.9 [232.6, 346.5]*	*0.76 [0.59, 0.98]*	*0.0318*
AEVP Cartridge based	*59*	*293.4 [243.6, 353.3]*	*0.78 [0.61, 1.01]*	*0.0644*
Soluble intercellular adhesion molecule-1 (ng/mL)				
AS	55	266.4 [239.3, 293.5]		
AEVP	117	224.5 [203.8, 245.2]	-41.9 [-76.1, -7.8]	0.0165
AEVP Tank based	*66*	*245.1 [216.7, 273.6]*	*-21.3 [-60.4, 17.9]*	*0.2853*
AEVP Cartridge based	*51*	*203.8 [173.2, 234.4]*	*-62.6 [-103.7, -21.5]*	*0.0030*

AEVP = adult e-vapor product users; AS = adult smokers; CI = confidence interval; Geo=geometric; LS = least squares; N = number of participants.

[a]*N* was the number of observations from each group used in the ANOVA.

[b]*p*-Value for comparison between groups from ANOVA.

[c]LS geometric mean and corresponding ratio was calculated (rather than LS mean).

Note: ANOVA model includes the biomarker as the response variable and study group, age group (30–<45, ≥45), gender, BMI group at PSC visit (<25, ≥25), race (black, non-black), study group by age group, and study group by BMI group at PSC visit as classification model terms.

The observations for the AEVP subgroups (Tank based and Cartridge based) are shown in italics. Since many of the participants in these sub-groups reported dual use of tank and cartridge based products, caution should be used when drawing any specific inferences regarding observations for the different types of EVPs

lower levels of BOE (total NNAL, urinary nicotine equivalents, 3-HMPA, and COHb) and BOPH (11-dehydrothromboxane B2, 8-epi-prostaglandin F2α, and sICAM-1) were observed in AEVP compared with AS. Although not statistically significant, favorable differences were observed for WBC count and HDL-C in AEVP compared with AS.

We demonstrate the feasibility of using a novel virtual study design for examining BOE and BOPH in a real-world setting.

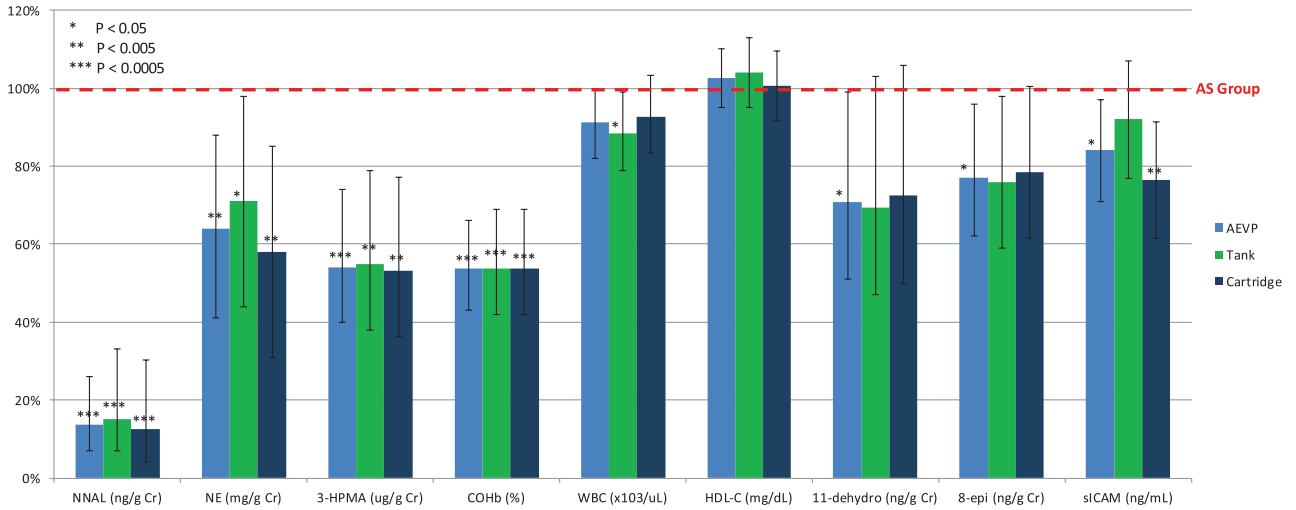

Figure 1. Biomarkers of exposure to HPHCs and biomarkers of potential harm among EVP users (represented as a percentage of AS). AEVP $N = 132$; Tank $N = 70$, Cartridge $N = 62$. COHb = carboxyhemoglobin; 11-dehydro = 11-dehydrothromboxane B2; 8-epi = 8-epi-prostaglandin F2α; HDL-C = high-density lipoprotein cholesterol; 3-HPMA = 3-hydroxypropylmercapturic acid; NE = nicotine equivalents; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and its glucuronides; sICAM-1 = soluble intercellular adhesion molecule-1; WBC = white blood cell count. Note: All *p*-values determined from the statistical model (Table 3); NE, HDL-C, and SICAM differences converted to ratios for illustrative purposes.

Utilization of the local laboratory network reduced the effort typically required for site recruitment and oversight by individual investigators while providing the opportunity to collect biomarker data among a reasonably sized sample population.

The mechanisms of diseases attributed to smoking are complex and multifaceted. Cigarette smoke consists of thousands of chemicals[31]; many of which are identified as contributing to the harmful effects of smoking.[1] Continuous exposure to HPHCs affects multiple organ systems, disease pathways, and mechanisms—inflammation, oxidative stress, platelet activation, and lipid metabolism to name a few—which eventually leads to the development of smoking-related diseases. In this study, we characterized the potential health impact of EVPs in adult smokers using these products for a minimum duration of 6 months. Specifically, we evaluated exposure to select chemicals identified by FDA as HPHCs. These select HPHCs are widely recognized, used in tobacco research, and are identified by FDA as toxicants of representative organ systems.[32] For instance, NNK is listed as a carcinogen, acrolein as a respiratory and cardiovascular toxicant, nicotine and carbon monoxide as reproductive or developmental toxicants, and nicotine as addictive. Cigarette smokes switching to exclusive EVP use should experience reductions in exposure to HPHCs; however, the potential exposure to inhaled EVP constituents (some that may not have yet been identified) is not represented through these BOEs. EVP users had statistically significant lower levels than AS for all BOEs measured in the study. These observations were comparable with those observed by Goniewicz et al.[11] and Hecht et al.[23] in similar cross-sectional studies. Substantially lower levels of exposure to the carcinogen NNK by approximately 86% as well as other HPHCs suggest that EVPs may present a lower likelihood of diseases upon switching completely from cigarette smoking. Indeed, several factors including duration and intensity of smoking, individual susceptibility will influence the ultimate disease outcome.

While levels of NNK have not been reportedly detectable in EVP aerosols,[17] some individuals had high levels of urinary NNAL approaching that for AS. These observations could be attributed to some level of smoking or some proportion of the NNAL levels could be from secondhand cigarette smoke exposure.[33] Further analysis indicated that majority of EVP users (62% tank, 55% cartridge) had COHb levels of ≤2% saturation confirming smoking abstinence. However, a small proportion of AEVP (17% tank, 25% cartridge) exhibited levels of COHb that exceeded 5% saturation. As EVP are non-combustible products and therefore do not generate carbon monoxide,[17] the observed levels of >5% saturation suggests that a select group of AEVP were not exclusive users and may have been smoking cigarettes. Similar observations have also been reported for urinary NNAL levels. An analysis of Wave 1 data from the PATH study by Goniewicz et al.[34] revealed that approximately 15% of surveyed individuals reporting exclusive EVP use had NNAL levels above the threshold determined by the authors as "no cigarette use" (14.5 pg/mg creatinine). Thus, poly-tobacco use in a small subset of AEVP users might explain the relatively large variability observed in this study with regards to total NNAL in AEVP relative to AS (%CV 152.1% and 99.7%, respectively). Some of the variability may also be attributed to the differences in the wide range of products with varying constituent yields. Nevertheless, evidence from this study indicates that AEVP, on average, were exposed to statistically significant and substantially lower levels of many HPHCs. Additionally, there were no noticeable trends observed between the tank-based and cartridge-based AEVP users. Given that AEVP users were dual users between the tank and cartridge-based products, no specific inference could be drawn from the observations for the different types of EVPs. Although AEVP were vaping with products that contained nicotine, the exposure to nicotine—as determined by urinary nicotine equivalents—was significantly lower (by ~35%) compared with AS in this study. Similar observations have been reported by others.[23,35,36] One of the reasons for the lower nicotine exposure in AEVP user could be that EVPs do not deliver as much nicotine as combustible cigarettes, thus resulting in lower exposure. Nevertheless, the long-term impact of lower nicotine exposure is unclear and EVPs should still be considered addictive.

This is the first systematic assessment of BOEs and BOPHs in a single study. We selected the BOPHs based on the underlying mechanistic principle that oxidative stress and chronic inflammation are

hallmarks of smoking-related diseases.[37–41] As mentioned in the 2010 Surgeon General's report on tobacco and smoking-attributable disease, these mechanisms are a common thread among the three major smoking-related diseases—lung cancer, chronic obstructive pulmonary disease, and cardiovascular disease.[41] And there is published evidence to support this notion. For example, a decrease in WBC count of 1,000 µL has been associated with a 14% decrease in the risk of cardiovascular disease death.[42] Furthermore, epidemiological studies suggest every 2% to 3% increase in HDL-C (independent of low-density lipoprotein cholesterol) is associated with a 2% to 4% reduction in cardiovascular disease events.[43] We observed statistically significantly lower levels in several BOPHs mechanistically linked to smoking-related diseases; for example, oxidative stress (8-epi-prostaglandin F2α), platelet activation (11-dehydrothromoxane B2), and endothelial dysfunction (sICAM-1) in AEVP relative to AS. The levels of these BOPHs trended in favorable directions, approaching that observed after smoking cessation.[44–47] We note that the BMI of AEVP users tended to be higher although not statistically significant (p = .066). Nominally higher proportions of individuals in the > 25 kg/m^2 group were observed amongst the AEVP users (~74%) compared with AS (~61%). While the smaller magnitude of difference in HDL cholesterol cannot be directly attributed to these differences in body weight, Aubin et al.,[48] based on a meta-analysis of published literature, reported a mean increase of 4–5 kg in body weight after 12 months of abstinence. However, given that baseline bodyweight of the AEVP group was not available before switching to EVP, we cannot definitively attribute the observations regarding HDL cholesterol to potential weight gain.

While it is difficult to make comparisons across different studies given the variabilities associated with difference between the two populations, the BOE and BOPH levels in AEVP users appear to be trending in the same direction as observed in AS after 6 months of smoking abstinence.[49] The observations related to BOPH corroborate with substantially low levels of BOEs to select HPHCs and suggest that if such low levels are sustained over a long enough time period, smoking-related disease risks could be lowered among AEVP compared with AS.

The conclusions drawn from this study should be considered in lieu of its limitations. Since this was a cross-sectional study, baseline assessment of BOE and BOPH was not available as would be in a controlled study; thus, changes in the levels of biomarkers over time could not be determined. The implications of this study on reduction of health risk should be approached with caution due to the uncertainty in the extent of possible in BOE and BOPHs in the AEVP users switching from smoking to exclusive EVP use. We attempted to offset this limitation by measuring BOEs and BOPHs in matched control group of participants (AEVP to AS). Group allocations for the study were based on self-reported product use, which are inevitable in any ambulatory study, but may be subject to reporting bias. Additionally, adherence to "exclusive use" prior to and during the study could not be verified until after the biomarker specimens were analyzed. Furthermore, there was no measure that captured the progression of product use over time. The potential for poly-use of tobacco products during the study, in combination with differences in ad libitum use patterns and rapidly evolving EVP designs, might lend support to the large coefficient of variation (>50%) observed for several biomarkers. Although the study was powered to detect differences in the BOE, urinary total NNAL, the study was not formally powered to detect differences in BOPHs; thus, the interpretation of these results may be limited. Since the sampling was not random, results from this study cannot be generalized to all EVP users and cigarette smokers. Given the dynamic transitions in tobacco use patterns, the results of this study may not be generalizable to other sub-populations of poly tobacco users (e.g., poly tobacco users of cigarettes and smokeless tobacco and/or heat-not-burn tobacco products). Nevertheless, the value of obtaining real-world evidence under actual use conditions with participants using their own products without any restrictions should be considered when assessing the limitations of this study.

This study adds to a growing body of evidence suggesting the relatively lower likelihood of smoking-related disease risks in adult-exclusive users of EVP versus adult smokers who continue to use combustible tobacco products. The results provide evidence that EVPs (former smokers of conventional cigarettes with at least 6 months of EVP use) are associated with lower exposure to specific smoke constituents (NNK, nicotine, acrolein, and carbon monoxide) and favorable differences in the biomarkers key to monitoring long-term effects of tobacco use (platelet activation, oxidative stress, and endothelial function). Taken together, we conclude that switching completely to EVPs may offer an opportunity to lower the harmful effects of smoking compared to continuing to use conventional cigarettes.

Funding

This work was funded by Altria Client Services LLC.

Declarations of Interests

MS is currently employed with Altria Client Services LLC. DO and QL are former employees of Altria Client Services LLC.

Acknowledgments

The authors thank Drs. Maria Gogova and Jeff Edmiston (Altria Client Services LLC), for their insightful discussions regarding the design and interpretation of results from this study. The authors also sincerely appreciate the contributions of the staff involved in the study. They also thank Teresa A. Oblak, PhD, CMPP, of Covance Market Access Services Inc., for providing research support, coordination assistance, and editorial contributions. Select data from the manuscript were presented in poster format at the 24th annual meeting of the Society of Research for Nicotine and Tobacco in Baltimore, Maryland, 21–24 February 2018.

References

1. 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.
2. Colilla SA. An epidemiologic review of smokeless tobacco health effects and harm reduction potential. *Regul Toxicol Pharmacol*. 2010;56(2):197–211.
3. Fairchild AL, Lee JS, Bayer R, Curran J. E-cigarettes and the harm-reduction continuum. *N Engl J Med*. 2018;378(3):216–219.
4. Gartner C, Hall W. The potential role of snus in tobacco harm reduction. *Addiction*. 2009;104(9):1586–1587.
5. Haziza C, de La Bourdonnaye G, Donelli A, et al. Reduction in exposure to selected harmful and potentially harmful constituents approaching those observed upon smoking abstinence in smokers switching to the menthol tobacco heating system 2.2 for three months (Part 1). *Nicotine Tob Res*. 2020;22(4):539–548.
6. Timberlake DS, Nikitin D, Johnson NJ, Altekruse SF. A longitudinal study of smokeless tobacco use and mortality in the United States. *Int J Cancer*. 2017;141(2):264–270.

7. Abrams DB, Glasser AM, Pearson JL, et al. Harm minimization and tobacco control: Reframing societal views of nicotine use to rapidly save lives. *Annu Rev Public Health*. 2018;39:193–213.
8. Nutt DJ, Phillips LD, Balfour D, et al. Estimating the harms of nicotine-containing products using the MCDA approach. *Eur Addict Res*. 2014;20(5):218–225.
9. Zeller M, Hatsukami D; Strategic Dialogue on Tobacco Harm Reduction Group. The strategic dialogue on tobacco harm reduction: A vision and blueprint for action in the US. *Tob Control*. 2009;18(4):324–332.
10. Gottlieb S, Zeller M. A nicotine-focused framework for public health. *N Engl J Med*. 2017;377(12):1111–1114.
11. Goniewicz ML, Gawron M, Smith DM, et al. Exposure to nicotine and selected toxicants in cigarette smokers who switched to electronic cigarettes: A longitudinal within-subjects observational study. *Nicotine Tob Res*. 2017;19(2):160–167.
12. Benowitz NL. Emerging nicotine delivery products. Implications for public health. *Ann Am Thorac Soc*. 2014;11(2):231–235.
13. Bhatnagar A, Whitsel LP, Ribisl KM, et al.; American Heart Association Advocacy Coordinating Committee, Council on Cardiovascular and Stroke Nursing, Council on Clinical Cardiology, and Council on Quality of Care and Outcomes Research. Electronic cigarettes: A policy statement from the American Heart Association. *Circulation*. 2014;130(16):1418–1436.
14. Hajek P, Etter JF, Benowitz N, Eissenberg T, McRobbie H. Electronic cigarettes: Review of use, content, safety, effects on smokers and potential for harm and benefit. *Addiction*. 2014;109(11):1801–1810.
15. Smith TT, Hatsukami DK, Benowitz NL, et al. Whether to push or pull? Nicotine reduction and non-combusted alternatives - Two strategies for reducing smoking and improving public health. *Prev Med*. 2018;117:8–14.
16. Goniewicz ML, Knysak J, Gawron M, et al. Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control*. 2014;23(2):133–139.
17. Wagner KA, Flora JW, Melvin MS, et al. An evaluation of electronic cigarette formulations and aerosols for harmful and potentially harmful constituents (HPHCs) typically derived from combustion. *Regul Toxicol Pharmacol*. 2018;95:153–160.
18. Bullen C, Howe C, Laugesen M, et al. Electronic cigarettes for smoking cessation: A randomised controlled trial. *Lancet*. 2013;382(9905):1629–1637.
19. Caponnetto P, Campagna D, Cibella F, et al. EffiCiency and Safety of an eLectronic cigAreTte (ECLAT) as tobacco cigarettes substitute: A prospective 12-month randomized control design study. *PLoS One*. 2013;8(6):e66317.
20. Carpenter MJ, Heckman BW, Wahlquist AE, et al. A naturalistic, randomized pilot trial of e-cigarettes: Uptake, exposure, and behavioral effects. *Cancer Epidemiol Biomarkers Prev*. 2017;26(12):1795–1803.
21. Cravo AS, Bush J, Sharma G, et al. A randomised, parallel group study to evaluate the safety profile of an electronic vapour product over 12 weeks. *Regul Toxicol Pharmacol*. 2016;81(Suppl 1):S1–S14.
22. Goniewicz ML, Smith DM, Edwards KC, et al. Comparison of nicotine and toxicant exposure in users of electronic cigarettes and combustible cigarettes. *JAMA Netw Open*. 2018;1(8):e185937.
23. Hecht SS, Carmella SG, Kotandeniya D, et al. Evaluation of toxicant and carcinogen metabolites in the urine of e-cigarette users versus cigarette smokers. *Nicotine Tob Res*. 2015;17(6):704–709.
24. Polosa R, Morjaria JB, Prosperini U, et al. Health effects in COPD smokers who switch to electronic cigarettes: A retrospective-prospective 3-year follow-up. *Int J Chron Obstruct Pulmon Dis*. 2018;13:2533–2542.
25. Sarkar M, Muhammad-Kah R, Liang Q, et al. Evaluation of spot urine as an alternative to 24h urine collection for determination of biomarkers of exposure to cigarette smoke in adult smokers. *Environ Toxicol Pharmacol*. 2013;36(1):108–114.
26. Sarkar M, Kapur S, Frost-Pineda K, et al. Evaluation of biomarkers of exposure to selected cigarette smoke constituents in adult smokers switched to carbon-filtered cigarettes in short-term and long-term clinical studies. *Nicotine Tob Res*. 2008;10(12):1761–1772.
27. Roethig HJ, Munjal S, Feng S, et al. Population estimates for biomarkers of exposure to cigarette smoke in adult U.S. cigarette smokers. *Nicotine Tob Res*. 2009;11(10):1216–1225.
28. Wang J, Liang Q, Mendes P, Sarkar M. Is 24 h nicotine equivalents a surrogate for smoke exposure based on its relationship with other biomarkers of exposure? *Biomarkers*. 2011;16(2):144–154.
29. Goniewicz ML, Havel CM, Peng MW, et al. Elimination kinetics of the tobacco-specific biomarker and lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. *Cancer Epidemiol Biomarkers Prev*. 2009;18(12):3421–3425.
30. Liu J, Liang Q, Frost-Pineda K, et al. Relationship between biomarkers of cigarette smoke exposure and biomarkers of inflammation, oxidative stress, and platelet activation in adult cigarette smokers. *Cancer Epidemiol Biomarkers Prev*. 2011;20(8):1760–1769.
31. Rodgman A, Perfetti T. *The Chemical Components of Tobacco and Tobacco Smoke*. Boca Rotan, FL: CRC Press, Taylor and Francis Group; 2009.
32. FDA US. Harmful and potentially harmful constituents in tobacco products and tobacco smoke; established list. *Federal Register*. 2012;77:20034–20037.
33. Kim J, Lee K, Kwon HJ, et al. Association between secondhand smoke in hospitality venues and urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol concentrations in non-smoking staff. *Int J Environ Res Public Health*. 2016;13:1101–1112.
34. Goniewicz ML, Smith DM. Are some E-cigarette users "blowing smoke"?: Assessing the accuracy of self-reported smoking abstinence in exclusive e-cigarette users. *Nicotine Tob Res*. 2019;21(5):699–700.
35. Round EK, Chen P, Taylor AK, Schmidt E. Biomarkers of tobacco exposure decrease after smokers switch to an e-cigarette or nicotine gum. *Nicotine Tob Res*. 2019;21(9):1239–1247.
36. Shahab L, Goniewicz ML, Blount BC, et al. Nicotine, carcinogen, and toxin exposure in long-term e-cigarette and nicotine replacement therapy users: A cross-sectional Study. *Ann Intern Med*. 2017;166(6):390–400.
37. Barnes PJ. New concepts in chronic obstructive pulmonary disease. *Annu Rev Med*. 2003;54:113–129.
38. Barnes PJ, Shapiro SD, Pauwels RA. Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. *Eur Respir J*. 2003;22(4):672–688.
39. Dourado VZ, Tanni SE, Vale SA, et al. Systemic manifestations in chronic obstructive pulmonary disease. *J Bras Pneumol*. 2006;32(2):161–171.
40. Milara J, Cortijo J. Tobacco, inflammation, and respiratory tract cancer. *Curr Pharm Des*. 2012;18(26):3901–3938.
41. U.S. Department of Health and Human Services. *How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease: A Report of the Surgeon General*. Atlanta, GA: Centers for Disease Control and Prevention; 2010.
42. Brown DW, Giles WH, Croft JB. White blood cell count: An independent predictor of coronary heart disease mortality among a national cohort. *J Clin Epidemiol*. 2001;54(3):316–322.
43. Charland SL, Malone DC. Prediction of cardiovascular event risk reduction from lipid changes associated with high potency dyslipidemia therapy. *Curr Med Res Opin*. 2010;26(2):365–375.
44. Flores L, Vidal M, Abian J, et al. The effects of smoking and its cessation on 8-epi-PGF2alpha and transforming growth factor-beta 1 in Type 1 diabetes mellitus. *Diabet Med*. 2004;21(3):285–289.
45. Palmer RM, Stapleton JA, Sutherland G, et al. Effect of nicotine replacement and quitting smoking on circulating adhesion molecule profiles (sICAM-1, sCD44v5, sCD44v6). *Eur J Clin Invest*. 2002;32(11):852–857.
46. van der Plas A, Pouly S, de La Bourdonnaye G, et al. Influence of smoking and smoking cessation on levels of urinary 11-dehydro thromboxane B2. *Toxicol Rep*. 2018;5:561–567.
47. Forey BA, Fry JS, Lee PN, Thornton AJ, Coombs KJ. The effect of quitting smoking on HDL-cholesterol - a review based on within-subject changes. *Biomark Res*. 2013;1(1):26.
48. Aubin HJ, Farley A, Lycett D, Lahmek P, Aveyard P. Weight gain in smokers after quitting cigarettes: Meta-analysis. *BMJ*. 2012;345:e4439.
49. L. Felber Medlin CTT, W. T. Ng, N. Lama, et al. A multicenter, multiregional study on biological and functional changes in healthy adult smokers during one year of continuous smoking abstinence. San Francisco, CA: Society for Research on Nicotine and Tobacco; 2019.