ORIGINAL ARTICLE
E-CIGARETTES

Systemic biomarkers in electronic cigarette users: implications for noninvasive assessment of vaping-associated pulmonary injuries

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ABSTRACT

Background: Electronic cigarettes (e-cigs) were introduced as electronic nicotine delivery systems, and have become very popular in the USA and globally. There is a paucity of data on systemic injury biomarkers of vaping in e-cig users that can be used as a noninvasive assessment of vaping-associated lung injuries. We hypothesised that characterisation of systemic biomarkers of inflammation, anti-inflammatory, oxidative stress, vascular and lipid mediators, growth factors, and extracellular matrix breakdown may provide information regarding the toxicity of vaping in e-cig users.

Methods: We collected various biological fluids, *i.e.* plasma, urine, saliva and exhaled breath condensate (EBC), measured pulmonary function and vaping characteristics, and assessed various biomarkers in e-cig users and nonusers.

Results: The plasma samples of e-cig users showed a significant increase in biomarkers of inflammation (interleukin (IL)-1β, IL-6, IL-8, IL-13, interferon (IFN)-γ, matrix metalloproteinase-9, intercellular cell adhesion molecule-1) and extracellular matrix breakdown (desmosine), and decreased pro-resolving lipid mediators (resolvin D_1 and resolvin D_2). There was a significant increase in growth factor (endothelial growth factor, vascular endothelial growth factor, β-nerve growth factor, platelet-derived growth factor-AA, stem cell factor, hepatocyte growth factor and placental growth factor) levels in plasma of e-cig users *versus* nonusers. E-cig users showed a significant increase in levels of inflammatory biomarker IFN-γ, oxidative stress biomarker 8-isoprostane and oxidative DNA damage biomarker 8-oxo-dG in urine samples, and of inflammatory biomarker IL-1β in saliva samples. EBC showed a slight increase in levels of triglycerides and 8-isoprostane in e-cig users compared with normal nonusers.

Conclusion: E-cig users have increased levels of biomarkers of inflammation and oxidative stress, reduced pro-resolving anti-inflammatory mediators, and endothelial dysfunction, which may act as risk factors for increasing susceptibility to systemic diseases. The identified noninvasive biomarkers can be used for determining e-cig vaping-associated lung injuries, and for regulatory and diagnostic aspects of vaping in humans.

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E-cig use adversely affects oxidative stress and inflammatory responses, and induces tissue remodelling. The identified biomarkers can be used for assessment of vaping-associated lung injuries, and for regulatory and diagnostic aspects of vaping in humans. http://bit.ly/2nxZQ8R

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Introduction

Electronic cigarettes (e-cigs) have gained recent popularity in the USA and throughout the world. Despite the perception among e-cig users that e-cig vaping is safer than cigarette smoking, there are large numbers of epidemiological studies showing its effects otherwise. E-cigs are battery-operated devices that generate inhalable aerosol known as vapour. This vapour is created by heating the "e-liquid mixture" that has been filled inside the e-cig. E-cig liquid contains propylene glycol, vegetable glycerin, nicotine in various concentrations, flavouring agents and other additive compounds [1]. Recently, various other additives including terpenes, mineral oil, organic compounds and adulterated compounds have been added to e-cig juices and various vaping products. The vapour generated by e-cigs is composed of ultrafine particles which contain numerous toxic chemicals, including acetaldehyde, acrolein, toluene and formaldehyde, in lower concentrations than in cigarette smoke [2]. Increased levels of aldehydes (*e.g.* formaldehyde, acrolein and acetaldehyde) were found in the exhaled breath of e-cig users during vaping [3].

Flavouring chemicals present in e-cig liquid, such as diacetyl (2,3-butanedione) and 2,3-pentanedione, have been associated with bronchiolitis obliterans syndrome (popcorn lung disease) [4]. Recent studies suggest that e-cig use can change vital signs and symptoms, such as heart rate and blood pressure, in users and affect the cardiovascular system [5–9]. A single use of e-cigs can produce oxidative stress and endothelial cell dysfunction [10, 11]. In addition, the rapid increase in circulating endothelial progenitor cells found in the blood of e-cig users was comparable to the cell numbers found in cigarette smokers, which may be a sign of acute endothelial cell dysfunction and vascular injury [12]. E-cig users have increased neutrophil activation, changes in mucin secretion and altered innate immune response in the lungs [13]. Moreover, e-cig users have impaired lung function and these changes exhibited peripheral obstructive airway involvement [14]. In a recent study, e-cig use has been found to be associated with lung inflammation through impaired systemic inflammation signalling [15]. E-cig users exposed to these harmful organic and inorganic compounds (including metals) are expected to be more susceptible to develop cancer than nonusers [16]. Furthermore, these chemical compounds in e-cigs may pose a risk of asthma [17, 18].

Studies on systemic biomarkers of inflammation, oxidative stress, platelet activation and systemic immune markers in cigarette smokers [19, 20] and systemic serum biomarkers in chronic obstructive pulmonary disease (COPD) patients are abundant [21–23]. However, there is a gap in knowledge on systemic biomarkers related to the aforementioned parameters in blood, urine, saliva and exhaled breath condensate (EBC) analysis in e-cig users. We hypothesise that detailed characterisation of various systemic noninvasive biomarkers of inflammation, oxidative stress, growth factors, vascular mediators, lipid mediators and extracellular matrix breakdown may provide information regarding molecular mechanisms through which e-cigs cause cellular/tissue damage. Hence, we determined selective biomarkers of inflammation, oxidative stress, pro-resolving mediators, triglycerides, growth factors and lung damage in plasma, urine, saliva and EBC of e-cig users and normal nonusers. We further implicated that these noninvasive markers may serve as biomarkers of vaping-associated lung injuries.

Methods

Participants and procedure

The study was conducted at the University of Rochester Medical Center (Rochester, NY, USA) (Institutional Review Board approval RSRB00064337). The study participants were recruited/enrolled at the General Clinical Research Center of the University of Rochester Medical Center through various local newspaper and magazine advertisements, word of mouth, and flyers posted in and around the university campus. The participants were selected based on a self-reported questionnaire containing information about demographic variables, e-cig vaping history and vaping behaviour.

All the potential participants identified were screened for eligibility by using the following criteria: 1) age 21–65 years, 2) normal subjects to have never used any tobacco products, 3) e-cig users to have not used other forms of tobacco products or smoking devices (*e.g.* cigarettes or waterpipes), 4) none of the participants to have a history of chronic disease, such as lung and heart diseases, diabetes and cancer, 5) none of the participants to have respiratory infections or be using any anti-inflammatory and/or corticosteroid drugs, and 6) female participants to not be currently breastfeeding or pregnant. Written informed consent was obtained from all study participants.

Study design

This study was planned to analyse various biomarkers in the biological samples of e-cig users. Before sample collection from each subject, body mass index (BMI) was calculated and lung function testing was performed. Collected biological samples included plasma, urine, saliva and EBC. Venous blood (20–25 mL) was collected and the plasma samples were stored immediately at −80°C until analysed. Urine

samples (20–25 mL) provided by participants were aliquoted and stored in a freezer at −20°C until analysed. SalivaBio Swabs (Salimetrics, Newmarket, UK) were used for the collection of saliva samples from participants for various analyses. The collection procedure was followed as per the vendor's instructions. The samples were stored at −80°C. The participants were asked to refrain from eating, drinking and vaping for at least 3 h prior to EBC collection, and samples were obtained prior to lung function assessments. EBC samples were collected during tidal breathing for 10 min using an RTube collection device (Respiratory Research, Charlottesville, VA, USA). Samples were aliquoted and stored at −80°C.

Demographics and e-cig vaping status

The participants provided demographic information which included their age, sex and ethnicity. They also provided information regarding e-cig vaping duration, vaping frequency and vaping sessions. The study participants were divided into two groups: normal nonusers (n=26) and e-cig users (n=22) (table 1).

Measurement of pulmonary function

Pulmonary function tests were performed using a portable spirometer (Vitalograph micro 6300; Vitalograph, Lenexa, KS, USA). Forced expiratory volume in 1 s (FEV_1), FEV_1 % pred, forced vital capacity (FVC) %, peak expiratory flow (PEF) % pred and forced expiratory ratio (FEV_1/FVC %) were recorded. The best of three attempts of the forced manoeuvre was measured.

Measurement of plasma cotinine

The plasma cotinine concentration was measured in plasma samples of participants to confirm the status of electronic nicotine delivery system/nicotine exposure by e-cigs using the Salimetrics high-sensitivity quantitative enzyme immunoassay kit (Salimetrics, Carlsbad, CA, USA) following the manufacturer's instructions.

Measurement of biomarkers by multiplex panel assay

Plasma and urine cytokine and chemokine biomarkers were assayed using the Luminex Human XL Cytokine Discovery Panel and a custom 9-plex Human Magnetic Luminex Assay (R&D Systems, Minneapolis, MN,

TABLE 1 Baseline demographic, anthropometric and spirometry characteristics of study participants: normal subjects and electronic cigarette (e-cig) users

	Normal subjects[#]	E-cig users[#]
Subjects	26	22
Age years		
Overall	33.88±14.07	35.54±12.21
Male	37.27±14.22	32.00±14.50
Female	31.40±13.91	38.50±9.58
Sex		
Male	11 (42.3)	10 (45.5)
Female	15 (57.7)	12 (54.5)
BMI kg·m^{-2}	24.86±5.06	27.39±5.39
Ethnicity		
White	18 (69.2)	11 (50.0)
African-American	3 (11.5)	6 (27.3)
Asian	4 (15.4)	3 (13.6)
Hispanic	1 (3.9)	2 (9.1)
E-cig vaping status		
Vaping duration years		2.00±1.64
Vaping frequency day^{-1}		7.75±3.14
Vaping session min		8.79±5.50
Lung function variables		
FEV_1 % pred	98.08±12.72	91.22±10.46
FVC %	99.17±11.69	95.72±13.20
FEV_1/FVC %	99.26±6.12	95.50±8.98
PEF % pred	84.56±18.48	76.90±19.82

Data are presented as n, mean±SD or n (%). BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PEF: peak expiratory flow. [#]: not all biological samples collected from normal subjects and e-cig users were used for each biomarker analysis.

USA). Growth factors were measured using a custom 12-plex Magnetic Luminex Assay (R&D Systems). The plasma and urine levels of interleukin (IL)-1α, IL-β, IL-6, IL-8, IL-10, IL-13, IL-33, interferon (IFN)-γ, tumour necrosis factor (TNF)-α, eotaxin, macrophage inflammatory protein (MIP)-1α, MIP-1β, granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor (GM-CSF), monocyte chemoattractant protein (MCP)-1, CXCL1, CXCL2 and RANTES were measured by the XL Cytokine Discovery Panel, while a custom 9-plex assay was used to measure S100A8, S100A9, plasminogen activator inhibitor (PAI)-1/serpine-1, matrix metalloproteinase (MMP)-9, myeloperoxidase, galectin-3, CC10/CC16, RAGE and EN-RAGE. Plasma growth factor levels of brain-derived neurotrophic factor (BDNF), bone morphogenetic protein (BMP)-2, endothelial growth factor (EGF), basic fibroblast growth factor (bFGF)/FGF2, hepatocyte growth factor (HGF), leukaemia inhibitory factor (LIF), β-nerve growth factor (NGF), platelet-derived growth factor (PDGF)-AA, placental growth factor (PIGF), stem cell factor (SCF)/c-kit ligand, transforming growth factor (TGF)-α and vascular endothelial growth factor (VEGF) were included in the custom 12-plex assay. The Luminex kits were used following the manufacturer's protocols.

Measurement of biomarkers by ELISA

Plasma levels of biomarkers were measured using commercially available ELISA kits: C-reactive protein (CRP) (Sigma, St Louis, MO, USA), desmosine (MyBiosource, San Diego, CA, USA), 8-isoprostane, prostaglandin E_2, thromboxane B_2, resolvin D_1, resolvin D_2, resolvin E_1, leukotriene E_4 (Cayman, Ann Arbor, MI, USA), fibrinogen (Abcam, Cambridge, MA, USA), intracellular adhesion molecule (ICAM)-1 (R&D Systems), 4-hydroxynonenal, malondialdehyde (Cell Biolabs, San Diego, CA, USA) and lipoxin A_4 (TSZ ELISA, Waltham, MA, USA). Urine samples were analysed for 8-isoprostane, leukotriene E_4, desmosine and 8-oxo-dG (HT 8-Oxo-dG Human ELISA; Trevigen, Gaithersburg, MD, USA). Salivary biomarkers IL-1β, IL-6, prostaglandin E_2, resolvin D_1 and resolvin D_2 were also measured. EBC samples were analysed for 8-isoprostane. All these biomarkers were measured quantitatively as per the manufacturers' instructions.

Triglyceride quantification in EBC

Triglyceride levels in EBC samples from normal nonusers and e-cig users were quantified using a fluorometric kit (BioVision, Milpitas, CA, USA) according to the manufacturer's instructions. In brief, the standards were prepared from 0–10 nmol using 0.02 mM triglyceride standard. 50 µL EBC sample and standards were pipetted into the wells and incubated with 2 µL lipase for 20 min. Subsequently, the triglyceride reaction mix was prepared with 0.4 µL·well^{-1} probe, enzyme mix and assay buffer. After adding 50 µL reaction mix to the samples and standards, the plate was incubated for 30 min prior to recording the fluorescence (excitation/emission 535/590 nm). Final triglyceride concentrations were presented in millimolar units in EBC samples.

Statistical analysis

Statistical analysis was performed using Prism version 8.0.1 (GraphPad, La Jolla, CA, USA). Categorical variables data are presented as percentages and continuous variables are presented as mean±SEM, unless otherwise indicated. Statistical significance was determined by the t-test. A p-value <0.05 was considered significant.

Results

The baseline demographic, BMI, e-cig vaping status and lung function variables are summarised in table 1. The study enrolled 48 subjects, of which 22 adult e-cig users and 26 normal subjects were examined. The mean age of e-cig users was higher compared with normal subjects. BMI was higher in e-cig users compared with normal subjects. E-cig users averaged 2.00±1.64 years of vaping for 7.75±3.14 times per day in 8.79±5.50 min sessions. The majority of participants were Caucasian, followed by African-American, Asian and Hispanic. Pulmonary function test parameters such as FEV_1 (% pred), FVC (%), FEV_1/FVC (%) and PEF (% pred) of e-cig users did not show significant changes compared with normal subjects. We did not find any significant Pearson correlation in vaping status and vaping frequency between male and female subjects in normal subjects or e-cig users.

Plasma cotinine levels were significantly higher in e-cig users (164.70±39.92 ng·mL^{-1}) compared with normal subjects (3.86±2.74 ng·mL^{-1}), indicating all users were actively vaping e-cigs containing nicotine.

Biomarkers of systemic inflammation, anti-inflammatory, oxidative stress, vascular and lipid mediators, and extracellular matrix breakdown in plasma

Plasma levels of IL-6, IL-8, IL-13 and MMP-9 were significantly higher, whereas levels of CXCL1 and RAGE were significantly lower, in e-cig users compared with normal subjects (figure 1). The levels of IL-1β, IL-33, TNF-α, IFN-γ, GM-CSF, EN-RAGE, galectin-3 and myeloperoxidase showed insignificant small decreases in e-cig users compared with normal subjects. Nonsignificant changes were observed in the levels of IL-1α, MCP-1, MIP-1α, MIP-1β, IL-10, CC10/CC16, RANTES, eotaxin, S100A8 and S100A9

(figure 1). The plasma of e-cig users showed a nonsignificant change in levels of CXCL2 (e-cig 2603±454.8 *versus* normal 3108±443.6 pg·mL^{-1}) and a significant change in levels of G-CSF (e-cig 10.86±2.03 *versus* normal 41.35±3.66 pg·mL^{-1}; p<0.001) compared with normal subjects.

The biomarkers for vascular function (ICAM-1) and extracellular matrix breakdown (desmosine) were significantly higher in e-cig users compared with normal subjects. Other biomarkers, such as fibrinogen,

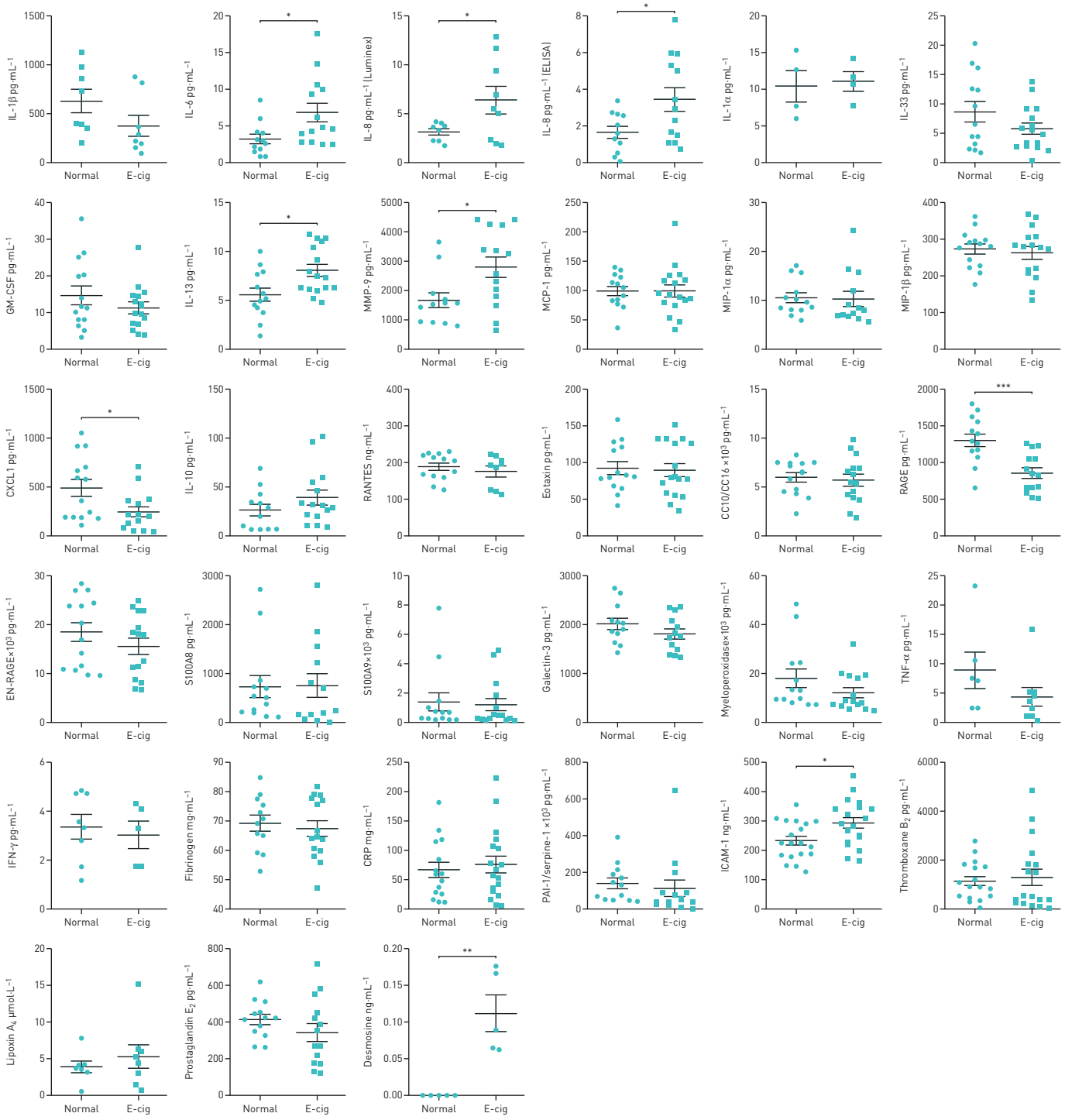

FIGURE 1 Plasma biomarkers of systemic inflammation, anti-inflammatory, vascular and lipid mediators, and extracellular matrix breakdown in normal subjects and e-cigarette (e-cig) users. IL: interleukin; GM-CSF: granulocyte–macrophage colony-stimulating factor; MMP: matrix metalloproteinase; MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; TNF: tumour necrosis factor; IFN: interferon; CRP: C-reactive protein; PAI: plasminogen activator inhibitor; ICAM: intracellular adhesion molecule. Data are presented as mean±SEM (n=4–20). *: p<0.05; **: p<0.01; ***: p<0.001.

CRP, PAI-1/serpine-1, thromboxane B_2, lipoxin A_4 and prostaglandin E_2, showed nonsignificant changes in e-cig users compared with nonusers (figure 1). Oxidative stress biomarkers in plasma, such as 8-isoprostane, 4-hydroxynonenal and malondialdehyde, showed no significant changes between the two groups (figure 2).

Biomarkers of lipid mediators for lung inflammation and resolution, such as pro-resolving resolvin D_1 and resolvin D_2, were also measured in plasma. These levels were significantly decreased in e-cig users compared with normal subjects (figure 2).

Growth factors levels in plasma samples

Plasma growth factors EGF, VEGF, β-NGF, PDGF-AA, SCF, HGF and PIGF were significantly increased in e-cig users compared with normal subjects, suggesting adverse health effects. However, the other growth factors, such as BDNF, BMP-2, TGF-α and bFGF/FGF2, showed no change in e-cig users compared with normal subjects (figure 3). The level of LIF in plasma was undetectable in both e-cig users and normal subjects.

Biomarkers of systemic inflammation, vascular mediators, oxidative stress, tissue injury repair, oxidative DNA damage and extracellular matrix breakdown in urine

Significant increases in levels of IFN-γ, 8-isoprostane and 8-oxo-dG were shown in urine samples of e-cig users compared with normal subjects. Nonsignificant increased levels of MIP-1α, GM-CSF, IL-33, RANTES and MMP-9, and decreased levels of IL-8, IL-10, IL-13 and S100A8, were observed in e-cig users compared with normal subjects. Other inflammatory biomarkers, such as MCP-1, eotaxin, MIP-1α, MIP-1β, CC10/CC16, leukotriene E_4, RAGE, EN-RAGE, S100A9 and PAI-1/serpine-1, showed no change in e-cig users compared with normal subjects (figure 4a). Urine levels of IL-1α, IL-1β, IL-6, TNF-α, G-CSF and CXCL2 were undetectable. However, no change was observed in galectin-3, myeloperoxidase and desmosine levels in urine samples of e-cig users compared with normal subjects (figure 4a).

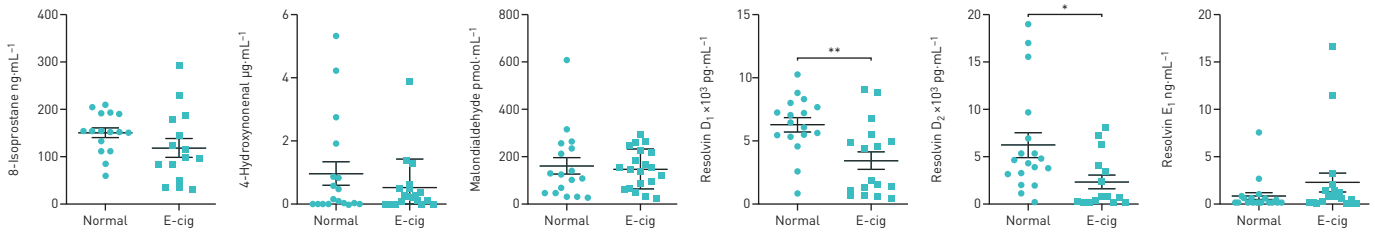

FIGURE 2 Plasma biomarkers of oxidative stress in normal subjects and e-cigarette (e-cig) users. Data are presented as mean±SEM (n=15–20). *: p<0.05; **: p<0.01.

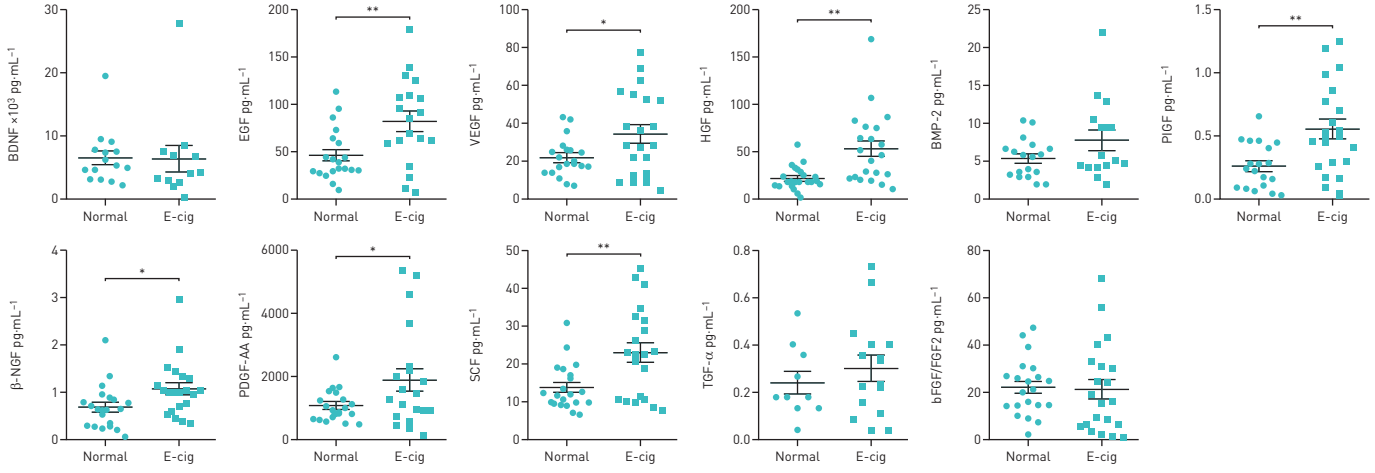

FIGURE 3 Plasma growth factors in normal subjects and e-cigarette (e-cig) users. BDNF: brain-derived neurotrophic factor; EGF: endothelial growth factor; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; BMP: bone morphogenetic protein; PlGF: placental growth factor; NGF: nerve growth factor; PDGF: platelet-derived growth factor; SCF: stem cell factor; TGF: transforming growth factor; bFGF: basic fibroblast growth factor. Data are presented as mean±SEM (n=10–21). *: p<0.05; **: p<0.01.

Biomarkers of systemic inflammation and lipid mediators in saliva

We extended our assessment of selected biomarkers, such as IL-1β, IL-6, prostaglandin E_2, resolvin D_1 and resolvin D_2, in saliva samples of e-cig users and normal subjects. The saliva samples of e-cig users showed significant increases in IL-1β compared with normal subjects (figure 4c). A nonsignificant increase of IL-6, resolvin D_2, and a decrease in resolvin D_1 and prostaglandin E_2, was observed in e-cig users compared with normal subjects (figure 4c).

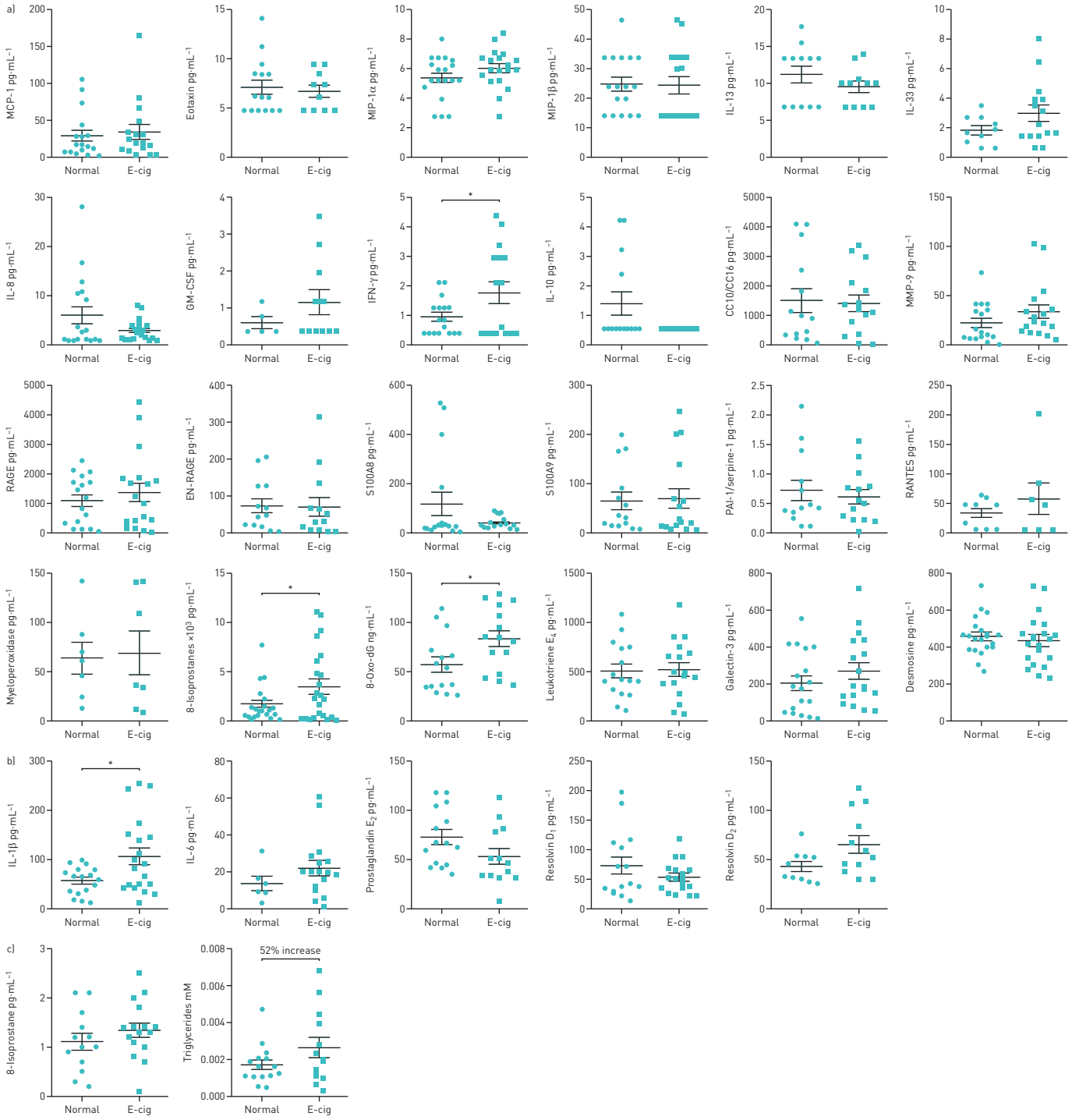

FIGURE 4 a) Urinary, b) salivary and c) exhaled breath condensate biomarkers of systemic inflammation, vascular mediators, oxidative stress, tissue injury repair, oxidative DNA damage, extracellular matrix breakdown and triglycerides in normal subjects and e-cigarette (e-cig) users. MCP: monocyte chemoattractant protein; MIP: macrophage inflammatory protein; IL: interleukin; GM-CSF: granulocyte–macrophage colony-stimulating factor; IFN: interferon; MMP: matrix metalloproteinase; PAI: plasminogen activator inhibitor. Data are presented as mean±SEM (n=5–23). *: $p<0.05$.

Biomarkers of oxidative stress in EBC

Biomarkers for oxidative stress (8-isoprostane) and lipids (triglycerides) were measured in EBC. There was a nonsignificant increase in the levels of 8-isoprostane (22% increase) and triglycerides (52% increase) in e-cig users compared with normal subjects (figure 4d).

Discussion

We carried out a comprehensive analysis of various systemic biomarkers of inflammation, oxidative stress, growth factors, vascular mediators, lipid mediators and extracellular matrix breakdown in biological samples of e-cig users and normal subjects. We found the levels of systemic biomarkers of inflammation, endothelial dysfunction, elastin breakdown, oxidative stress, pro-resolving lipid mediators and triglycerides were altered in e-cig users in various biological fluids. Plasma cotinine levels were significantly increased in e-cig users, indicating they were exposed to nicotine during vaping e-cigs.

It is perceived that e-cig users have significantly increased levels of oxidative stress marker 8-isoprostane and oxidative DNA damage marker 8-oxo-dG. 8-isoprostane is formed *in vivo* by free radical peroxidation of arachidonic acid and levels in biological fluids indicate oxidative stress *in vivo*. Cigarette smoke induces oxidative stress and airway inflammation, which leads to COPD [23–25]. Prolonged oxidative exposure may produce various diseases, such as cardiovascular disease, lung fibrosis, blood and lung cancer [26]. Furthermore, observed increases in 8-isoprostane (22% increase) and triglycerides (52% increase) in EBC suggest that oxidative stress induces alteration in epithelial lining fluid metabolites in e-cig users. Triglycerides are formed due to fatty acid (endogenous or exogenous) breakdown/oxidation, possibly due to tetrahydrocannabinol (THC) oil in some e-cigs becoming oxidised *via* aerosol-mediated oxidative stress. It is important to consider that some e-cig users may be vaping other products that are rich in organic oils, leading to the accumulation of lipids in macrophages (lipoid/foamy macrophages). Our data showing a modest increase in EBC triglyceride levels as a noninvasive biomarker highlight the importance of further studies, with the potential to cause acute lung injury (*i.e.* lipoid pneumonia by vaping-associated pulmonary disease) [27–30].

Several studies have reported that cigarette smoke can increase oxidative stress and increase the release of 8-isoprostane in human respiratory epithelial cells [31–34]. Cigarette smoke-induced oxidative stress activates the inflammatory response by upregulating cytokines, such as IL-6 and IL-8 [35, 36]. IL-6 has been linked to inflammation, ageing, immunity and various chronic diseases [37, 38], whereas IL-8 has been linked to the pathogenesis of chronic inflammation and cancer [39]. IL-13 plays a role in various inflammatory diseases, including lung fibrosis, asthma, allergy and cancer [40, 41]. IL-1β is a marker of inflammatory status. Another study reported similar findings of increased levels of salivary IL-1β in e-cig users [42]. The inflammatory mediator IFN-γ was increased in urine samples of e-cig users compared with nonusers, which was also seen in tobacco smokers [43]. Supporting the observations in our study, pro-inflammatory effects of e-cig condensate on human alveolar macrophages *in vitro* have been reported in another study [44]. Similarly, primary airway smooth muscle cells from COPD patients have a greater release of inflammatory mediators compared with those of non-COPD patients, suggesting a greater risk from e-cigs in COPD patients [45].

Corroborating the increased IL-6 data in e-cig users in this study, Lerner *et al.* [46] demonstrated significantly increased IL-6 cytokine levels in bronchoalveolar lavage fluid (BALF) in a 3-day acute Blu e-cig side-stream aerosol C57BL/6J mouse model. In contrast, another group showed reduced IL-6 levels after a 2-week exposure to NJOY e-cig vapour in a mouse model [47]. Consistent with Lerner *et al.* [46], a study with C57BL/6J mice exposed to V4L CoolCart vapour for 3 days showed increased lung IL-6 mRNA levels and pulmonary oedema, with a 5.5-fold wet-to-dry ratio [48]. These outcomes may suggest that the inflammatory response depends on the frequency and the e-cig brand used. CD-1 mice exposed to e-cig aerosols for 1 month showed increased KC and triggering receptor expressed on myeloid cells-1 (TREM-1) pro-inflammatory mediators in BALF, bolstering the hypothesis that exposure to e-cig aerosols induces an inflammatory response in the lung [49]. BALF from mice exposed to NJOY e-cig vapour for 2 weeks prior to challenging with *Streptococcus pneumonia* had significantly greater bacterial growth compared with their uninfected counterparts [47]. These findings suggest that exposure to e-cig aerosols may cause inflammation and may reduce the ability to clear a bacterial infection effectively. This dampened immunity had also been observed in a human study in which users self-reported their e-cig use [50].

E-cig plasma samples had a significant increase in MMP-9 levels. Similarly, a significant increase in MMP-9 levels was observed in induced sputum samples from e-cig users and cigarette smokers [13]. MMP-9 plays a role in breaking down the extracellular matrix in injured tissues. MMP-9 is involved in several diseases, including cigarette smoke-induced emphysema, coronary heart disease and COPD [51–53]. Our data suggest that exposure to e-cigs may induce acute pulmonary tissue injury. The increase of ICAM-1 in plasma samples of e-cig users suggests altered vascular permeability. This biomarker has also been associated with inflammation and progression of lung cancer [54].

RAGE plays a role in regulating cardiovascular and pulmonary disease progression in smokers [55, 56]. We observed lower levels of RAGE in the plasma of e-cig users compared with normal subjects. Although reports of serum levels of RAGE are variable in smokers [55], other studies have shown decreased levels [56–58].

Our results showed significant increases in plasma levels of desmosine in e-cig users compared with normal subjects. Plasma desmosine is a biomarker of elastin degradation, and is associated with cardiovascular risk and severity of COPD. Chronic cigarette smoking causes elastin degradation in the alveolar wall by proteases, leading to emphysema, cardiovascular risk and COPD [59, 60]. E-cigs containing nicotine cause arterial stiffness and increases in blood pressure contributing to cardiovascular events [61]. Hence, long-term use may make e-cig users more susceptible to cardiovascular and pulmonary diseases [62, 63].

The levels of growth factors EGF, VEGF, β-NGF, PDGF-AA, SCF, HGF and PIGF were significantly increased in e-cig users compared with normal subjects, suggesting systemic effects of compensatory responses against other pro-inflammatory mediators. However, it is known that cigarette smoke alters the levels of VEGF, TGF-β1 and FGFs [64–66]. This may be due to cigarette smoke-mediated alteration of other mediators directly/indirectly affecting endothelium/monocytes to alter growth factors. For example, cigarette smoking causes perturbation in VEGF-induced endothelial cell functions. Other growth factors, such as BDNF, BMP-2, TGF-α and bFGF/FGF2, showed no changes in e-cig users, suggesting that the e-cig effect is selective. Nevertheless, a long-term study is required to determine the mechanism of impact of e-cigs on angiogenesis and/or endothelium/monocyte regulation.

Pro-resolving anti-inflammatory mediators, such as resolvins D_1 and D_2, play a major role in dampening inflammation [67, 68]. The levels of resolvins D_1 and D_2 were significantly decreased in plasma samples of e-cig users compared with normal subjects, suggesting downregulation of natural protecting mechanisms against inflammation and tissue repair. Thus, prolonged inflammation may lead to cardiovascular and pulmonary diseases.

Increased lung infiltration of macrophages, lymphocytes and neutrophils may occur as a result of chronic and subchronic e-cig use. A cross-sectional study suggested inflammasome complex proteins, caspase-1 and apoptosis-associated speck-like protein containing a caspase activation and recruitment domain, which promotes cellular pyroptosis, are elevated in the BALF of e-cig users [69]. Consistent with our observations in e-cig users, our study showed that pulmonary toxicity of e-cig aerosol exposure can induce inflammation, reduce/dampen host defence and protease-mediated lung injury. In a study correlating e-cig use with chronic bronchitis, the risk of disease symptoms (odds ratio) was increased approximately twofold in current and past e-cig users, and the risk was also increased with the frequency of vaping [70].

In summary, our study showed that e-cig users have increased levels of systemic biomarkers of inflammation, such as IL-1β, IL-6, IL-8, IL-13, IFN-γ, RAGE and MMP-9. Levels of ICAM-1, a vascular biomarker, were also increased. Levels of desmosine, a biomarker for elastin breakdown, were also increased, suggesting extracellular matrix breakdown occurs rapidly in e-cig vapour-exposed lung tissue. Furthermore, levels of growth factors, such as EGF, VEGF, β-NGF, PDGF-AA, SCF, HGF and PIGF, were significantly increased. In addition, pro-resolving lipid mediators resolvin D_1 and resolvin D_2 were at low levels in e-cig users, indicating compromised anti-inflammatory, immunity and tissue repair mechanisms. The oxidative stress biomarker 8-isoprostane, oxidative DNA damage biomarker 8-oxo-dG and fatty acid oxidation product triglycerides were also increased in e-cig users, suggesting e-cig vaping produces oxidative stress leading to damage in organ systems.

It is important to highlight that the levels of pro-inflammatory mediator IL-1β were increased by vaping across the biological fluids, whereas other inflammatory mediators (IL-6, IL-8 and IL-13), endothelial dysfunction (ICAM-1) and extracellular matrix breakdown and remodelling products (desmosine and MMP-9) were increased only in plasma, and oxidative stress biomarkers in urine of e-cig users. On the other hand, pro-resolving lipid mediators (resolvin D_1 and D_2) were decreased in other biological fluids in response to e-cig vaping. No correlation was found between biomarker levels when compared with each other in various biofluids.

Conclusions

This study suggests that e-cig use adversely affects oxidative stress, inflammatory responses and induces tissue remodelling. Prolonged activation of such mediators may lead to the progression of cardiovascular and pulmonary diseases. Some of the identified mediators across various biological fluids may be considered as hallmark noninvasive biomarkers for e-cig vaping. These biomarkers can be used for the assessment of e-cig vaping-associated lung injuries, and for regulatory and diagnostic aspects of vaping in humans.

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experimental plans/assays. N.A. Khan and I. Rahman recruited the volunteers. G. Lawyer, D. Ye, K.P. Maremanda and S.R. McDonough performed the experiments and analysed data. S. McIntosh designed the vaping surveys. All authors contributed to manuscript preparation and approved the final version before the submission.

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