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ORIGINAL ARTICLE

Acute effects of e-cigarette vaping on pulmonary function and airway inflammation in healthy individuals and in patients with asthma

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ABSTRACT

Background and objective: **The acute effects of e-cigarettes have not been scientifically demonstrated yet. The aim of this study was to assess the acute changes in pulmonary function and airway inflammation in patients with asthma after vaping one e-cigarette.**

Methods: **Twenty-five smokers suffering from stable moderate asthma according to GINA guidelines with no other comorbidities and 25 healthy smokers matched with the baseline characteristics of the asthmatic patients were recruited. PFT, IOS, FeNO and EBC were performed before and after vaping one e-cigarette with nicotine. pH and concentrations of IL-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17A, TNF-α, ISO8 and LTB4 were measured in EBC.**

Results: **FFEV1/FVC ratio and PEF were reduced in asthmatic patients after e-cigarette. Z5Hz and R5Hz, R10Hz and R20Hz increased in both groups. FeNO and EBC pH increased by 3.60 ppb (P = 0.001) and 0.15 (P = 0.014) in asthmatic patients after e-cigarette, whereas they decreased in control group by 3.28 ppb (P < 0.001) and 0.12 (P = 0.064), respectively. The concentrations of IL-10, TNF-α and ISO8 in EBC increased in asthmatic patients after e-cigarette and the changes in concentrations of IL-1β and IL-4 differed significantly between the two groups.**

Conclusion: **E-cigarette vaping resulted in acute alteration of both pulmonary function and airway inflammation in stable moderate asthmatic patients.**

Clinical trial registration: ISRCTN89151172I at ISRCTN registry http://isrctn.com

SUMMARY AT A GLANCE

The e-cigarette is rapidly gaining ground on tobacco cigarettes and is a hot topic worldwide, but its safety has not been scientifically proved. This is the first study that investigates the acute effects of e-cigarettes on both lung function and airway inflammation in asthmatic patients.

Key words: airway inflammation, asthma, e-cigarette, impulse oscillometry system, pulmonary function tests.

INTRODUCTION

Asthma is a chronic inflammatory airway disease,[1] and several factors have been implicated in the aggravation this inflammation, with cigarette smoke being one of the key factors.[1] Smoking prevalence in asthmatic patients is relatively close to that of the general population.[2] Asthmatic smokers present with worse lung function, more symptoms, more exacerbations and worse prognosis than non-smoking asthmatic patients.[1,3] Non-invasive techniques as the assessment of the fractional exhaled nitric oxide (FeNO) and exhaled breath condensate (EBC) are increasingly used for analysing airway inflammation. The effect of acute exposure to cigarette smoke on the respiratory system using EBC has not been studied extensively,[4–7] especially in asthmatic patients.[8–11] Smokers with asthma are more sensitive to the inflammatory effects of conventional cigarette compared to healthy smokers,[9] and they develop a different type of airway inflammation with more cells and higher percentage of neutrophils in induced sputum, lower pH and diverse inflammatory mediators' profiles in EBC.[8–11]

Several smokers attempt to quit smoking using electronic cigarette (e-cigarette) as an alternative. The

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studies on the acute effects of e-cigarette on lung function are few,[12–15] and regarding asthmatic patients, very few studies examined pulmonary function,[16–18] and none to date investigated airway inflammation evaluated by EBC.

The aim of this study was to investigate the acute effects of e-cigarette on lung function and airway inflammation in a group of asthmatic smokers and compare them with a matched sample of healthy smokers.

METHODS

The protocol of this study was approved by the Ethics Committee of the Medical School, Aristotle University of Thessaloniki, reference 369-8/22.2.2017, before the initiation of enrolment and all participants gave their written informed consent. The protocol of the study was registered in ISRCTN registry (ISRCTN89151172). Participants of both groups were current every-day smokers.[19] The inclusion criteria for the asthmatic patients were: (i) patients suffering from moderate persistent stable asthma well controlled with long-acting β2-agonists (LABA) and low dose of inhaled corticosteroids (ICS) (step 3)[1] from the asthma clinic, (ii) not systematic use of e-cigarette,[19] (iii) age ≥18 years, (iv) absence of another chronic or acute disease necessitating any kind of treatment, (v) absence of acute asthma exacerbation or use of systemic corticosteroids 1 month prior to the study and (vi) absence of administration of any other medication 2 weeks before the study. Asthmatic patients were asked not to refrain from their medications before the study in order to better reflect real-life conditions and the last dose of ICS was administered after their morning awakening. From 967 asthmatic patients assessed for eligibility, 580 were excluded because they had mild or severe asthma, 271 because they were not every-day smokers and 29 because they did not meet the rest of inclusion criteria. Of the 87 patients eligible, 25 were selected using a random number generator. Healthy smokers visiting the smoking cessation clinic were recruited for the control group. The inclusion criteria for the control group were: (i) age ≥18 years, (ii) not systematic use of e-cigarette,[19] (iii) absence of any chronic or acute disease and (iv) abstinence of any medication 2 weeks before participating in the study. Controls were matched for age (±2 years), gender, body mass index (BMI) (±2 kg/m^2) and the number of pack/years (±2 pack/years) with asthmatic patients.

Sample size was calculated using the G*Power software (Die Heinrich Heine Universität, Düsseldorf, Germany). The difference of the changes in FeNO before and after e-cigarette between the two groups was used as the primary endpoint of the study. Sample size was calculated considering a statistically significant change of 20% in FeNO.[20] The expected effect size was calculated at 0.94, with a power at 0.95 and a level of statistical significance at 0.05.

Before the initiation of the study, exhaled carbon monoxide was measured in all the participants (piCO Smokerlyzer; Bedfont-Scientific-Ltd, Kent, UK) to confirm smoking abstinence (at least 8 h). Measurements less than 6 parts per million were acceptable (Fig. 1).

All participants underwent pulmonary function tests (PFT)[21] (Master Screen PFT; Jaeger, Wurzburg, Germany) and total respiratory resistance measurement with an impulse oscillometry system (IOS)[22,23] (Master Screen IOS; Viasys Jaeger, Hoechberg, Germany). Forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC ratio, peak expiratory flow (PEF), maximal expiratory flow at 25%, 50% and 75% of vital capacity, functional residual capacity (FRC), total lung capacity (TLC), residual volume (RV), expiratory reserve volume (ERV), alveolar ventilation (VA) and diffusion lung capacity for carbon monoxide (DL_{CO}) were measured. Respiratory impedance at 5 Hz (Z5Hz); respiratory resistance at 5, 10 and 20 Hz (R5Hz, R10Hz and R20Hz, respectively); reactance at 5, 10 and 20 Hz; and resonant frequency were assessed with IOS.

Airway inflammation was assessed by FeNO measurement[24] (Denox88; ECOMEDICS, Dürnten, Switzerland) and EBC collection[25] (EcoScreen-device; Jaeger, Wurzburg, Germany). EBC pH measurement was performed by pH meter[25] (OrionStar-A121 pH portable meter kit with triode pH/ATC electrode solutions meter armour and field; ThermoScientific, Waltham, MA, USA). EBC concentrations of 8-isoprostane (ISO8) and leukotriene B4 (LTB4) were performed by enzyme-linked immunosorbent assay (ELISA)[25] (VICTOR X3 Spectrophotometer; PerkinElmer, Waltham, MA, USA), and EBC concentrations of interleukin (IL)-1β, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17A and tumour necrosis factor-alpha (TNF-α) were detected by flow cytometry (bead-technology)[26] (FACSCalibur-BD-Biosciences, Jhotwara, Rajasthan, India).

All participants were provided an e-cigarette of the same company with the same concentration of nicotine in the cartridge (medium nicotine content; NOBACCO, Halandri, Greece), powered by a lithium battery with 1.2 Ω coil resistance. Its chemical composition has been analysed in a previous study.[13] A new cartridge and atomizer was used for each participant. Participants of both groups vaped the e-cigarette for 5 min (10 puffs with 30-s inter-puff intervals, 1.0–1.5 mL of e-liquid). Vaping protocol was strictly followed to reassure similar inhalation process in both groups. No serious adverse events were observed, except for two asthmatic patients experiencing mild wheezing after vaping which resolved quickly without bronchodilators. In 15 min after vaping, participants were subjected again to PFT and IOS measurements and in 30 min after vaping to FeNO measurement and EBC collection. The same procedure and timeline were followed for all measurements both before and after e-cigarette. All measurements were performed by paramedical technicians who were blinded to the research.

Statistical analysis was performed using the SPSS (version-20; IBM-SPSS, Armonk, NY, USA). Continuous variables were presented as mean ± SD. $P < 0.05$ was accepted as statistically significant. To separate parametric from non-parametric variables, normality test using the Shapiro–Wilk test was performed. Paired samples t-test for parametric or Wilcoxon signed-ranks test for non-parametric variables were

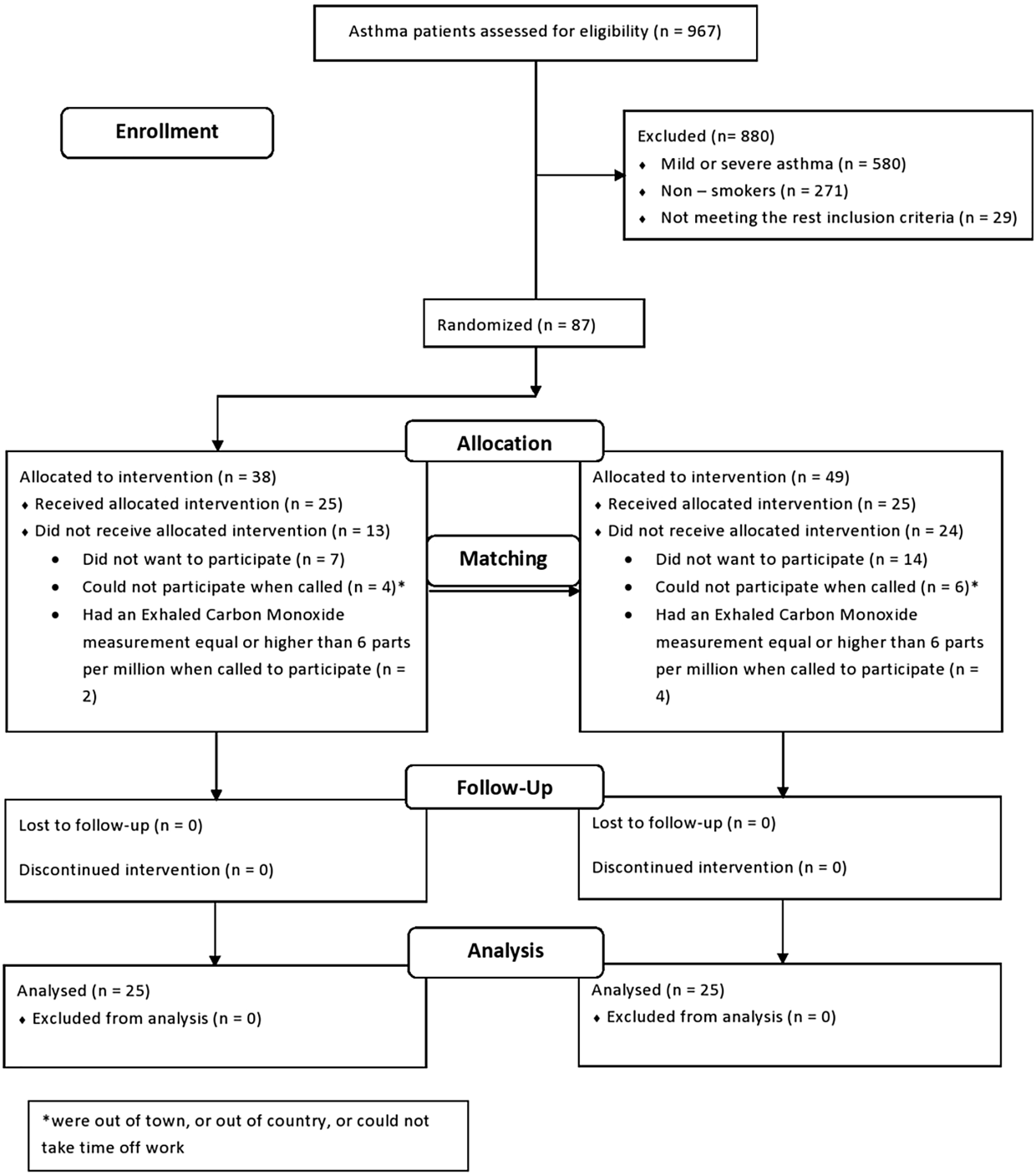

Figure 1 Study flow chart. †Were out of town, or out of country or could not take time off work from the art work

used to detect significant differences in the variables measured before and after the e-cigarette for each group. To detect significant differences between the two groups in baseline characteristics and to compare the differences in the variables measured before and after e-cigarette between the two groups, independent samples t-rest and the Mann–Whitney U-test were used for parametric and non-parametric variables, respectively.

RESULTS

The two groups were matched for gender, age, BMI and number of pack/years (Table 1). After e-cigarette, FEV1/FVC and PEF significantly decreased in asthmatic patients, but not in controls (Tables 2,3). In addition, Z5Hz significantly increased in asthmatic patients, whereas in controls the respective increase was not significant (Tables 2,3). R5Hz,

Table 1 Comparison of baseline characteristics and values between the two groups (mean ± SD)

Variables	Group of healthy controls	Group of asthmatic patients	*P*-value
Gender (M (%)/F (%))	8 (32%)/17 (68%)	13 (52%)/12 (48%)	0.152
Age (years)	39.88 ± 10.24	40.64 ± 10.81	0.503
Height (m)	1.69 ± 0.11	1.75 ± 0.11	0.092
Weight (kg)	76.72 ± 17.48	80.20 ± 22.09	0.648
BMI (kg/m^2)	26.46 ± 3.82	25.99 ± 5.03	0.541
Pack/years (*n*)	15.26 ± 13.80	14.82 ± 18.63	0.506
FVC (L)	4.02 ± 0.91	4.45 ± 1.15	0.145
FVC (% predicted)	104.61 ± 15.17	104.61 ± 14.2	0.999
FEV1 (L)	3.42 ± 0.79	3.43 ± 0.90	0.948
FEV1 (% predicted)	105.20 ± 16.67	95.94 ± 13.18	0.008
FEV1/FVC (%)	82.63 ± 6.95	75.19 ± 8.23	<0.001
FEV1/FVC (% predicted)	101.83 ± 7.60	93.26 ± 9.25	0.001
PEF (L/s)	7.42 ± 1.75	7.58 ± 2.02	0.772
PEF (% predicted)	98.80 ± 21.51	92.03 ± 19.55	0.250
RV (L)	1.51 ± 0.43	1.87 ± 0.53	0.009
RV (% predicted)	87.30 ± 14.91	100.43 ± 26.64	0.114
ERV (L)	1.08 ± 0.48	1.44 ± 0.65	0.027
ERV (% predicted)	87.52 ± 36.43	108.88 ± 39.00	0.054
TLC (L)	5.56 ± 0.95	6.20 ± 1.33	0.057
TLC (% predicted)	97.41 ± 9.60	97.52 ± 12.4	0.973
VA (L)	5.39 ± 0.93	6.02 ± 1.31	0.057
VA (% predicted)	97.12 ± 10.28	97.12 ± 12.91	0.999
DL_{CO} (mmol/min/kPa)	8.25 ± 1.73	8.70 ± 2.32	0.322
DL_{CO} (% predicted)	85.63 ± 8.60	84.23 ± 15.29	0.692
K_{CO} (mmol/min/kPa/L)	1.53 ± 0.14	1.45 ± 0.26	0.190
K_{CO} (% predicted)	91.07 ± 10.44	89.64 ± 15.35	0.854
Z5Hz (kPa/L/s)	0.440 ± 0.098	0.431 ± 0.121	0.779
R5Hz (kPa/L/s)	0.426 ± 0.099	0.419 ± 0.115	0.803
R10Hz (kPa/L/s)	0.382 ± 0.096	0.376 ± 0.104	0.844
R20Hz (kPa/L/s)	0.367 ± 0.097	0.362 ± 0.101	0.865
FeNO (ppb)	15.12 ± 6.48	14.88 ± 11.60	0.232
pH	6.40 ± 0.29	6.25 ± 0.21	0.037
IL-1β (pg/mL)	4.14 ± 6.37	1.06 ± 2.32	0.017
IL-4 (pg/mL)	2.15 ± 2.62	0.80 ± 1.98	0.019
IL-6 (pg/mL)	0.99 ± 1.36	1.19 ± 3.93	0.330
IL-8 (pg/mL)	0.40 ± 1.85	0.01 ± 0.04	0.574
IL-10 (pg/mL)	17.49 ± 0.76	17.15 ± 0.47	0.103
IL-13 (pg/mL)	1.14 ± 0.28	1.04 ± 0.32	0.127
IL-17A (pg/mL)	1.51 ± 2.01	0.81 ± 1.72	0.123
TNF-α (pg/mL)	0.42 ± 0.72	0.10 ± 0.30	0.014
ISO8 (pg/mL)	12.59 ± 24.03	1.36 ± 4.26	0.009
LTB4 (pg/mL)	243.12 ± 256.08	92.74 ± 70.84	0.005

BMI, body mass index; DL_{CO}, diffusion lung capacity for carbon monoxide; ERV, expiratory reserve volume; F, female; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL, interleukin; ISO8, 8-isoprostane; K_{CO}, DL_{CO}/VA; LTB4, leukotriene B4; M, male; PEF, peak expiratory flow; ppb, parts per billion; R5Hz, R10Hz, R20Hz, respiratory resistance at 5, 10 and 20 Hz; RV, residual volume; TLC, total lung capacity; TNF-α, tumour necrosis factor-alpha; VA, alveolar ventilation; Z5Hz, respiratory impedance at 5 Hz.

R10Hz and R20Hz increased in both groups (Tables 2,3).

Regarding airway inflammation, initially, there were statistically significant differences in EBC pH and concentrations of IL-1β, IL-4, TNF-α, ISO8 and LTB4 between the two groups (Table 1). After e-cigarette, FeNO and EBC pH increased significantly in asthmatic patients, whereas in controls they decreased (Tables 2,3). After e-cigarette, IL-10, TNF-α and ISO8 increased significantly in asthmatic patients, whereas no significant differences were observed in controls (Tables 2,3). Furthermore, the changes in concentrations of IL-1β and IL-4 before and after e-cigarette between the two groups differed significantly (Table 4).

DISCUSSION

The main outcome of our study was that both pulmonary function and airway inflammation were altered after e-cigarette vaping for 5 min in asthmatic patients despite the regular use of ICS, while in healthy controls

Table 2 Changes in pulmonary function and inflammatory mediators before and after e-cigarette in the asthmatic group (mean ± SD)

Variables	Before e-cigarette	After e-cigarette	Mean difference	*P*-value
FVC (L)	4.45 ± 1.15	4.43 ± 1.17	−0.02	0.534
FVC (% predicted)	104.61 ± 14.2	103.88 ± 13.62	−0.73	0.726
FEV1 (L)	3.43 ± 0.90	3.39 ± 0.91	−0.04	0.113
FEV1 (% predicted)	95.94 ± 13.18	94.64 ± 14.29	−1.30	0.067
FEV1/FVC (%)	75.19 ± 8.23	74.58 ± 7.96	−0.61	**0.040**
FEV1/FVC (% predicted)	93.26 ± 9.25	92.52 ± 9.01	−0.74	**0.042**
PEF (L/s)	7.58 ± 2.02	7.12 ± 2.08	−0.46	**0.003**
PEF (% predicted)	92.03 ± 19.55	84.84 ± 19.02	−7.19	**0.001**
RV (L)	1.87 ± 0.53	1.89 ± 0.44	+0.02	0.772
RV (% predicted)	100.43 ± 26.64	101.69 ± 21.59	+1.26	0.738
ERV (L)	1.44 ± 0.65	1.29 ± 0.57	−0.15	0.051
ERV (% predicted)	108.88 ± 39.00	96.69 ± 28.97	−12.19	0.053
TLC (L)	6.20 ± 1.33	6.13 ± 1.28	−0.07	0.141
TLC (% predicted)	97.52 ± 12.4	96.58 ± 11.33	−0.94	0.187
VA (L)	6.02 ± 1.31	5.96 ± 1.26	−0.06	0.148
VA (% predicted)	97.12 ± 12.91	96.16 ± 11.78	−0.96	0.186
DL_{CO} (mmol/min/kPa)	8.70 ± 2.32	8.56 ± 2.05	−0.14	0.165
DL_{CO} (% predicted)	84.23 ± 15.29	82.94 ± 12.40	−1.29	0.173
K_{CO} (mmol/min/kPa/L)	1.45 ± 0.26	1.44 ± 0.24	−0.01	0.620
K_{CO} (% predicted)	89.64 ± 15.35	89.26 ± 13.64	−0.38	0.662
Z5Hz (kPa/L/s)	0.431 ± 0.121	0.464 ± 0.149	+0.033	**0.040**
R5Hz (kPa/L/s)	0.419 ± 0.115	0.449 ± 0.142	+0.030	0.054
R10Hz (kPa/L/s)	0.376 ± 0.104	0.403 ± 0.128	+0.027	**0.043**
R20Hz (kPa/L/s)	0.362 ± 0.101	0.386 ± 0.114	+0.024	**0.026**
FeNO (ppb)	14.88 ± 11.60	18.48 ± 13.38	+3.60	**0.001**
pH	6.25 ± 0.21	6.40 ± 0.30	+0.15	**0.014**
IL-1β (pg/mL)	1.06 ± 2.32	1.13 ± 1.32	+0.07	0.084
IL-4 (pg/mL)	0.80 ± 1.98	1.42 ± 2.95	+0.62	0.328
IL-6 (pg/mL)	1.19 ± 3.93	0.92 ± 1.77	−0.27	0.394
IL-8 (pg/mL)	0.01 ± 0.04	0.17 ± 0.62	+0.16	0.080
IL-10 (pg/mL)	17.15 ± 0.47	17.49 ± 0.67	+0.34	**0.002**
IL-13 (pg/mL)	1.04 ± 0.32	1.14 ± 0.32	+0.10	0.074
IL-17A (pg/mL)	0.81 ± 1.72	1.70 ± 2.48	+0.89	0.061
TNF-α (pg/mL)	0.10 ± 0.30	0.43 ± 0.68	+0.33	**0.028**
ISO8 (pg/mL)	1.36 ± 4.26	12.79 ± 34.50	+11.43	**0.008**
LTB4 (pg/mL)	92.74 ± 70.84	168.02 ± 253.44	+75.28	0.189

DL_{CO}, diffusion lung capacity for carbon monoxide; e-cigarette, electronic cigarette; ERV, expiratory reserve volume; F, female; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL, interleukin; ISO8, 8-isoprostane; K_{CO}, DL_{CO}/VA; LTB4, leukotriene B4; M, male; PEF, peak expiratory flow; ppb, parts per billion; R5Hz, R10Hz, R20Hz, respiratory resistance at 5, 10 and 20 Hz; RV, residual volume; TLC, total lung capacity; TNF-α, tumour necrosis factor-alpha; VA, alveolar ventilation; Z5Hz, respiratory impedance at 5 Hz.

the changes in airway inflammation markers were not significant.

Previous studies showed that e-cigarette is associated with the deterioration of respiratory symptoms and lung function and with increased flow resistance in healthy but also in smokers with obstructive pulmonary disease, such as asthma.[12–17] However, other studies have shown that the use of e-cigarettes free of nicotine, with a mixture of propylene glycol and glycerol, did not significantly affect the pulmonary function or symptoms of both healthy and asthmatic subjects.[18] In our study, the pulmonary function (especially FEV1/FVC ratio and PEF) was reduced only in asthmatic patients but flow resistance increased in both groups. This finding is in accordance with previous studies in healthy adults,[13,16,17] but also in asthmatic patients.[16,17] Z5Hz was significantly increased only in asthmatic patients after e-cigarette. In a recent study on the acute effects (i.e. immediately and 15 and 30 min) of e-cigarettes on the pulmonary function of smokers with mild asthma, Z5Hz was found to be increased in both asthmatic and healthy smokers with a more significant increase in asthmatic patients.[16] In that study, similarly with ours, both large (R20Hz) and total airway resistance (R5Hz) increased in both groups indicating acute bronchoconstriction. In our study, the difference was not significantly higher in asthmatic patients probably because we performed only one measurement 15 min after e-cigarette.

Concerning airway inflammation, an acute FeNO and pH increase was found 30 min after e-cigarette in asthmatic patients, whereas a decrease was measured

Table 3 Changes in pulmonary function and inflammatory mediators before and after e-cigarette in the control group (mean ± SD)

Variables	Before e-cigarette	After e-cigarette	Mean difference	*P*-value
FVC (L)	4.02 ± 0.91	4.03 ± 0.90	+0.01	0.696
FVC (% predicted)	104.61 ± 15.17	104.74 ± 13.62	+0.13	0.873
FEV1 (L)	3.42 ± 0.79	3.39 ± 0.79	−0.03	0.267
FEV1 (% predicted)	105.20 ± 16.67	104.06 ± 14.29	−1.14	0.125
FEV1/FVC (%)	82.63 ± 6.95	81.80 ± 6.38	−0.83	0.169
FEV1/FVC (% predicted)	101.83 ± 7.60	100.82 ± 6.98	−1.01	0.175
PEF (L/s)	7.42 ± 1.75	7.23 ± 2.17	−0.19	0.321
PEF (% predicted)	98.80 ± 21.51	94.78 ± 22.40	−4.02	0.141
RV (L)	1.51 ± 0.43	1.53 ± 0.50	+0.01	0.590
RV (% predicted)	87.30 ± 14.91	88.32 ± 18.03	+1.02	0.757
ERV (L)	1.08 ± 0.48	1.06 ± 0.49	−0.02	0.818
ERV (% predicted)	87.52 ± 36.43	84.84 ± 32.09	−2.68	0.583
TLC (L)	5.56 ± 0.95	5.59 ± 0.97	+0.03	0.277
TLC (% predicted)	97.41 ± 9.60	97.88 ± 8.08	+0.47	0.426
VA (L)	5.39 ± 0.93	5.43 ± 0.95	+0.04	0.292
VA (% predicted)	97.12 ± 10.28	97.60 ± 8.75	+0.48	0.434
DL_{CO} (mmol/min/kPa)	8.25 ± 1.73	8.12 ± 1.74	−0.13	0.289
DL_{CO} (% predicted)	85.63 ± 8.60	84.15 ± 7.25	−1.48	0.207
K_{CO} (mmol/min/kPa/L)	1.53 ± 0.14	1.49 ± 0.14	−0.04	0.070
K_{CO} (% predicted)	91.07 ± 10.44	89.23 ± 10.51	−1.8	0.122
Z5Hz (kPa/L/s)	0.440 ± 0.098	0.461 ± 0.106	+0.021	0.063
R5Hz (kPa/L/s)	0.426 ± 0.099	0.450 ± 0.105	+0.024	**0.034**
R10Hz (kPa/L/s)	0.382 ± 0.096	0.402 ± 0.098	+0.020	**0.038**
R20Hz (kPa/L/s)	0.367 ± 0.097	0.388 ± 0.098	+0.021	**0.034**
FeNO (ppb)	15.12 ± 6.48	11.84 ± 5.19	−3.28	**<0.001**
pH	6.40 ± 0.29	6.28 ± 0.29	−0.12	0.064
IL-1β (pg/mL)	4.14 ± 6.37	1.70 ± 2.68	−2.44	0.064
IL-4 (pg/mL)	2.15 ± 2.62	1.27 ± 1.96	−0.88	0.058
IL-6 (pg/mL)	0.99 ± 1.36	0.78 ± 2.35	−0.21	0.231
IL-8 (pg/mL)	0.40 ± 1.85	0.03 ± 0.17	−0.37	0.345
IL-10 (pg/mL)	17.49 ± 0.76	17.59 ± 0.77	+0.10	0.401
IL-13 (pg/mL)	1.14 ± 0.28	1.21 ± 0.37	+0.07	0.493
IL-17A (pg/mL)	1.51 ± 2.01	1.28 ± 1.30	−0.23	0.940
TNF-α (pg/mL)	0.42 ± 0.72	0.46 ± 0.63	+0.04	0.737
ISO8 (pg/mL)	12.59 ± 24.03	28.67 ± 75.07	+16.08	0.530
LTB4 (pg/mL)	243.12 ± 256.08	250.97 ± 289.39	+7.85	0.901

DL_{CO}, diffusion lung capacity for carbon monoxide; e-cigarette, electronic cigarette; ERV, expiratory reserve volume; F, female; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL, interleukin; ISO8, 8-isoprostane; K_{CO}, DL_{CO}/VA; LTB4, leukotriene B4; M, male; PEF, peak expiratory flow; ppb, parts per billion; R5Hz, R10Hz, R20Hz, respiratory resistance at 5, 10 and 20 Hz; RV, residual volume; TLC, total lung capacity; TNF-α, tumour necrosis factor-alpha; VA, alveolar ventilation; Z5Hz, respiratory impedance at 5 Hz.

in controls. Previously published studies have shown that FeNO decreased immediately after passive, active smoking[27–29] and e-cigarette use in healthy and asthmatic smokers,[13,16] although those reductions reversed after 15 and 30 min.[16] However, in other studies no significant changes were observed,[30–32] and in one study FeNO was increased in adult smokers 5 days after switching from tobacco to e-cigarette.[33] In our study, FeNO decreased in the controls 30 min after e-cigarette while it increased in asthmatic patients. This finding complies with the changes observed in the concentrations of T-helper-type-2 (Th2) cytokines, such as IL-4 and IL-13, in EBC before and after e-cigarette. IL-4 is important for Th2 differentiation and IL-13 for IgE formation; their increase results in pulmonary eosinophil activation, B-cell IgE production and recruitment of mast cells. FeNO has been associated with the presence of Th2-mediated asthma, reflecting eosinophilic inflammation. In a previous study, IL-4 and IL-5 decreased in EBC of active and passive smokers 30 min after indoor waterpipe smoking.[34] It is already known that FeNO is reduced after corticosteroid and antileukotriene treatment.[35] Our asthmatic patients received treatment with LABA and ICS. In our point of view, the FeNO difference between the two groups after vaping is due to the different pathophysiology of airway inflammation. E-cigarette acted as a more potent stimulus in asthmatic patients compared to controls and despite the use of ICS the FeNO increased.

EBC pH has been found low in patients with stable asthma compared with healthy controls, with further pH reduction during asthma exacerbations and

Table 4 Comparison of differences before and after e-cigarette between two groups (mean ± SD)

Variables	Control group	Asthma group	Mean difference	*P*-value
FVC (L)	+0.01 ± 0.16	−0.02 ± 0.14	0.03	0.480
FVC (% predicted)	+0.13 ± 3.97	−0.73 ± 3.71	0.86	0.977
FEV1 (L)	−0.03 ± 0.12	−0.04 ± 0.13	0.01	0.628
FEV1 (% predicted)	−1.14 ± 3.57	−1.30 ± 3.40	0.16	0.865
FEV1/FVC (%)	−0.83 ± 2.91	−0.61 ± 2.03	0.22	0.677
FEV1/FVC (% predicted)	−1.01 ± 3.62	−0.75 ± 2.47	0.26	0.684
PEF (L/s)	−0.19 ± 0.97	−0.46 ± 1.05	0.27	0.467
PEF (% predicted)	−4.02 ± 13.20	−7.19 ± 11.44	3.17	0.600
RV (L)	+0.01 ± 0.14	+0.02 ± 0.35	0.01	0.946
RV (% predicted)	+1.02 ± 7.75	+1.26 ± 18.64	0.24	0.900
ERV (L)	−0.02 ± 0.30	−0.15 ± 0.37	0.13	0.157
ERV (% predicted)	−2.68 ± 24.01	−12.18 ± 29.91	9.50	0.221
TLC (L)	+0.03 ± 0.17	−0.07 ± 0.21	0.10	0.066
TLC (% predicted)	+0.48 ± 2.94	−0.93 ± 3.43	1.41	0.126
VA (L)	+0.04 ± 0.17	−0.06 ± 0.21	0.10	0.072
VA (% predicted)	+0.48 ± 3.02	−0.96 ± 3.51	1.44	0.127
DL_{CO} (mmol/min/kPa)	−0.13 ± 0.58	−0.14 ± 0.50	0.01	0.911
DL_{CO} (% predicted)	−1.48 ± 5.69	−1.29 ± 4.58	0.19	0.898
K_{CO} (mmol/min/kPa/L)	−0.04 ± 0.09	−0.01 ± 0.07	0.03	0.242
K_{CO} (% predicted)	−1.84 ± 5.75	−0.38 ± 4.34	1.46	0.316
Z5Hz (kPa/L/s)	+0.021 ± 0.054	+0.033 ± 0.077	0.012	0.515
R5Hz (kPa/L/s)	+0.024 ± 0.053	+0.030 ± 0.075	0.006	0.712
R10Hz (kPa/L/s)	+0.020 ± 0.046	+0.027 ± 0.064	0.007	0.668
R20Hz (kPa/L/s)	+0.021 ± 0.046	+0.024 ± 0.051	0.003	0.816
FeNO (ppb)	−3.28 ± 4.00	+3.60 ± 5.97	6.88	**<0.001**
pH	−0.12 ± 0.30	+0.15 ± 0.29	0.27	**0.002**
IL-1β (pg/mL)	−2.44 ± 6.17	+0.07 ± 2.43	2.51	**0.017**
IL-4 (pg/mL)	−0.88 ± 2.22	+0.62 ± 3.25	1.50	**0.009**
IL-6 (pg/mL)	−0.21 ± 2.58	−0.27 ± 4.28	0.06	0.239
IL-8 (pg/mL)	−0.37 ± 1.87	+0.16 ± 0.62	0.53	0.091
IL-10 (pg/mL)	+0.10 ± 0.60	+0.34 ± 0.72	0.24	0.123
IL-13 (pg/mL)	+0.07 ± 0.29	+0.10 ± 0.37	0.03	0.749
IL-17A (pg/mL)	−0.23 ± 2.17	+0.89 ± 2.37	1.12	0.549
TNF-α (pg/mL)	+0.04 ± 0.80	+0.33 ± 0.67	0.29	0.241
ISO8 (pg/mL)	+16.08 ± 64.71	+11.43 ± 33.14	4.65	0.683
LTB4 (pg/mL)	+7.85 ± 248.64	+75.28 ± 242.98	67.43	0.600

DL_{CO}, diffusion lung capacity for carbon monoxide; e-cigarette, electronic cigarette; ERV, expiratory reserve volume; F, female; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; IL, interleukin; ISO8, 8-isoprostane; K_{CO}, DL_{CO}/VA; LTB4, leukotriene B4; M, male; PEF, peak expiratory flow; ppb, parts per billion; R5Hz, R10Hz, R20Hz, respiratory resistance at 5, 10 and 20 Hz; RV, residual volume; TLC, total lung capacity; TNF-α, tumour necrosis factor-alpha; VA, alveolar ventilation; Z5Hz, respiratory impedance at 5 Hz.

increase after treatment with ICS.[36] EBC pH of asymptomatic smokers with more than 10 pack/years was found to be significantly reduced and pH reduction correlated with smoking history.[4,36] Smoking a single cigarette after overnight abstinence increased EBC pH in healthy smokers,[37] while the opposite applied for asthmatic smokers in another study.[9] However, the aforementioned studies evaluated conventional cigarette not e-cigarette. The changes of EBC pH, in our study, could be partially explained by the changes of the concentrations of some inflammatory mediators with strongly basal isoelectric point in the EBC of the two groups before and after e-cigarette, possibly due to different e-cigarette components. More specifically, LTB4 increased markedly after e-cigarette in asthmatic patients compared to controls and IL-4 increased in asthmatic patients and decreased in controls and the difference between the two groups was significant. Previous studies have demonstrated that substances with strongly basal isoelectric point in EBC may affect its pH.[25]

Studies have identified several inflammatory mediators such as nitric oxide, IL-4, IL-5, IL-13 and various eicosanoids (leukotrienes, prostaglandins and thromboxanes), which play a role in airway inflammation.[9,38] The concentrations of some inflammatory mediators were found to vary in EBC of patients with asthma especially in case of aggravation of airway inflammation.[10,39] IL-4, IL-5, IL-6, IL-8, IL-10, IL-17, ISO8 and TNF-α were found to be increased in EBC of stable asthmatic patients.[36] ICS may affect EBC biomarkers in these patients by decreasing ISO8 and IL-4.[35] In our study, EBC inflammatory biomarkers were higher in controls at baseline as both groups had a significant

history of tobacco exposure; however, in the asthmatic patients, the expression of these indicators was suppressed by ICS.

IL-1β and TNF-α are pro-inflammatory cytokines which amplify and orchestrate the inflammatory response in asthma and determine its severity. IL-10 is a cytokine derived from Th2 cells while ISO8 is a biomarker of oxidative stress. In our study, IL-10, TNF-α and ISO8 were significantly increased in asthmatic patients after e-cigarette, whereas no significant differences existed in the controls. IL-1β and IL-4 increased in asthmatic patients, compared to baseline values, but not significantly, whereas they were reduced in controls. However, the changes in concentrations before and after e-cigarette between the two groups differed significantly. To our knowledge, this is the first study examining the acute effects of e-cigarette in EBC of healthy and asthmatic smokers. In previous studies assessing the effect of tobacco cigarette or waterpipe smoke on EBC biomarkers, a reduction of IL-4, IL-5, IL-10, IL-17 and INF-γ, and an increase of ISO8 and TNF-α were found.[7,9,34,36] In addition, in a recent study, an increase in IL-1β and IL-6 was found on mice exposed to tobacco-flavoured e-cigarette as in accordance with our study, as far as IL-1β.[40]

The main limitation of our study was that we assessed the pulmonary function and FeNO and EBC only once after 15 and 30 min, respectively. It would be interesting to assess the acute effects of e-cigarette for a longer duration. Another limitation was that our participants were not exposed to a sham-vaping procedure or different types of liquids with and without nicotine. We used only a specific brand of e-cigarette and evaluated patients with asthma treated with low doses of ICS + LABA. However, the effects of e-cigarette had not been studied previously in patients treated with ICS. It is well established that ICS control but not eliminate airway inflammation and the majority of asthmatic patients receive ICS treatment. Therefore, the group of asthmatic patients in our study reflects better real-life conditions. The asthmatic patients recruited belonged to Global Initiative for Asthma (GINA) 'step-3' group and used only one specific brand of e-cigarette, in order to achieve the best possible homogeneity. We used only patients with stable asthma, well controlled with low doses of ICS + LABA to avoid outsized deviations in which patients with poorly controlled asthma or without ICS treatment may have been susceptible at, something that could be a potentially confounding factor on our results.

In conclusion, the use of an e-cigarette for 5 min in asthmatic smokers treated with low doses of ICS + LABA altered acutely both pulmonary function and airway inflammation compared to a sample of matched healthy smokers. This study adds to the very limited published information concerning the short-term impact of e-cigarette on lung function and particularly on airway inflammation evaluated by EBC. As e-cigarette is becoming more popular, there is an urgent need for more future studies in larger populations of smokers both with and without pulmonary disease as asthma and for more prolonged or chronic use of e-cigarette, aiming to better understand the pathophysiological mechanisms and effects of e-cigarette on lung function and airway inflammation.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Abbreviations: DL_{CO}, diffusion lung capacity for carbon monoxide; e-cigarette, electronic cigarette; EBC, exhaled breath condensate; ERV, expiratory reserve volume; FeNO, fractional exhaled nitric oxide; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; Ig, immunoglobulin; IL, interleukin; IOS, impulse oscillometry system; ISO8, 8-isoprostane; K_{CO}, DL_{CO}/VA; LABA, long-acting β2-agonist; LTB4, leukotriene B4; PEF, peak expiratory flow; PFT, pulmonary function test; ppb, parts per billion; R5Hz, R10Hz and R20Hz, respiratory resistance at 5, 10 and 20 Hz, respectively; RV, residual volume; Th2, T-helper-type-2; TLC, total lung capacity; TNF-α, tumour necrosis factor-alpha; VA, alveolar ventilation; Z5Hz, respiratory impedance at 5 Hz.

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