

Journal of Asthma

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/ijas20

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To cite this article: Berna Duman, Sermin Borekci, Nilgun Akdeniz, Sema Bilgic Gazioglu, Gunnur Deniz & Bilun Gemicioglu (2022) Inhaled corticosteroids' effects on biomarkers in exhaled breath condensate and blood in patients newly diagnosed with asthma who smoke, Journal of Asthma, 59:8, 1613-1620, DOI: 10.1080/02770903.2021.1962341

To link to this article: https://doi.org/10.1080/02770903.2021.1962341

Published online: 14 Aug 2021.

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JOURNAL OF ASTHMA
2022, VOL. 59, NO. 8, 1613–1620
https://doi.org/10.1080/02770903.2021.1962341

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BIOMARKERS

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Inhaled corticosteroids' effects on biomarkers in exhaled breath condensate and blood in patients newly diagnosed with asthma who smoke

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ABSTRACT

Objective: Exposure to cigarette smoke complicates the treatment and management of asthma through a variety of inflammatory effects. This study aimed to investigate the differences between newly diagnosed cases of asthma in smokers and nonsmokers in terms of localized and systemic biomarkers following treatment with inhaled corticosteroids (ICS) or ICS in combination with a long-acting β2 agonist (LABA).

Methods: Specimens of exhaled breath condensate (EBC) from newly diagnosed patients with asthma were used to quantify inflammation in the airways, while blood samples were used to assess systemic inflammation. In both samples, the levels of IL-6, LTB4, LTD4, and 8-isoprostane were measured and these were repeated after 3 months of treatment with ICS or ICS + LABA.

Results: Of the 20 patients, 10 (50%) were nonsmokers with asthma (NSA) and 10 (50%) smokers with asthma (SA). There was no statistically significant difference in the blood or EBC levels of IL-6, LTB4, LTD4, or 8-isoprostane between the groups prior to treatment. Only the decrease in 8-isoprostane level in the EBC samples was found to be significantly greater in the NSA group after treatment (for smokers, the change was 2.91 ± 23.22, while for nonsmokers it was −22.72 ± 33.12, $p = 0.022$). Post-treatment asthma control was significantly better in the NSA group ($p = 0.033$).

Conclusion: Monitoring the alterations in 8-isoprostane levels in EBC in patients with asthma who smoke may be helpful in deciding on therapeutic management and switching treatments. Asthma control was better in nonsmokers than in smokers.

ARTICLE HISTORY
Received 27 April 2021
Revised 12 July 2021
Accepted 26 July 2021

KEYWORDS
Asthma; smoking; biomarkers; 8-isoprostane; exhaled breath condensate; IL-6; LTB4; LTD4

Introduction

Asthma is a heterogeneous disorder encompassing different phenotypes and endotypes, with the unifying characteristics of airway inflammation and a variable degree of airway obstruction (1). Since smoking makes it difficult to differentiate between asthma and chronic obstructive pulmonary disease (COPD), patients with asthma who smoke are frequently excluded from studies. Nonetheless, in asthmatic patients, smoking is not at a level where it may be disregarded, because its frequency approaches 25% (2,3). Smoking is known to have negative effects on the severity of disease, disease control, treatment response, increased loss of respiratory function, and worsened emergency admission, number of flare-ups, hospitalization, symptoms, and quality of life in patients with asthma (4–7).

Various mediators contribute to the formation of inflammation in asthma, including leukotrienes B4, C4, D4, and E4, which play an important role owing to their potent biological effects (8,9). Among leukotrienes, the synthesis of eicosanoids depends on the metabolic action of the 5-lipoxygenase enzyme. This group includes LTC4 and its metabolites, LTD4 and LTE4, which are collectively referred to as the "cysteinyl leukotrienes" (10,11). The most well-understood action of cysteinyl leukotrienes is the contraction of smooth muscles within the airways. In addition, their effects include producing hypersensitivity of the airways, increasing microvascular permeability, and promoting mucous hypersecretion (11). The key biological role of LTB4 is the chemotactic attraction of leukocytes, including neutrophils. It has been demonstrated that LTB4 stimulates IL-5 synthesis, which plays a

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role in eosinophilia. Furthermore, it potentiates the effect of IL-4, by which B lymphocytes are able to synthesize IgE (12).

Interleukin 6 (IL-6) is a pro-inflammatory cytokine secreted by epithelial cells and macrophages and is a key member of the IL-6 cytokine family consisting of several different cytokines (13,14). This family of cytokines has been implicated in the etiopathogenesis of many diseases involving the hematological, rheumatological, neurological, and gastrointestinal systems, particularly in the control of the autoimmune response. Some studies also indicate elevated IL-6 levels in patients with asthma (13,15). We believe that as smoking induces IL-6 by neutrophilic inflammation, the results of asthma patients who smoke or do not smoke will provide some clues to the differences in asthma phenotypes.

The 8-isoprostane is a prostaglandin-$F_2\alpha$ isomer formed by the peroxidation of arachidonic acid. It is a noninvasive marker of oxidative stress and has been shown to be elevated in adult respiratory distress syndrome, smokers, idiopathic pulmonary fibrosis, cystic fibrosis, and COPD (16–18). It is preferable to other isoprostanes as a marker because it reflects oxidative stress levels much better under *in vivo* conditions than the other isoprostanes. It is considered an important marker of oxidative damage in asthma (19,20), and it is generally accepted that inflammatory markers are especially important for monitoring the response to ICS treatment.

A number of noninvasive methods are used to indicate inflammation in the airways in asthma, such as sputum samples and various markers in exhaled breath samples (10,21,22). Exhaled breath condensate (EBC) is one of these methods (10,19–22).

Smoking is known to have a negative effect on asthma in multiple ways (4–7). However, in this particular group of patients, the differences in inflammatory response underlying the less favorable response to treatment and asthma control compared to nonsmokers, and how these differences result in the need for higher doses of inhaled corticosteroids, are currently largely unknown and remain open to investigation (4).

In this study, specimens of EBC and blood from patients who had been newly diagnosed with asthma were used to quantify inflammation. These measurements were repeated after 3 months of treatment with ICS or ICS + LABA.

Methods

This study was planned as a prospective cross-sectional study. The ethics committee approval for the study was obtained from the faculty's ethics committee (approval reference number:11112012–35438).

Study population

All patients who attended a polyclinic within a six-month period were evaluated. These patients had not previously been diagnosed with asthma. They had a history compatible with asthma and were shown to have reversible obstruction on pulmonary function testing or had normal pulmonary function but a positive response to the bronchial provocation test. All patients were diagnosed with asthma (1). Besides the fact that the patients had newly received a diagnosis of asthma, the following inclusion and exclusion criteria were used:

Inclusion criteria

The inclusion criteria were as follows: no previous treatment with any agent, for any reason whatsoever, that may interfere with the treatment of asthma, namely systemic steroids, ICS, ICS plus LABA, ICS, and long-acting muscarinic antagonist (LAMA); no other disease; either being a nonsmoker or, in the smoker group, having smoked every day for at least one year; and agreement and consent to participate in the study.

Exclusion criteria

The exclusion criteria were as follows: previous treatment with any agent, for any reason that may interfere with the treatment of asthma, namely systemic steroids, ICS, ICS plus LABA, ICS, and long-acting anticholinergics; being in a flare-up at the time of diagnosis; presence of another disease or pathological results on testing apart from those involved in spirometry and allergy testing; exposure to passive smoking in the immediate environment; being an ex-smoker; and not agreeing to participate in the study.

Patient history and physical examination results were documented on the asthma polyclinic record cards, and routine examinations (spirometry, chest radiography, full blood count, and allergic skin prick tests) were performed. EBC and blood samples were taken from each patient twice, before and three months after the initiation of treatment, and IL-6, LTB4, LTD4, and 8-isoprostane levels were obtained for each sample. Treatment was initiated in newly diagnosed asthma patients either with a low dose of ICS used alone or in combination with an LABA, in accordance with the treatment guidelines (1). After

three months, the checkups were performed in accordance with the Asthma Control Test (ACT) and by undertaking measurements of the study parameters. The groups were compared statistically in terms of intergroup differences in these parameters and in the values obtained before and after treatment.

Respiratory function tests

Spirometry tests were performed using a spirometry device (ZAN 100 Flow Handy II, Germany). The tests were performed in accordance with the European Respiratory Society and American Respiratory Society joint criteria (23). Forced vital capacity (FVC), FVC percentage (FVC%), forced expiratory volume in one second (FEV_1), FEV_1 percentage (FEV_1%), forced mid-expiratory flow (MEF_{25-75}), and FEV_1/FVC values were recorded.

Evaluation of asthma control ACT

This study is based on the asthma control ACT. The ACT is a questionnaire consisting of five domains enquiring about diurnal and nocturnal manifestations of asthma, any use of rescue drugs, and any restriction to daily living activities resulting from asthma. A validated Turkish version was used in this study. In interpreting ACT scores, a result between 20 and 25 was considered to indicate asthma was "controlled" while a result below 20 indicated "uncontrolled" (1,24).

Collection and storage of EBC

Two samples of EBC were taken from newly diagnosed asthma patients, one prior to, and one following treatment. The samples were obtained according to ATS/ERS criteria (10). To account for the normal differences owing to circadian rhythms, this procedure was performed on a previously agreed day at a specific time (between 08:00 and 10:00 am). Patients were instructed to arrive at the hospital after overnight fasting. The procedure was performed when the Turbo DECCS 09 device (Medivac, Italy) reached a temperature of −5 °C. The patient was made to sit in an upright position and breathe using their tidal volume and wearing a nose clip for 10 min. Approximately 1–2 ml of breath was collected into a falcon tube, which was then spun for one min in a centrifuge at 1000 rpm. The obtained material was transferred to an Eppendorf tube and stored at −80 °C.

Blood collection and storage

Blood samples were obtained from the patients prior to treatment and 3 months after treatment, once the EBC had been obtained. The blood was centrifuged for 5 min at 3000 rpm, and the serum obtained was stored at −80 °C until testing for IL-6, 8-isoprostane, LTB4, and LTD4.

Processing of the EBC and serum samples

On the day of processing, the EBC and serum samples were taken out of storage at −80 °C. IL-6 levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit produced by Diaclone, while LTB4, LTD4, and 8-isoprostane levels were measured using an ELISA kit produced by Bio-Medical Assay.

Statistical analysis

While evaluating the study data, in addition to descriptive statistical methods (mean, standard deviation, median, frequency, ratio, minimum, and maximum), the Mann-Whitney U test was used for the comparison of those parameters that did not show normal distribution in the comparison of quantitative data. The Wilcoxon signed-rank test was used for pre- and post-treatment comparisons of parameters that did not show a normal distribution. Spearman's rank-order correlation coefficient was used to evaluate the relationships between parameters. Significance was tested at p values of <0.01 and <0.05. SPSS v0.21 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Fifty-eight patients who attended a chest disease polyclinic within a 6-month period were screened, and 23 patients were enrolled in the study. However, since three patients failed to attend their second visit, the number of individuals completing the study was 20 (Figure 1).

Demographic data and pulmonary function test values of the patients are summarized in Table 1.

Seven (35%) of the 20 patients were on low-dose ICS, while 13 (65%) were on low-dose ICS in combination with LABA. In the smokers' group, seven patients (70%) were on low-dose ICS in combination with LABA, and three patients (30%) were using low-dose ICS as monotherapy. In the nonsmoker group, six patients (60%) were on low-dose ICS in

combination with LABA, while four patients (40%) were using low-dose ICS only.

The SA and NSA groups were compared in terms of blood levels of IL-6, LTB4, LTD4, 8-isoprostane, and EBC levels of IL-6, LTB4, LTD4, and 8-isoprostane, and no statistically significant difference was observed in pretreatment. The blood results had p values of 0.414, 0.744, 0.935, and 0.121, respectively. For EBC, the corresponding p values were 0.935, 0.935, 0.743, and 0.220, respectively.

The smokers and nonsmokers groups were compared in terms of alterations in pulmonary function test parameters after treatment. The only parameter that showed a change in statistical significance was the FVC% ($p = 0.045$) (Table 2).

Following treatment, all 10 nonsmokers (100%) had asthma under control, while only five (50%) of the10 smokers had asthma that was under control. This difference between the two groups in terms of asthma control was statistically significant ($p = 0.033$) (Table 2).

The complete set of patients was also compared in terms of pre-and post-treatment values. No statistically significant difference was observed in the blood levels of IL-6, LTB4, LTD4, and 8-isoprostane ($p = 0.601$, $p = 0.573$, $p = 0.528$, and $p = 0.243$, respectively). In addition, no statistically significant difference was observed in the EBC levels of IL-6, LTB4, LTD4, and 8-isoprostane compared in this way ($p = 0.295$, $p = 0.398$, $p = 0.469$, and $p = 0.131$, respectively).

No statistically significant differences were noted in the blood levels of IL-6, LTB4, LTD4, or 8-isoprostane after treatment ($p = 0.369$, $p = 0.142$, $p = 0.683$, $p = 0.102$, respectively). Additionally, for the EBC values, no statistically significant differences were noted at the alteration in IL-6, LTB4 or LTD4 ($p = 0.806$, $p = 0.369$, $p = 0.236$, respectively). However, there was a statistically significant decrease in the EBC levels of 8-isoprostane when the SA and NSA groups were compared ($p = 0.022$) (Table 3).

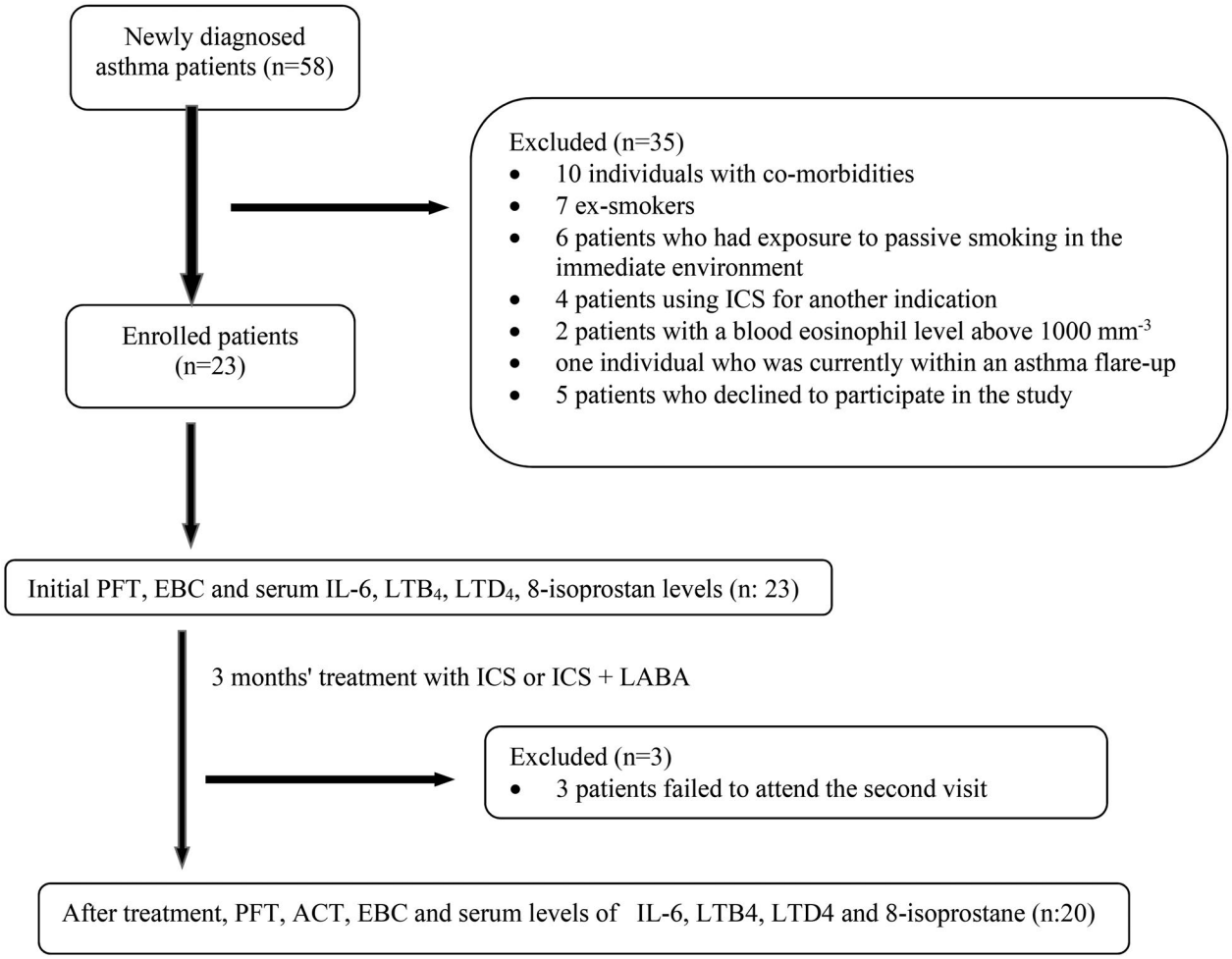


Figure 1. Flow Chart. PFT: Pulmonary Function Test; ACT: Asthma Control Test: EBC: Exhaled Breath Condensate; IL: Interleukin; LT: leukotriene, ICS: inhaled corticosteroid, LABA: long-acting beta agonist.

No significant association was found between blood and EBC levels of 8-isoprostane and the ACT score ($p = 0.337$ and $p = 0.214$, respectively).

Discussion

Our findings showed that asthma control was better in nonsmokers than in smokers, that the levels of IL-6, LCTB4, LCTD4 and 8-isoprostane were similar in both groups, and that, following 3 months' treatment consisting of ICS alone or ICS in combination with LABA, the nonsmokers had a greater decrease in the level of 8-isoprostane in the EBC samples. Furthermore, we found that no such decrease occurred in smokers.

IL-6, a pro-inflammatory cytokine, may play a key role in airway remodeling that occurs in asthma (13). Yokoyama et al. established that the blood levels of IL-6 were higher in asthmatic patients than in healthy controls (15). Ghaffari et al. compared blood levels in 21 cases of severe and 30 of mild asthma but found no significant difference between the two groups (25). Carpagnano et al. conducted a study with 28 patients with persistent asthma, with a control group of 15 individuals. The levels of IL-6 in EBC samples were higher in the asthmatic group. After 6 months of treatment with ICS, there was no statistically significant decrease in IL-6 levels (26). Similarly, to the Carpagnano study, our study showed no statistically significant difference in the IL-6 levels following 3 months' treatment with ICS. Carpagnano et al. undertook a study with 21 smokers and 14 nonsmoking, healthy subjects, in which it was demonstrated that, in the group of smokers, IL-6 levels in the EBC

Table 1. The demographic data and the pulmonary function test values of the patients.

Parameter		n	%
Female		11	55
Male		9	45
Smokers with asthma		10	50
Nonsmokers with asthma		10	50
	Minimum–Maximum	Mean	SD
Age	20–70	35.55	13.54
Smoking history of smokers with asthma (package-year)	1–10	4.67	3.31
FEV_1 (ml)	1480–4420	2776.31	983.30
FEV_1 %	53–103	80.42	14.60
FVC (ml)	2120–6180	4093.68	1275.89
FVC%	76–131	101.16	14.39
FEV_1 / FVC %	43–85	67.58	8.55
FEF_{25-75} (ml)	630–4180	1865.26	1005.78
FEF_{25-75} %	15–84	43.42	17.49

Note. FEV_1: forced expiratory volume in 1 s, FVC: forced vital capacity, FEF: forced expiratory flow, SD: standard deviation.

Table 2. Changes in pulmonary function parameters and ACT after treatment in smokers and nonsmokers with asthma.

		Smoker with asthma (SA) ($n = 10$)	Nonsmoker with asthma (NSA) ($n = 10$)	p
ΔFEV_1 (ml)	Min/Max (Median) Mean ± SD	−70/360 (60) 144.44 ± 181.32	−280/2450 (585) 637.00 ± 836.32	0.236
ΔFEV_1 (%)	Min/Max (Median) Mean ± SD	−2/13 (2) 4.22 ± 5.52	−8/62 (24) 21.60 ± 23.58	0.153
ΔFVC (ml)	Min/Max (Median) Mean ± SD	−80/240 (30) 44.44 ± 110.13	−180/1330 (260) 398.00 ± 527.46	0.141
ΔFVC (%)	Min/Max (Median) Mean ± SD	−2/8 (0.5) 1.11 ± 3.33	−3/44 (7.5) 12,50 ± 14,86	**0.045***
ΔFEV_1/FVC (%)	Min/Max (Median) Mean ± SD	−1/10 (2) 2.78 ± 3.80	−11/30 (9,5) 9.30 ± 12.25	0.130
ΔFEF_{25-75} (ml)	Min/Max (Median) Mean ± SD	−100/960 (290) 350.00 ± 369.12	−590/2800 (870) 1055.00 ± 1200.89	0.253
ΔFEF_{25-75} (%)	Min/Max (Median) Mean ± SD	−3/18 (6) 7.56 ± 7.70	−15/73 (25) 28.20 ± 29.74	0.121
ACT > 20	n (%)	5 (50)	10 (100)	**0.033***

Note. Δ: post treatment change; *: $p < 0.05$.
ACT: Asthma Control Test, EBC: Exhaled Breath Condensate, FEV1: forced expiratory volume in 1 s, FVC: forced vital capacity, FEF: forced expiratory flow, Min: Minimum, Max: Maximum, SD: Standard deviation.

Table 3. Changes in IL-6, Leukotriene B4, Leukotriene D4 and 8-isoprostane levels in blood and EBC samples after treatment, in smokers and nonsmokers with asthma.

Parameters (pg/ml)		Smoker with asthma (SA) ($n = 10$)	Nonsmoker with asthma (NSA) ($n = 10$)	p
Blood ΔIL-6	Min/Max (Median) Mean ± SD	−2354.64/3981.71 (−39.68) 343.58 ± 1873.06	−3994.94/2367.86 (−363.78) −727.09 ± 2280.06	0.369
Blood ΔLTB_4	Min/Max (Median) Mean ± SD	−643.53/1570,52 (176.21) 277.50 ± 633.19	−559,26/1700,76 (−42,14) 52.69 ± 610.23	0.142
Blood ΔLTD_4	Min/Max (Median) Mean ± SD	−731.6/815.56 (9) 18.99 ± 412.76	−200.89/506.9 (40,48) 67.18 ± 212.4	0.683
Blood Δ8-isoprostan	Min/Max (Median) Mean ± SD	−1028.95/1714.91 (−192.24) 125.01 ± 1013.66	−4782.1/5085.76 (−808.31) −721.57 ± 2534.82	0.102
EBC ΔIL-6	Min/Max (Median) Mean ± SD	−4140,45/2566,29 (−754.02) −848.08 ± 2169.34	−4987.06/5317.77 (−423.31) −488,12 ± 3163,91	0.806
EBC ΔLTB_4	Min/Max (Median) Mean ± SD	−416.64/444.34 (38.31) 44.41 ± 226.93	−865.71/130.24 (18.54) −118.84 ± 315.40	0.369
LTD_4	Min/Max (Median) Mean ± SD	−10,19/3,06 (−2.04) −2.83 ± 4.93	−20.36/9.17 (1.53) −0.41 ± 9.30	0.236
EBC Δ8-isoprostan	Min/Max (Median) Mean ± SD	−41.5/43.7 (−2.18) 2.91 ± 23.22	−104.86/15.29 (−14.2) −22.72 ± 33.12	**0.022***

Note. Δ: post treatment change; * $p < 0.05$.
EBC: Exhaled breath condensate, LT: Leukotriene, pg: picogram.

samples increased in proportion to the number of cigarettes smoked daily (27). Wood et al. found a positive correlation between smoking and blood IL-6 levels (28). In our study, in contrast with the studies of Carpagnano and Wood, no statistically significant difference was found between the levels of IL-6 in blood and in the EBC samples in the smoker and nonsmoker groups. This may be owing to the number of cigarettes smoked by asthmatic patients in our study was less compared to in Wood and Carpagnano's study.

There are different results from studies involving blood levels of LTB4, LTC4, LTD4, and LTE4, which are among the most studied molecules in the pathogenesis of asthma, and cause smooth muscle contraction. While there was no significant difference in blood LTB4 levels between the control group and patients with asthma in a study undertaken by Seggev et al., in a study conducted by Mansour et al., blood cysteinyl leukotriene and LTB4 levels were found to be higher in asthmatic patients than in the control group (29,30). Since there was no control group in our study, this comparison was not possible. In studies investigating leukotrienes in EBC samples, leukotriene levels were found to be higher in asthmatic patients than in the control group (11,31). In the studies undertaken by Samitas et al., leukotriene levels were found to be significantly higher in patients with moderate and severe asthma than in patients with mild asthma or in control groups (11). In our study, no statistically significant decrease was found in the levels of LTB4 and LTC4 in blood or EBC samples before and after treatment. The detection of high leukotrienes in moderate and severe asthma in the Samitas study, despite treatment with ICS, and the absence of a significant decrease in leukotrienes following ICS treatment in our study may be because steroid treatment failed to change the levels of leukotrienes. From the literature, it is clear that smoking causes neutrophilic inflammation. Neutrophils, however, do not synthesize cysteinyl leukotrienes, the synthesis, which is increased via unknown pathophysiological mechanisms (32). In a study undertaken by Carpagnano et al., LTB4 levels in EBC were found to be significantly higher in healthy smokers than in nonsmokers (27); in a study by Çelik et al., EBC LTD4 levels were found to be higher in asthmatic patients who smoked than in nonsmoking patients (33). In our study, no statistically significant difference was observed in blood or EBC levels of LTB4 and LTD4 between smoking and nonsmoking asthma patients. This may be owing to the low number of cases and the fact that cigarette consumption was not as high as it was in other studies.

8-isoprostane is considered an important marker of oxidative damage in asthma (16). In a study conducted by Wood et al., the plasma levels of 8-isoprostane were elevated in asthma (34). A study undertaken by Mak et al. demonstrated that plasma levels of 8-isoprostane increased during acute exacerbations and decreased during remission, although the level remained higher in asthmatic patients than in healthy controls, even during periods of remission (35). In a study by Montuschi et al., compared with levels in healthy controls, the 8-isoprostane level in EBC samples was twice higher in mild-moderate asthma and three times higher in severe asthma (31). In a study by Samitas et al., a 3-fold increase in mild-to-moderate asthma and a 4-fold increase in severe asthma was demonstrated (11). The high level of 8-isoprostane despite oral steroid and/or ICS treatment can be explained by the non-responsiveness of 8-isoprostane to steroids. In a study by Piotrowski et al., 8-isoprostane levels were compared in EBC samples obtained from 25 nonsmokers with severe asthma, 11 nonsmokers, healthy controls, and 16 newly diagnosed mild asthma patients. No statistically significant differences were found between the groups in 8-isoprostane levels. There was no significant correlation between 8-isoprostane levels and ACT scores (36). In our study, in contrast to the results obtained by Piotrowski et al., a statistically significant decrease in 8-isoprostane levels in EBC samples was noted in smokers compared to nonsmokers following treatment with ICS. This situation may indicate that the burden of oxidative stress in the airways of nonsmoking patients with asthma decreases rapidly and effectively following ICS treatment, unlike in smokers. This result may also allow us to predict that smokers with asthma will not respond well to ICS treatment.

Considering that 8-isoprostane is a noninvasive indicator of oxidative stress that plays an important role in asthma, the use of antioxidants derived from food and medicinal plants for patients with asthma is a good hypothesis, but this issue is a complete mystery, new studies are needed to clarify this (37). Another mystery point is antioxidative genes, genetic variants of antioxidative genes, especially variants of glutathione S-transferases (GSTT1 and GSSM1), which play a significant role in detoxification processes in asthma (38). We believe that in the future, antioxidant foods and antioxidative genes will be more involved in asthma treatment.

In our study, we studied IL-6, LTB4, LTD4, and 8-isoprostane levels in blood and EBC samples and found that many biomarkers and metabolomics can be studied in blood and EBC samples to identify

metabolic signatures specific to asthma and sub-types (39). Metabolomics, the study of low-molecular-weight metabolites, is a rising star for future asthma studies, especially to clarify potential pathogenic mechanisms. In addition to blood and EBC samples, nasal samples are also a guide in asthma studies and provide important clues for the pathogenesis of asthma (40).

In our study, a higher rate of asthma control was achieved in nonsmokers with asthma, and this result was statistically significant. However, no relationship was established between the degree of asthma control and IL-6, LTB4, LTD4, or 8-isoprostane levels in blood or in EBC samples.

The limitations of our study stem from the small number of cases, the fact that only a single center was involved, and that ICS treatment was only of a brief duration. Measuring the level of multiple markers and the cost of reagents to the study budget were limited to the number of cases. Its strengths are that it included both smokers and nonsmokers in a group of newly diagnosed asthma patients and simultaneously investigated IL-6, LTB4, LTD4, and 8-isoprostane levels in blood and EBC samples.

Conclusion

In conclusion, the similarity of IL-6, LTB4, LTD4, and 8-isoprostane levels in blood and EBC samples at the time of asthma diagnosis in both smokers and nonsmokers suggests that the inflammatory pathways are similar in both groups. The significantly greater decrease in 8-isoprostane levels in EBC samples following 3 months of ICS treatment in the nonsmoker group indicates the existence of phenotypic differences. Monitoring changes in 8-isoprostane levels in SA may help to guide the development of new treatments by informing them about treatment management and asthma suppression.

Declaration of interest

The authors report no conflicts of interest.

Funding

This study was conducted with the support of the university scientific research project.

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References

1. Global strategy for asthma management and prevention. 2020. https://ginasthma.org/wp-content/uploads/2020/04/GINA-2020-full-report_-final-_wms.pdf. [last accessed 2 February 2021].
2. Thomson NC, Chaudhuri R. Asthma in smokers: challenges and opportunities. Curr Opin Pulm Med. 2009;15(1):39–45. doi:10.1097/MCP.0b013e32831da894.
3. Yildiz F, Dursun AB, Disçi R. Prevalence of asthmatic smokers: Turkish experience (PASTE Study). Clin Respir J. 2014;8(3):350–356. doi:10.1111/crj.12079.
4. Sprio AE, Ciprandi G, Riccardi E, Giannoccaro F, Carriero V, Bertolini F, Ricciardolo FLM. The influence of smoking on asthma in the real-life. Respir Med. 2020;170:106066. doi:10.1016/j.rmed.2020.106066.
5. Tommola M, Ilmarinen P, Tuomisto LE, Haanpää J, Kankaanranta T, Niemelä O, Kankaanranta H. The effect of smoking on lung function: a clinical study of adult-onset asthma. Eur Respir J. 2016;48(5):1298–1306. doi:10.1183/13993003.00850-2016.
6. Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, Menzies-Gow AN, Mansur AH, McSharry C. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. J Allergy Clin Immunol. 2013;131(4):1008. doi:10.1016./j.jaci.2012.12.1574.
7. Aanerud M, Carsin A-E, Sunyer J, Dratva J, Gislason T, Jarvis D, deMarco R, Raherison C, Wjst M, Dharmage SC, et al. Interaction between asthma and smoking increases the risk of adult airway obstruction. Eur Respir J. 2015;45(3):635–643. doi:10.1183/09031936.00055514.
8. TS, Hallstrand WR. HendersonJr. An update on the role of leukotrienes in asthma. Curr Opin Allergy Clin Immunol. 2010;10(1):60–66. doi:10.1097/ACI.0b013e32833489c3.
9. Devillier P, Baccard N, Advenier C. Leukotrienes, leukotriene receptor antagonists and leukotriene synthesis inhibitors in asthma: an update. Part II: clinical studies with leukotriene receptor antagonists and leukotriene synthesis inhibitors in asthma. Pharmacol Res. 1999;40(1):15–29. doi:10.1006/phrs.1998.0461.
10. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJC, Corradi M, Dekhuijzen R, et al. Exhaled breath condensate: methodological recommendations and unresolved questions. Eur Respir J. 2005;26(3):523–548. doi:10.1183/09031936.05.00029705.
11. Samitas K, Chorianopoulos D, Vittorakis S, Zervas E, Economidou E, Papatheodorou G, Loukides S, Gaga M. Exhaled cysteinyl-leukotrienes and 8-isoprostane in patients with asthma and their relation to clinical severity. Respir Med. 2009;103(5):750–756. doi:10.1016/j.rmed.2008.11.009.
12. Claesson HE, Dahlen SE . Asthma and leukotrienes: antileukotrienes as novel anti-asthmatic drugs. J Intern Med. 1999;245(3):205–227. doi:10.1046/j.1365-2796.1999.00418.x.
13. Mikuniya T, Nagai S, Tsutsumi T, Morita K, Mio T, Satake N, Izumi T. Proinflammatory or regulatory cytokines released from BALF macrophages of healthy

smokers. Respiration. 1999;66(5):419–426. doi:10.1159/000029425.
14. Kang S, Narazaki M, Metwally H, Kishimoto T. Historical overview of the interleukin-6 family cytokine. J Exp Med. 2020;217(5):e20190347. doi:10.1084/jem.20190347.
15. Yokoyama A, Kohno N, Fujino S, Hamada H, Inoue Y, Fujioka S, Ishida S, Hiwada K. Circulating interleukin-6 levels in patients with bronchial asthma. Am J Respir Crit Care Med. 1995;151(5):1354–1358. doi:10.1164/ajrccm.151.5.7735584.
16. Montuschi P, Barnes P, Roberts LJ . Insights into oxidative stress: the isoprostanes. Curr Med Chem. 2007;14(6):703–717. doi:10.2174/092986707780059607.
17. Montuschi P, Collins JV, Ciabattoni G, Lazzeri N, Corradi M, Kharitonov SA, Barnes PJ. Exhaled 8-isoprostane as an in vivo biomarker of lung oxidative stress in patients with COPD and healthy smokers. Am J Respir Crit Care Med. 2000;162(3):1175–1177. doi:10.1164/ajrccm.162.3.2001063.
18. Shoman YWild P, Hemmendinger M, Graille M, Sauvain JJ, B Hopf N, Guseva Canu I. Reference ranges of 8-isoprostane concentrations in exhaled breath condensate (EBC): a systematic review and meta-analysis. Int J Mol Sci. 2020;21:3822. doi:10.3390/ijms21113822.
19. Montuschi P, Corradi M, Ciabattoni G, Nightingale J, Kharitonov SA, Barnes PJ. Increased 8-isoprostane, a marker of oxidative stres, in exhaled condansate of asthma patients. Am J Respir Crit Care Med. 1999;160(1):216–220. doi:10.1164/ajrccm.160.1.9809140.
20. Peel AM, Crossman-Barnes CJ, Tang J, Fowler SJ, Davies GA, Wilson AM, Loke YK. Biomarkers in adult asthma: a systematic review of 8-isoprostane in exhaled breath condensate. J Breath Res. 2017;11(1):016011. doi:10.1088/1752-7163/aa5a8a.
21. Gemicioglu B, Musellim B, Dogan I, Guven K. Fractional exhaled nitric oxide (FeNo) in different asthma phenotypes. Allergy Rhinol (Providence) 2014;5(3):157–161. doi:10.2500/ar.2014.5.0099.
22. Montuschi P . Analysis of exhaled breath condensate in respiratory medicine: methodological aspects and potential clinical applications. Ther Adv Respir Dis. 2007;1(1):5–23. doi:10.1177/1753465807082373.
23. Culver BH, Graham BL, Coates AL, Wanger J, Berry CE, Clarke PK, Hallstrand TS, Hankinson JL, Kaminsky DA, MacIntyre NR, et al. Recommendations for a standardized pulmonary function report. An official american thoracic society technical statement. Am J Respir Crit Care Med. 2017;196(11):1463–1472. doi:10.1164/rccm.201710-1981ST.
24. Uysal MA, Mungan D, Yorgancioglu A, Yildiz F, Akgun M, Gemicioglu B, Turktas H. The validation of the Turkish version of asthma control test. Qual Life Res. 2013;22(7):1773–1779. doi:10.1007/s11136-012-0309-1.
25. Ghaffari J, Rafiei AR, Ajami A, Mahdavi M, Hoshiar B. Serum interleukins 6 and 8 in mild and severe asthmatic patients, is it difference. Caspian J Intern Med. (2)2011;2:226–228.
26. Carpagnano GE, Foschino Barbaro MP, Resta O, Gramiccioni E, Valerio NV, Bracciale P, Valerio G. Exhaled markers in the monitoring of airways inflammation and its response to steroid's treatment in mild persistent asthma. Eur J Pharmacol. 2005;519(1–2):175–181. doi:10.1016/j.ejphar.2005.06.034.
27. Carpagnano GE, Kharitonov SA, Foschino-Barbaro MP, Resta O, Gramiccioni E, Barnes PJ . Increased inflammatory markers in the exhaled breath condensate of cigarette smokers. Eur Respir J. 2003;21(4):589–593. doi:10.1183/09031936.03.00022203.
28. Wood LG, Baines KJ, Fu J, Scott HA, Gibson PG. The neutrophilic inflammatory phenotype is associated with systemic inflammation in asthma. Chest. 2012;142(1):86–93. doi:10.1378/chest.11-1838.
29. Seggev JS, Wiessner JH, Thornton WH, Jr, Edes TE. Comparison of serum and plasma leukotriene B4 levels in normal and asthma subjects. Ann Allergy Asthma Immunol. 1995;75(4):365–368.
30. Mansour M, Farouk N, el Maragy A, Radwan I, el-Ahmady O . Elevated plasma level of leukotrienes in bronchial asthma patients: a possible clinical relevance . Dis Markers. 1995(2);12:117–122. doi:10.1155/1994/121453.
31. Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. J Allergy Clin Immunol. 2002;109(4):615–620. doi:10.1067/mai.2002.122461.
32. Garey KW, Neuhauser MM, Robbins RA, Danziger LH, Rubinstein I. Markers of inflammation in exhaled breath condansate of young healthy smokers. Chest. 2004;125(1):22–26. doi:10.1378/chest.125.1.22.
33. Celik D, Doruk S, Koseoglu HI, Sahin S, Celikel S, Erkorkmaz U . Cysteinyl leukotrienes in exhaled breath condensate of smoking asthmatics. Clin Chem Lab Med. 2013;51(5):1069–1073. doi:10.1515/cclm-2012-0479.
34. Wood LG, Fitzgerald DA, Gibson PG, Cooper DM, Garg ML. Lipid peroxidation as determined by plasma isoprostanes is related to disease severity in mild asthma. Lipids. 2000;35(9):967–974. doi:10.1007/s11745-000-0607-x.
35. Mak JCW, Ho SP, Ho ASS, Law BKW, Cheung AHK, Ho JCM, Ip MSM, Chan-Yeung MMW . Sustained elevation of systemic oxidative stress and inflammation in exacerbation and remission of asthma. ISRN Allergy. 2013;2013:561831. doi:10.1155/2013/561831.
36. Piotrowski WJ, Majewski S, Marczak J, Kurmanowska Z, Górski P, Antczak A . Exhaled breath 8-isoprostane as a marker of asthma severity. Arch Med Sci. 2012;8(3):515–520. doi:10.5114/aoms.2012.28639.
37. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Zhang JJ, Li HB. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int J Mol Sci. 2017;18:96. doi:10.3390/ijms18010096.
38. Liang S, Wei X, Gong C, Wei J, Chen Z, Chen X, Wang Z, Deng J. Significant association between asthma risk and the GSTM1 and GSTT1 deletion polymorphisms: an updated meta-analysis of case-control studies. Respirology. 2013;18(5):774–783. doi:10.1111/resp.12097.
39. Papamichael MM, Katsardis C, Sarandi E, Georgaki S, Frima ES, Varvarigou A, Tsoukalas D. Application of metabolomics in pediatric asthma: prediction, diagnosis and personalized treatment. Metabolites. 2021;11:251. doi:10.3390/metabo11040251.
40. Krantz C, Janson C, Alving K, Malinovschi A. Nasal nitric oxide in relation to asthma characteristics in a longitudinal asthma cohort study. Nitric Oxide. 2021;106:1–8. doi:10.1016/j.niox.2020.09.002.