Changes in eotaxin-2 and periostin levels in patients with bronchial asthma according to their smoking status: a cross-sectional study

Mohammed F. Abdelghany[a], Atef F. El-Karn[a], Mahmoud F. Sherif[b], Mohamed I. Seddik[c], Safaa A. Eid[a], Sahar F. Youssif[a]

Departments of [a]Chest Diseases and Tuberculosis, [b]Pathology, [c]Clinical Pathology, Assiut University, Assiut, Egypt

Correspondence to Safaa A. Eid, MD, Department of Chest Disease and Tuberculosis, Assiut University, Assiut 71515, Egypt
Tel: +20 100 256 9966;
Fax: (088) 2080228 - 2423476;
e-mail: safaa_gayed@yahoo.com

Received: 05 August 2022
Revised: 24 October 2022
Accepted: 25 October 2022
Published: 13 July 2023

The Egyptian Journal of Chest Diseases and Tuberculosis 2023, 72:305–312

Background
Smoking influences the nature of airway inflammation in patients with bronchial asthma though synthesis of certain cytokines. Patterns of bronchial asthma are differentiated clinically, functionally, and regarding inflammatory biomarkers.
Aim
The research aimed to study the clinical, functional, sputum cytological differences, and serum eotaxin-2 and periostin levels in asthmatic patients regarding smoking status.
Patients and methods
The research was a cross-sectional study. The collection of cases began in August 2018 and ended in January 2020 at the Chest Department, Assiut University Hospital. We studied 117 asthmatic patients who were classified regarding their smoking status (45 nonsmokers, 42 smokers, and 30 former smokers) for serum eotaxin-2 and periostin by enzyme-linked immunosorbent assay. The effects of smoking were analyzed on inflammatory cells including eosinophilic and neutrophilic percentages in sputum and serum eotaxin-2 and periostin levels.
Results
Smokers with asthma had worse clinical and functional outcomes. Asthmatic smokers had mainly neutrophilic phenotype. Serum eotaxin-2 level was higher in smokers compared with nonsmokers and former smokers. However, serum periostin level was higher in nonsmokers compared with smokers and former smokers. Serum eotaxin-2 had a positive correlation with smoking index and eosinophilic and neutrophilic count in sputum, whereas serum periostin was correlated negatively with smoking index and positively with eosinophilic count.
Conclusion
Asthmatic smokers had worse clinical and functional outcomes with increased neutrophils in the sputum. The inflammatory biomarkers seen in smokers with asthma showed low serum periostin and increased serum eotaxin-2 levels.

Keywords:
asthma, biomarkers, eosinophil, eotaxin-2, neutrophil, periostin, smoker

Egypt J Chest Dis Tuberc 2023, 72:305–312

2090-9950

Background

Smoking influences bronchial asthma. Asthmatic smokers have distinct characteristics with worse prognosis, poor outcomes, and definite therapeutic implications. The incidence of asthma is higher in smokers, and asthmatic smokers may consider a particular phenotype of asthma [1].

Asthma can be categorized into phenotypes according to clinical, functional, and cytological features. Personalized asthma treatment regarding the clinical phenotype is helpful as the diagnosis of asthma is usually on a clinical basis [2]. Researchers make best effort to categorize asthmatics into clusters and identifying endotypes according to clinical features, prognosis, and treatment response. Recently, new asthma treatment development depend on its phenotype and endotypes [3].

Asthmatic smokers have less serum periostin concentrations, and periostin levels are not related to asthma control indices such as asthma control questionnaire (ACQ) score and pulmonary function, smoking index, or asthma severity in all asthma groups, including never smokers. An elevated serum periostin level in asthmatic smokers may use a predictor of

response to Th2 inflammation target therapies; future studies are needed [4].

Asthmatic smokers, compared with asthmatic never-smokers, show elevated levels of eotaxin-2 in serum, sputum, and bronchoalveolar lavage (BAL) with no significant increase in eosinophilic count. The eotaxin levels were associated with eosinophilic and neutrophilic counts in all examined tissue. This study suggested that smoking stimulates eotaxin production and alters airway inflammation in asthmatic patients [5].

The present research aimed to study levels of serum eotaxin-2 and periostin in asthmatic patients regarding their smoking status, and to correlate their level in serum with the sputum eosinophilic and neutrophilic proportion.

Patients and methods

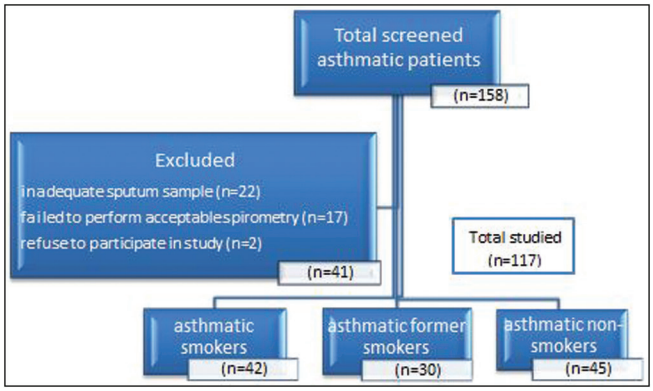
Study design

The research was cross-sectional and registered (ClinicalTrials.gov ID: NCT03207620). The collection of cases began in August 2018 and ended in January 2020 at the Chest Department, Assiut University Hospital. The Scientific Ethics Committee of the Faculty of Medicine, Assiut University, approved the research. Informed consent was obtained from the studied groups.

Patient selection

We studied 117 asthmatic patients classified regarding their smoking status (45 nonsmokers, 42 smokers, and 30 former smokers) randomly chosen from patients who visited the chest outpatient clinic, Assiut University Hospital (Fig. 1).

Figure 1



Selection process of patients.

Diagnostic criteria of asthma according to GINA guidelines 2021

Variability of respiratory symptoms

Cough, wheezing, shortness of breath, and chest tightness were assessed. Moreover, variability of symptoms over time and in intensity was measured. Symptoms worsened at night, on exertion, or with viral infections. Triggers by exercise, allergens, or cold air were assessed.

Variability of expiratory airflow limitation

A report that the forced expiratory volume in one second/forced vital capacity (FEV1/FVC) ratio was below the lower limit of normal and FEV1 was low was obtained at least once for evident diagnosis. Variability in lung function was recorded if FEV1 increased by more than 200 ml and more than 12% of the baseline value after bronchodilators, which means significant bronchodilator reversibility [6]. Inclusion criteria were as follows: stable asthmatic patients included current and former smokers. Stable asthmatic denoted no emergency department visit, hospital admission, systemic corticosteroid therapy, or altering the treatment of asthma in the last month. Current smoking denoted smoking five or more cigarettes per day and a smoking history of five pack-years or more [7]. Former smoker denoted quit smoking for more than 6 months. Male patients aged between 18 and 45 years old were included. All current therapies were allowed such as inhaled corticosteroids (ICS), leukotriene receptor antagonists, long-acting and short-acting b2-agonists, long-acting muscarinic antagonists, and theophylline. Exclusion criteria were as follows: acute attacks of asthma, other causes of obstructive airway diseases, age younger than 18 years and older than 45 years old, obese patients with BMI above 35, a requirement for treatment with systemic steroids in the past month, and the patients who stopped smoking in duration less than 6 months. Eligibility for the research demanded a confirmation by reversible expiratory airflow limitation [postbronchodilator FEV1 improved by >12% (and >200 ml)] [8]. International consensus guidelines were considered in all pulmonary function test assessments.

Sample size calculation

G* Power 3 software was used for calculating the sample size [9]. The sample size was at least 109 asthmatic patients, depending on the prevalence of bronchial asthma in the Egyptian population was 7.6%, determined by Tarraf *et al.* [10] with a probability of 0.05 and 80% power on a two-tailed test with a 90% confidence interval.

Baseline data: all studied groups were assessed using the following:

(1) Clinical assessment: asking about smoking status and severity, symptoms, and asthma control assessment by ACQ score of GINA guidelines [8].
(2) Spirometry: measuring postbronchodilator FEV1, FEV1/FVC, and forced expiratory flow at 25–75%. Moreover, total lung capacity (TLC), residual volume/TLC, and diffusing capacity of the lung for carbon monoxide (DLco) were measured. The lung function analyzers used were ZAN 300 (Nspire Health GMBH Co., Oberthulba, Lower Franconia, Germany).
(3) Sputum cytology: sputum samples collection. The patient was informed of cough with the maximum force and depth to expectorate. The sputum amount required was about 5–10 ml. The sputum was collected in the early morning. Specimen was collected in a sterile container. Each specimen saved in a sealed plastic container with correct label for transport and storage. Hypertonic saline was used for sputum induction on demand [11]. The 'pick and smear' method was used for sputum processing [12].
(4) Serum periostin level: commercially available Human Periostin (POSTN) enzyme-linked immunosorbent assay kit [13] (SinoGeneClon Biotech Co. Ltd, Hangzhou, China) was used for the quantitative measurement of Human POSTN concentrations following the manufacturer's instructions (Catalog No: SG-10345). Specimen collection: the standard laboratory methods were followed for blood sampling. Approximately 3 ml of venous blood was sampled from patients using a complete aseptic technique.
(5) Serum Eotaxin-2 level: commercially available human eotaxin-2 (CCL24) enzyme-linked immunosorbent assay kit [14] (SinoGeneClon Biotech Co. Ltd, Hangzhou, China) was used following the manufacturer's instructions (Catalog No: SG-10249).

Research outcome measures

Primary (main) outcome was to detect serum eotaxin-2 and periostin levels.

Statistical analysis

Statistical Package for the Social Sciences, version 20 (SPSS; IBM, Armonk, New York, USA) was applied for data analysis. The mean±SD and median (range) were used for expressing continuous data. Frequency (percentage) was used for expressing nominal data. The nominal data of different groups were compared using χ^2 test. The continuous data of two groups were compared using Student *t* test. However, the continuous data of more than two groups were compared using analysis of variance test followed by Bonferroni post-hoc analysis. The correlations between eotaxin-2 and periostin with different parameters were determined using Pearson correlation. The confidence level was kept at 95%, and the *P* value was considered significant if less than 0.05.

IRB approval

The Local Scientific Ethics Committee of the Faculty of Medicine, Assiut University, approved the research. IRB no: 17200038. The provisions of the Declaration of Helsinki were followed.

Results

This research included 117 patients having bronchial asthma. They were classified according to their smoking status into 45 nonsmokers, 42 smokers, and 30 former smokers.

In the current study, most asthmatic smokers and former smokers were cigarette smokers. The range of the smoking index was between 100 and 1240, with a mean of 500 in the case of smokers. However, it was between 100 and 700 with a mean of 300 in the case of former smoker. Cigarette smoking was frequently present in both groups. Three former smokers and two smokers were cigarette and hookah smokers.

Table 1 shows that there was no statistical significance regarding clinical data among the three studied groups ($P>0.05$). However, it was described that wheezes were higher among asthmatic smokers and former smokers compared with nonsmokers.

Based on the ACQ, the majority of asthmatic smokers and former smokers were uncontrolled compared with asthmatic nonsmokers. However, the majority of asthmatic nonsmokers were well controlled.

A total of 26 asthmatic patients of the smoker group, 17 asthmatic patients of the former smoker group, and 10 asthmatic patients of the nonsmoker group were on ICS therapy. The percentage of patients who use ICS was superior in asthmatic smokers [26 (61.9%)] and former smokers [17 (56.7%)] compared with nonsmokers [10 (22.2%)] ($P<0.001$). The ICS dose was superior in 13 smokers and 10 former smokers. However, the ICS dose was lower in all nonsmokers.

The postbronchodilators FEV1/FVC was lower with a statistically significant difference ($P=0.02$) among asthmatic smokers and former smokers compared with nonsmokers. Moreover, postbronchodilators FEV1 was significantly lower ($P=0.04$) among asthmatic smokers and former smokers compared with nonsmokers. Regarding the TLC, it was significantly higher among former smokers compared with nonsmokers. The

Table 1 Clinical data in asthmatic patients regarding their smoking status

	Smoker (*N*=42)	Former smoker (*N*=30)	None (*N*=45)	*P*	*P*1	*P*2	*P*3
Cough	39 (92.9)	28 (93.3)	39 (86.7)	0.51	0.65	0.27	0.30
Sputum production	37 (88.1)	26 (86.7)	37 (82.2)	0.72	0.56	0.32	0.43
Persistent sputum	10 (23.8)	9 (30)	10 (22.2)	0.73	0.62	0.13	0.21
Dyspnea	42 (100)	30 (100)	42 (93.3)	0.08	–	0.05	0.05
Wheezes	40 (95.2)	29 (96.7)	37 (82.2)	0.04	0.66	0.02	0.03
Paroxysmal attacks	41 (97.6)	29 (96.7)	41 (91.1)	0.34	0.28	0.07	0.33
Nocturnal attack	33 (78.6)	21 (70)	28 (62.2)	0.25	0.37	0.53	0.31
ACQ				0.01	0.46	0.04	0.02
Well controlled	5 (11.9)	3 (10)	25 (55.6)				
Partly controlled	11 (26.2)	12 (40)	10 (22.2)				
Uncontrolled	26 (61.9)	15 (50)	10 (22.2)				

Data were expressed as frequency (percentage).
ACQ, asthma control questionnaire.
P compares between different three groups; *P*1 compares between smokers and former smokers; *P*2 compares between smokers and non-smokers; *P*3 compares between former smokers and nonsmokers. *P* was significant if less than 0.05.

Table 2 Pulmonary function tests in asthmatic patients regarding their smoking status

	Smoker (*N*=42)	Former smoker (*N*=30)	None (*N*=45)	*P*	*P*1	*P*2	*P*3
FEV1/FVC							
Pre-BD	66.62±11.79	69.28±15.20	71.11±14.93	0.73	0.71	0.42	0.74
Post-BD	70.07±8.95	72.70±20.11	81.89±12.14	0.02	0.91	0.03	0.03
Forced expiratory volume-1							
Pre-BD	69.59±24.35	70.16±23.98	71.11±25.07	0.85	0.92	0.65	0.62
Post-BD	71.61±24.70	74.66±10.18	84.63±8.87	0.04	0.56	0.03	0.02
Forced vital capacity							
Pre-BD	85.13±12.36	86.24±10.57	84.88±12.61	0.96	0.83	0.95	0.79
Post-BD	94.69±4.96	94.33±5.28	84.68±8.64	0.19	0.07	0.26	0.34
FEF25–75							
Pre-BD	47.28±33.42	48.41±29.21	49.28±34.45	0.90	0.65	0.82	0.77
Post-BD	53.83±24.71	52.55±46.55	55.23±30.69	0.49	0.39	0.77	0.24
Total lung capacity (%)	113±37.06	135.30±5.56	103.60±22.38	0.04	0.11	0.13	0.03
RV/TLC (%)	45±16.37	44±20.49	46.28±24.93	0.97	0.88	0.95	0.82
DICo (%)	83.90±23.80	86.11±21.34	90.87±25.57	0.83	0.54	0.79	0.65

Data were expressed as mean (SD).
BD: bronchodilator; FEF25–75, forced expiratory flow at 25–75%; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; RV, residual volume; TLC, total lung capacity.
P compares between different three groups; *P*1 compares between smokers and former smokers; *P*2 compares between smokers and non-smokers; *P*3 compares between former smokers and nonsmokers.
P was significant if less than 0.05.

DLco showed no significant difference among the three groups, as shown in Table 2.

It was noticed that sputum eosinophils were significantly higher (*P*=0.04) in asthmatic nonsmokers compared with those former smokers and smokers. However, sputum neutrophils were superior in asthmatic smokers and former smokers compared with nonsmokers (*P*=0.01), as shown in Table 3.

As shown in Fig. 2, serum periostin was superior in nonsmokers compared with smokers and former smokers (19.97±3.89 vs. 11.58±6.15 and 13.36±9.73, respectively; *P*=0.03).

As shown in Fig. 3, serum eotaxin-2 was significantly higher among smokers (462.43±109.58) in comparison with nonsmokers and former smokers (114.94±25.87 and 164.15±38.93, respectively; *P*=0.03).

Serum periostin in asthmatic smokers had a significantly negative correlation with the smoking index (*r*=-0.49; *P*<0.001). Moreover, serum periostin in all asthmatic patients had a significant positive correlation with eosinophil count (*r*=0.21; *P*=0.04), as shown in Table 4.

As shown in Table 5, serum eotaxin-2 had a significant positive correlation with age, smoking index, sputum eosinophilic count, and sputum neutrophilic count of all asthmatic patients. However, serum eotaxin-2 had a negative correlation with FEV1 in all studied groups.

Discussion

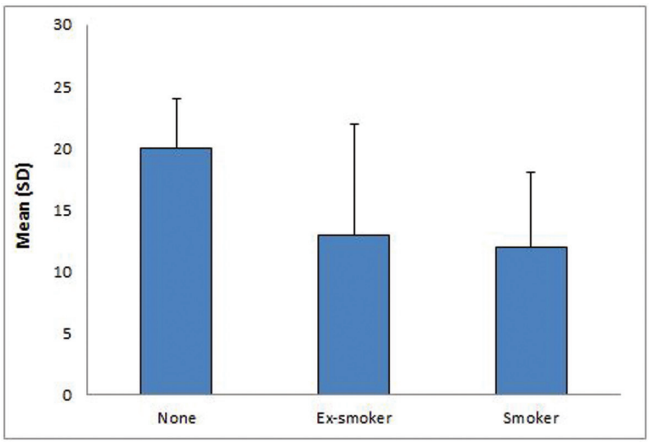
Regarding respiratory symptoms in the current study, wheezes were more frequent among smokers and former smokers than nonsmokers. Çolak *et al.* [15] stated that 'asthmatic smokers had more respiratory

Table 3 Sputum cytology in asthmatic patients regarding their smoking status

	Smoker (*N*=42)	Former smoker (*N*=30)	None (*N*=45)	*P*	*P*1	*P*2	*P*3
Sputum cellularity							
No	5 (11.9)	2 (6.7)	23 (51.1)	0.04	0.09	0.04	0.03
Mild	16 (38.1)	13 (43.3)	15 (33.3)				
Moderate	1 (2.4)	1 (3.3)	3 (6.7)				
High	20 (47.6)	14 (46.7)	4 (8.9)				
Alveolar macrophages	19 (45.2)	15 (50)	23 (51.1)	0.71	0.06	0.16	0.72
Eosinophils (%)	3.09±1.39	5.67±1.65	7.88±2.52	0.04	0.65	0.04	0.02
Neutrophils (%)	23.67±3.03	19.67±9.79	10.11±3.90	0.01	0.94	0.02	0.03
Histiocytes (%)	16.42±6.30	17±2.23	12.33±2.57	0.64	0.92	0.43	0.41
Lymphocytes (%)	11.30±6.84	9.67±1.08	9.77±3.49	0.94	0.77	0.76	0.98

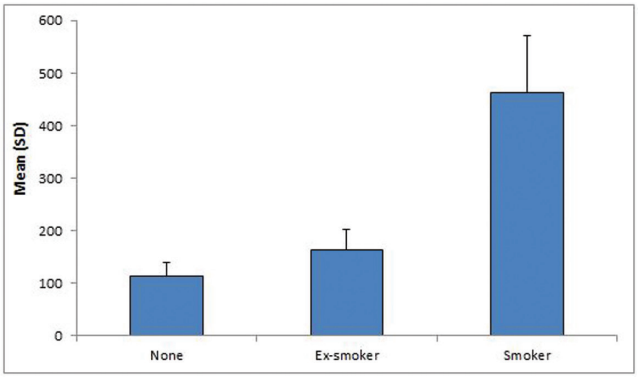
Data were expressed as frequency (percentage), mean (SD).
P compares between different three groups; *P*1 compares between smokers and former smokers; *P*2 compares between smokers and non-smokers; *P*3 compares between former smokers and nonsmokers.
P was significant if less than 0.05.

Figure 2



Mean periostin among different studied groups.

Figure 3



Mean eotaxin-2 among different studied groups.

symptoms than former and nonsmokers.' They found that wheeze was more frequent in current smokers than former smokers and nonsmokers.

In this study, the level of asthma control using ACQ was significantly different between the different groups. The majority of former smokers and smokers were uncontrolled. In contrast, the majority of nonsmokers were well controlled. The same results were determined by Thomson *et al.* [4], who described that 'The ACQ score was higher in asthmatic smokers compared with never smokers with asthma ($P<0.001$).' Moreover, these results had similarities with the results determined by Pilcher *et al.* [16], who stated that 'the asthmatic smokers had worse asthma control (i.e. higher ACQ scores) compared with former and nonsmokers.'

It was noteworthy that postbronchodilator FEV1/FVC and postbronchodilator FEV1 were significantly lower among asthmatic smokers and former smokers than nonsmokers. Jaakkola *et al.* [17] provided 'the evidence that smoking affected lung function and worsened its level among newly-onset asthmatic adult. Their study also showed that 'smoking had worse prebronchodilator and postbronchodilator lung function parameters of both large and small airways.' Moreover, Thomson *et al.* [4] found that 'postbronchodilator FEV1/FVC and FEV1%, and DLco were reduced in asthma smokers compared with nonsmokers.' The same result was reported by Çolak *et al.* [15], who stated that 'asthmatic smokers had more prevalent respiratory symptoms and worse airflow limitation compared with never smokers.'

Smoking alters cellular characteristics in blood and sputum and influences the nature of airway inflammation in patients with severe bronchial asthma. In smokers, the noneosinophilic sputum phenotype dominates, whereas former smokers and nonsmokers with severe asthma have an eosinophilic phenotype and, also, predominantly have higher sputum neutrophilic count [2]. In the current study, it was found that sputum neutrophils were significantly higher in asthmatic smokers and former smokers compared with never smokers. In this study, it was found that sputum neutrophils were superior in asthmatic smokers and former smokers compared with never smokers. However, the sputum eosinophils were significantly higher in nonsmokers than those former smokers and

Table 4 Correlations of periostin with clinical and laboratory parameters

	All patients	Nonsmokers	Former smokers	Smokers
	r (*P*)	*r* (*P*)	*r* (*P*)	*r* (*P*)
Age	0.23 (0.02)	0.11 (0.49)	0.23 (0.13)	0.24 (0.17)
BMI	0.13 (0.23)	0.30 (0.09)	0.21 (0.40)	-0.14 (0.48)
Duration of asthma	0.10 (0.32)	-0.04 (0.98)	0.23 (0.29)	0.05 (0.76)
Smoking index	-0.23 (0.03)	–	0.04 (0.85)	-0.49 (<0.001)
Dose of ICS	0.20 (0.21)	0.14 (0.58)	0.32 (0.27)	-0.12 (0.77)
Duration of ICS	0.02 (0.81)	-0.24 (0.35)	0.02 (0.99)	0.06 (0.98)
FEV1/FVC	0.19 (0.81)	0.14 (0.42)	-0.09 (0.71)	-0.04 (0.83)
FEV1	-0.03 (0.77)	0.07 (0.67)	-0.17 (0.49)	-0.06 (0.73)
FVC	-0.13 (0.24)	-0.05 (0.74)	-0.22 (0.36)	-0.19 (0.33)
FEF25–75	0.07 (0.62)	0.13 (0.56)	-0.42 (0.21)	-0.10 (0.75)
TLC (%)	0.29 (0.14)	0.24 (0.37)	0.39 (0.51)	0.52 (0.23)
RV/TLC (%)	0.11 (0.57)	0.26 (0.34)	0.30 (0.61)	-0.50 (0.30)
DICo (%)	0.12 (0.65)	0.09 (0.79)	0.11 (0.98)	0.97 (0.13)
Eosinophils (%)	0.21 (0.04)	-0.08 (0.64)	0.05 (0.98)	0.23 (0.04)
Neutrophils (%)	-0.16 (0.12)	-0.22 (0.18)	-0.17 (0.42)	-0.08 (0.65)
Histiocytes (%)	-0.04 (0.66)	-0.20 (0.23)	0.24 (0.26)	-0.19 (0.28)
Lymphocytes (%)	0.18 (0.07)	0.21 (0.20)	0.02 (0.92)	0.37 (0.03)

Data were expressed as *r* (degree of agreement), *P* (significance of agreement).
FEF25–75, forced expiratory flow at 25–75%; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroid; RV, residual volume; TLC, total lung capacity.
P value was if less than 0.05).

Table 5 Correlations of eotaxin-2 with clinical and laboratory parameters

	All patients	Nonsmokers	Former smokers	Smokers
	r (*P*)	*r* (*P*)	*r* (*P*)	*r* (*P*)
Age	0.29 (<0.001)	0.09 (0.59)	0.31 (0.15)	0.32 (0.07)
BMI	0.13 (0.23)	0.16 (0.38)	0.25 (0.30)	-0.24 (0.22)
Duration of asthma	0.10 (0.32)	-0.13 (0.44)	0.13 (0.55)	0.55 (< 0.001)
Smoking index	0.23 (0.03)	–	0.10 (0.64)	0.28 (0.03)
Dose of ICS	0.20 (0.21)	0.24 (0.34)	0.14 (0.64)	-0.18 (0.11)
Duration of ICS	0.11 (0.09)	-0.19 (0.45)	0.26 (0.37)	-0.19 (0.63)
FEV1/FVC	0.02 (0.81)	0.03 (0.86)	-0.16 (0.52)	0.26 (0.18)
FEV1	-0.27 (0.04)	-0.05 (0.78)	-0.28 (0.24)	-0.42 (0.02)
FVC	-0.02 (0.81)	-0.03 (0.85)	-0.35 (0.14)	0.37 (0.05)
FEF25–75	0.07 (0.63)	0.25 (0.25)	-0.34 (0.14)	0.15 (0.16)
TLC (%)	0.05 (0.78)	0.03 (0.90)	0.04 (0.93)	0.42 (0.34)
RV/TLC (%)	0.02 (0.89)	0.29 (0.15)	0.53 (0.35)	-0.48 (0.33)
DICo (%)	0.27 (0.30)	0.08 (0.22)	0.18 (0.12)	0.11 (0.08)
Eosinophils (%)	0.25 (0.04)	-0.06 (0.97)	0.19 (0.39)	0.31 (0.04)
Neutrophils (%)	0.21 (0.03)	-0.12 (0.49)	-0.04 (0.83)	0.40 (0.03)
Histiocytes (%)	-0.03 (0.73)	-0.13 (0.44)	0.01 (0.93)	-0.12 (0.51)
Lymphocytes (%)	-0.05 (0.58)	-0.11 (0.52)	0.09 (0.97)	-0.18 (0.32)

Data were expressed as *r* (degree of agreement).
FEF25–75, forced expiratory flow at 25–75%; FEV1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroid; RV, residual volume; TLC, total lung capacity.
P (significance of agreement). *P* value was if less than 0.05.

smokers. The same results were determined by Siew *et al.* [18] who described that 'the asthmatic smokers had significantly elevated levels of the submucosal neutrophils in the airways compared with the asthmatic never smokers.' Similarly, Thomson *et al.* [19] found that 'proportions of sputum neutrophils were higher in smokers and former smokers than nonsmokers, whereas proportions of sputum eosinophils were lower in smokers versus ex-smokers and nonsmokers.'

Clinical features and some biomarkers and cytokines can categorize bronchial asthma into phenotypes. The asthma biomarkers usually used are exhaled breath nitric oxide (eNO), eosinophils in sputum and blood, and serum periostin [20]. A specific endotype identified using biomarkers in blood, sputum, BAL, and bronchial biopsies. It may suggest better endotype-specific therapies response. For instance, raised serum periostin levels, induced by interleukin-4 and interleukin-13 in

the epithelium of airways, categorize asthma endotype patients with increased lebrikizumab responsiveness [21].

The essential outcome of this research was that serum periostin was significantly higher in nonsmokers than former smokers and smokers. The same results were determined by Kimura *et al.* [22] who described that 'smoking status affected serum periostin levels significantly (P=0.027). Serum periostin had lower levels in smokers asthmatics than those nonsmokers asthmatics.' The same result was observed by James *et al.* [23] and Thomson *et al.* [4] who found that 'current smoking was associated with lower periostin levels.' In contrast with this study, Takahashi *et al.* [24] observed that 'periostin levels were not significantly correlated with smoking history.' It might be explained that the studied group in their study had a lower smoking index (0.78 ± 2.09 pack/year).

Moreover, in the current study, serum periostin in all asthmatic patients had a significant positive correlation with eosinophils count. This was similar to the results of Asano *et al.* [25], Simpson *et al.* [26], and Bobolea *et al.* [27], who found that 'patients with eosinophilic asthma had significantly higher both serum and sputum periostin levels compared with noneosinophilic asthma.' Moreover, Hoshino *et al.* [13] described that 'serum periostin in asthmatic patients had a significant positive correlation with the percentage of sputum eosinophils in patients with steroid-naïve asthma.'

This study detected that serum periostin had a significant positive correlation with the age of all studied patients regardless smoking status. These results are in harmony with Takahashi *et al.* [24], who described that 'serum periostin levels were positively correlated with age in well-controlled asthma (r=0.2347, P<0.001).' The same results were obtained by James *et al.* [23], who observed that 'serum periostin levels were positively correlated with age (r=2.7 (1.0–4.4) P=0.002).'

Another crucial biomarker in this study was serum eotaxin-2, which was significantly higher among smokers than nonsmokers and former smokers. The same results were determined by Krisiukeniene *et al.* [5], who stated that 'asthmatic smokers had increased eotaxin-2 levels in serum, sputum, and BAL compared with asthmatic nonsmokers. These data suggested that smoking stimulated the production of eotaxins so may result in changes in asthmatic airway inflammation.'

Serum eotaxin-2 in all asthmatic patients had a significant positive correlation with eosinophilic count and neutrophilic count in sputum. The same results were observed by Krisiukeniene *et al.* [5], who described that 'both sputum neutrophils and eosinophils of asthmatic smokers were significantly correlated with the sputum eotaxin-2 concentration.' Moreover, Coleman *et al.* [28] found that 'in patients with asthma, airway epithelial cells (AEC) eotaxin-2 mRNA and protein positively correlated with sputum eosinophils.'

In the current study, serum eotaxin-2 had a negative correlation with FEV1 in all asthmatic patients. Moreover, Krisiukeniene *et al.* [5] found that 'eotaxin-2 had an inverse correlation with FEV1/FVC ratio in asthmatic never-smokers.'

Strengthens of this study: smoking outcomes in asthmatic patients and its effect on airway inflammation and asthma control have been studied by a relatively limited number of studies.

Limitations of this study: only patients who performed acceptable and reproducible pulmonary function tests were enrolled. Adequate sputum is difficult to collect from all patients; hence, patients who expectorated sufficient specimens were only enrolled.

Recommendations: further prospective research is needed to study the role of categorizing asthma clinically and using biomarkers such as serum eotaxin-2 and periostin into endotypes to predict endotype-specific therapy responses.

Conclusion

Asthmatic smokers had the worse outcomes. They showed neutrophilic inflammation with low eosinophils and increased serum eotaxin-2 and low serum periostin; this asthma phenotype requires specific therapeutic intervention aimed at preventing and reducing the remodeling, and the most promising therapies may need further studies.

Acknowledgements

Authors' contributions: all authors provided the final approval of the version to be published and agreed to be accountable for all aspects of the work to ensure that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All the authors participated in conception and design. S.F.Y. and S.A.E. collected the data and samples. A.F.E., M.F.A., S.F.Y., S.A.E., M.I.S., and M.F.S. were responsible for analysis and interpretation of data. M.F.A. and S.F.Y. were responsible for drafting the article. A.F.E., M.F.A., and S.F.Y. revised it critically for final approval of the version to be published. All authors have read and approved the manuscript.

Financial support and sponsorship

Funding was obtained for this study from the Faculty of Medicine's research grant office of Assiut University. Award number: 28/03/2017|Recipient: Sahar Farghly Youssif, PhD.

Conflicts of interest

There are no conflicts of interest.

References

1 Chatkin JM, Dullius CR. The management of asthmatic smokers. Asthma Res Pract 2016; 2:10.

2 Zedan M, Osman A. Clinical asthma phenotypes; a challenging but promising spectrum. Immunol Disord Immunother 2016; 01:1020.

3 Braido F, Tiotiu A, Kowal K, Mihaicuta S, Novakova P, Oguzulgen IK. Phenotypes/endotypes-driven treatment in asthma. Curr Opin Allergy Clin Immunol 2018; 18:184–189.

4 Thomson CN, Chaudhuri R, Spears M, Haughney J, McSharry C. Serum periostin in smokers and never smokers with asthma. Respir Med 2015; 109:708–715.

5 Krisiukeniene A, Babusyte A, Stravinskaite K, Lotvall J, Sakalauskas R, Sitkauskiene B. Smoking affects eotaxin levels in asthma patients. J Asthma 2009; 46:470–476.

6 Youssif SF, El-Karn AF, Sherif MF, Seddik MI, Abdelgayed S, Abdelghany MF. Inhaled corticosteroid response in smoker versus non-smoker asthmatic patients: a cross-sectional study. Egypt J Bronchol 2021; 15:1–9.

7 Mcsharry C, Spears M, Chaudhuri R, Cameron EJ, Husi H, Thomson NC. Increased sputum endotoxin levels are associated with an impaired lung function response to oral steroids in asthmatic patients. J Allergy Clin Immunol 2014; 134:1068–1075.

8 Rajan S, Gogtay NJ, Konwar M, Thatte UM. The global initiative for asthma guidelines (2019): change in the recommendation for the management of mild asthma based on the SYGMA-2 trial–A critical appraisal Lung India: Official Organ of Indian Chest Society 2020;37:169.

9 Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Research Meth 2007;39175–91.

10 Tarraf H, Aydin O, Mungan D, Albader M, Mahboub B, Doble A, *et al.* Prevalence of asthma among the adult general population of five Middle Eastern countries: results of the SNAPSHOT program. BMC Pulm Med 2018; 18:1-4.

11 Thomson NC, Chaudhuri R, Messow CM, Spears M, MacNee W, Connell M, *et al*. Chronic cough and sputum production are associated with worse clinical outcomes in stable asthma. Respir Med 2013; 107:1501–1508.

12 Issues C. Guidelines of the Papanicolaou Society of Cytopathology for the Examination of Cytologic Specimens Obtained from the Respiratory Tract. Diagn Cytopathol 1998; 21:61–69.

13 Hoshino M, Ohtawa J, Akitsu K. Effect of treatment with inhaled corticosteroid on serum periostin levels in asthma. Respirology 2016; 21:297–303.

14 Ulambayar B, Lee S-H, Yang E-M, Ye Y-M, Park H-S. Association between epithelial cytokines and clinical phenotypes of elderly asthma. Allergy Asthma Immunol Res 2019; 11:79.

15 Çolak Y, Afzal S, Nordestgaard BG, Lange P. Characteristics and prognosis of never-smokers and smokers with asthma in the copenhagen general population study: a prospective cohort study. Am J Respir Crit Care Med 2015; 192:172–181.

16 Pilcher J, Patel M, Reddel HK, Pritchard A, Black P, Shaw D, *et al*. Effect of smoking status on the efficacy of the SMART regimen in high risk asthma. Respirology 2016; 21:858–866.

17 Jaakkola JJK, Hernberg S, Lajunen TK, Sripaijboonkij P, Malmberg LP, Jaakkola MS. Smoking and lung function among adults with newly onset asthma. BMJ Open Respir Res 2019; 6:1–10.

18 Siew LQC, Wu S-Y, Ying S, Corrigan CJ. Cigarette smoking increases bronchial mucosal IL-17A expression in asthmatics, which acts in concert with environmental aeroallergens to engender neutrophilic inflammation. Clin Exp Allergy 2017; 47:740–750.

19 Thomson NC, Chaudhuri R, Heaney LG, Bucknall C, Niven RM, Brightling CE, *et al*. Clinical outcomes and inflammatory biomarkers in current smokers and exsmokers with severe asthma. J Allergy Clin Immunol 2013; 131:1008–1016.

20 Schofield JPR, Burg D, Nicholas B, Strazzeri F, Brandsma J, Staykova D, *et al*. Stratification of asthma phenotypes by airway proteomic signatures. J Allergy Clin Immunol 2019; 144:70–82.

21 Harris JM, Maciuca R, Bradley MS, Cabanski CR, Scheerens H, Lim J, *et al*. A randomized trial of the efficacy and safety of quilizumab in adults with inadequately controlled allergic asthma. Respir Res 2016; 17:1–11.

22 Kimura H, Konno S, Makita H, Taniguchi N, Kimura H, Goudarzi H, *et al*. Serum periostin is associated with body mass index and allergic rhinitis in healthy and asthmatic subjects. Allergol Int 2018; 67:357–363.

23 James A, Janson C, Malinovschi A, Holweg C, Alving K, Ono J, *et al*. Serum periostin relates to type-2 inflammation and lung function in asthma: Data from the large population-based cohort Swedish GA(2)LEN. Allergy 2017; 72:1753–1760.

24 Takahashi K, Meguro K, Kawashima H, Kashiwakuma D, Kagami SI, Ohta S, *et al*. Serum periostin levels serve as a biomarker for both eosinophilic airway inflammation and fixed airflow limitation in well-controlled asthmatics. J Asthma 2019; 56:236–243.

25 Asano T, Kanemitsu Y, Takemura M, Yokota M, Fukumitsu K, Takeda N, *et al*. Serum periostin as a biomarker for comorbid chronic rhinosinusitis in patients with asthma. Ann Am Thorac Soc 2017; 14:667–675.

26 Simpson JL, Yang IA, Upham JW, Reynolds PN, Hodge S, James AL, *et al*. Periostin levels and eosinophilic inflammation in poorly-controlled asthma. BMC Pulm Med 2016; 16:67.

27 Bobolea I, Barranco P, Del Pozo V, Romero D, Sanz V, López-Carrasco V, *et al*. Sputum periostin in patients with different severe asthma phenotypes. Allergy 2015; 70:540–546.

28 Coleman JM, Naik C, Holguin F, Ray A, Ray P, Trudeau JB, *et al*. Epithelial eotaxin-2 and eotaxin-3 expression: relation to asthma severity, luminal eosinophilia and age at onset. Thorax 2012; 67:1061–1066.