Induced Sputum Cell Profile in Mild to Severe Stable Asthmatics and Healthy Adults

Zuhal Karakurt, MD[1]; Berrin Ceyhan, MD[2]; Sait Karakurt, MD[2]; Hatice Türker, MD[1]

[1]*Department of Chest Diseases, SSK Sureyyapasa Center for Chest Diseases and Cardiovascular- Thoracic Surgery, İstanbul, Turkey*
[2]*Department of Chest Diseases, Marmara University Hospital, İstanbul, Turkey*

Abstract

Induced sputum cell counts provide a relatively noninvasive method to evaluate the presence, type and degree of inflammation in asthma. The objective was to examine the total and differential cell counts in induced sputum samples and blood samples of mild and severe stable asthmatics and to compare with those of controls and correlate them with the severity of the disease. Severe stable asthmatics (n=14, 4 males, 10 females, mean age 44.2±12.2 years), mild stable asthmatics (n= 15, 5 males, 10 females, mean age 36.8±11.4 years) and healthy adults control group (n= 13, 4 males, 9 females, mean age 36.6±11.9 years); a total 42 nonsmoking subjects were enrolled into the study. To obtain a sample of induced sputum, all subjects inhaled hypertonic saline solution (3-5%). Asthmatic patients were pretreated with 200 μg inhaled salbutamol. Adequate induced sputum samples from all subjects were processed within 2 hours.

Results: The mean total cell counts were as $x10^6$ cells/ml and $x10^6$ cells/g in controls, mild and severe asthmatics groups, $2.8\pm3.1x10^6$ cells/ml and $3.0\pm3.4x10^6$ cells/g, $1.9\pm1.9x10^6$ cells/ml and $1.58\pm1.36x10^6$ cells/g, 3.2 ± 2.6 $x10^6$ cells/ml and 3.0 ± 2.4 $x10^6$ cells/g respectively. They did not result in any statistically significant change. The percentages and absolute counts of eosinophils in induced sputum in mild and severe asthmatics were higher than healthy control groups (8±5.3% and $0.14\pm0.15x10^6$ cells/g, 9.0±4.5% and $0.29\pm0.32x10^6$ cells/g, 0.2±0.4% and $0.005\pm0.013x10^6$ cells/g, respectively). They were higher than those of controls ($p=0.00001$) but there was no difference between two asthmatic groups. The percentages of blood eosinophils in mild, severe asthmatics and control groups were 5.6±3.4%, 8.2±3.6%, 2.3±1.0% respectively. They were similarly higher in mild and severe asthmatics than those of control groups ($p<0.0002$).

Conclusions: This study has identified values for total and differential cell counts in induced sputum of stable asthmatics and healthy adults. Total cell counts ($x10^6$ cells per ml and per gram) showed no differences among groups and only the portions of eosinophils were significantly higher in mild and severe stable asthmatics than controls ($p<0.00001$). And, we also showed that patients with asthma in comparison with control subjects had a higher numbers of blood eosinophils ($p<0.0002$). We conclude that induced sputum cell analysis is a helpful technique to analyse asthmatic airway inflammation, however, we could not correlate cell profile with severity of airway inflammation.

Turkish Respiratory Journal, 2001;2 (3):22-27

Key words: *induced sputum, asthma*

Introduction

Airway inflammation is considered to be the primary cause of asthma and other airway diseases, and is associated with exacerbations (1) and airway remodeling (2). The measurement of airway inflammation has been facilitated by examination of induced sputum for cell and fluid-phase constituents. The method is relatively noninvasive and safe. The cell count results are repeatable, valid as illustrated by differences between

Correspondence: *Dr. Zuhal Karakurt*
Bağlarbaşı Soyak Evleri B-3 Daire 18
81270 Üsküdar, İstanbul, Türkiye

diseases of different pathogenesis, and responsive to change induced by pro- and anti-inflammatory stimuli (3-5). The composition of induced sputum in asthmatics has been compared to other direct markers of airway inflammation including bronchial wash (BW), bronchoalveolar lavage (BAL) or bronchial biopsies. From the data currently available, it appears that sputum contains more neutrophils and eosinophils, but less macrophages and lymphocytes than BW or BAL (6-9). However, relative eosinophil numbers correlate well between sputum and BW or BAL, but the correlation between eosinophil numbers and bronchial biopsies is somewhat less clear (6-9). This technique is used in a large number of applications. From a research perspective, analyses of components of supernatant and cellular fraction allow further characterization of the whole inflammatory network underlying asthma. From a more clinical point of view, sputum could help in discriminating between asthma and other diseases such as chronic obstructive lung disease (COPD) (10,11). Another area of particular interest relates long-term follow-up of asthma treatment. Preliminary data have been presented on a number of clinical studies that have compared different treatment regimens in asthma, using repeated analysis of induced sputum in addition to clinical and lung function criteria as outcome measures (10-13). From these studies, it should also emerge whether or not the analysis of sputum can provide additional information to that from the currently used outcome measures, thus allowing more individual adaptations of asthma treatment (12-18).

In our country, so far the results of induced sputum examinations have only been published for small numbers of asthmatic patients in whom the characteristics were often not well defined. We, therefore, aimed to examine sputum from well-defined asthmatic patients and healthy nonsmoking adults. Our objectives were: (I) to determine total and differential cell counts of the inflammatory cells; (II) to examine the cellular differentiability in patients with mild to severe stable asthma; (III) to compare cellular number and differential cells in sputum with those measured in peripheral blood; and (IV) to compare all those results with healthy nonsmoking adults.

Methods

Subjects

Three groups (2 asthma groups, 1 healthy control group) participated in the study. A total of 42 adult subjects (14 adults with severe stable asthma (SSA), 15 adults with mild stable asthma (MSA), and 13 healthy nonsmoking adults (C) were recruited from outpatient clinics in Marmara University Hospital and SSK Süreyyapaşa Center for Chest Diseases and Cardio-Thoracic Surgery. Fourteen SSA (5 males, 9 females mean age 44±12 years (range 20 to 65 years) and 15 MSA (5 males, 10 females, mean age 37±11 years (range 22 to 62 years) and 13 C (4 males, 9 females, mean age 37±12 years (range 24 to 63 years) were enrolled into the study. Classification of asthma severity was based on history, symptoms, clinical features, and medication requirement according to the international guidelines (19). All asthmatic patients had reversible airway obstruction characterized by an increase of 15% in their FEV_1 value and absolute FEV_1 value of at least 200 ml after inhalation of at least 200 μg of salbutamol. Asthmatic subjects (15 MSA) by symptoms of episodic wheezing, chest tightness, dyspnea with FEV_1 >70% predicted were hyperresponsive to inhaled metacholine (MCh) as shown by a provocative consentration causing 20% fall in FEV_1 (PC_{20} MCh) of less than 8 mg/ml (20). The clinical severity of chronic asthma was based on the step system of the Global Initiative for Asthma (GINA), which is used to grade chronic asthma from mild intermittent (step 1) to severe persistent (step 4) (19). The patients were considered as having stable asthma if the disease had been fully controlled for at least one month (GINA definition). The duration of asthma was established on the basis of the patient's history, followed by a careful clinical examination. The diagnosis of allergies was based on clinical history and on skin prick tests to common inhalant allergens. All asthmatic patients were atopic as indicated by one or more positive skin tests. None of the asthmatic subjects was a current or previous smoker, and none had a history of severe exacerbation of asthma requiring hospitalization during the month preceding the study.

Healthy adults (C) had no nasal or chest symptoms and no past history of asthma or other chronic respiratory disease. No subject was a previous or current smoker. All had normal spirometry (FEV_1 ≥80% predicted, ratio of FEV_1 to vital capacity (FEV_1/FVC) ≥80%), and normal MCh airway responsiveness (PC_{20} ≥16 mg/ml) and none was atopic as indicated with a negative history and negative skin prick tests.
The study was approved by the Research Committee of our hospital and all subjects gave written informed consent.

Design

After subjects were seen in our hospital's outpatient clinics, characteristics of patients were documented by a questionnaire, spirometry test was performed, and the patients kept a diary to record morning and evening PEF measurements during seven days before study. Eligible patients were chosen and at the eight-day MCh test and allergy skin tests were performed and on the other day, sputum induction was performed.

Lung function, skin test and sputum induction procedures

Spirometry was performed according to GINA (19) by

using Sensormedics (S3513-Ca, USA). Baseline FEV_1 was expressed as the best of three reproducible values. Metacholine (MCh) inhalation tests were carried out by the method described by Sterk and colleagues (20), and the results expressed as PC_{20} in non-cumulative units. The MCh challenge test was performed in MSA (n=13) and C groups (n=13). Skin prick tests were performed (if not done during the preceding year) using a modified prick technique (21). A positive skin test was indicated by a wheal ≥3 mm. We defined atopy as one or more positive skin test against inhalants.

Sputum was induced by the method described by Pizzichini and coworkers (4), by inhaling increasing concentrations of hypertonic saline (3%, 5%) generated by a Fisoneb Ultrasonic nebulizer (Canadian Medical Products, Ltd., Markham, Ontario, Canada) with an output of 0.87 ml/min and particle size of 5.58 μm aerodynamic mass median diameter. The aerosol was inhaled for 1 or 2 minutes according to the severity of airflow obstruction, at the baseline and after each period of inhalation (for 5 minutes and repeated the cycle no more than four times), PEF was measured in each cycle for safety. Subjects were asked to inhale hypertonic saline with a normal tidal volume (until the reduction of 20% PEF values) and to blow through their nose, rinse their mouth, and swallow the water to minimize contamination from postnasal drip and saliva. Then, they were instructed to cough sputum into a sterile container.

Sputum examination

The sputum was examined within 2 hours as described previously (4). Sputum was selected from saliva and weighted and was treated by adding four volumes of 0.1% dithiothreitol (DTT) and four volumes of phosphate-buffered saline (PBS). The suspension was filtered and the filtrate was centrifuged at 790 x g for 10 minutes, and the supernatant was aspirated. The cell pellet was resuspended in D-PBS, 200 to 600 μl, depending on macroscopic size, and a total cell count of leukocytes were determined as 10^6 cells per ml and 10^6 xcells per gram. The cell suspension was adjusted to 1.0 x 10^6 cell/ml, placed into cups of cytocentrifuge and two coded cytospins were prepared, air dried, and stained by Wright's stain. At least 200 intact nonsquamos cells were counted on the best stained cytospin.

Blood measurements

Serum was collected and stored at -40°C to measure blood Ig E levels by using chemiluminescence (PDC, Ca, USA), later the total counts and differential count of white cells were performed in peripheral blood.

Statistical analysis

The differences in age, spirometry results, cells in the blood and sputum among three groups were investigated by using ANOVA test. If there was a significant difference, Tukey test was used as *post hoc test*. T student test was used to investigate differences in asthma duration and bronchodilator response in patients with asthmatics. Cell counts and differentials were expressed as mean (±SD, standart deviation). A p value of 0.05 or less was considered significant.

Results

Characteristics of subjects

Demographic and clinical characteristics of the groups are shown in Table 1. The age, sex and the number of subjects were similar in three groups (p >0.05). There was no significant difference in the duration of disease in two asthmatic groups. The mean FEV_1 (% pred) and FEV_1/FVC (%) values in mild, severe asthmatics and in control groups were 90.0±12.5%, 91.0±9.5%; 46.6±12.4%, 54.9±6.9% and 102.7±9.0%, 104.4±7.2% respectively. The mean total IgE levels were 191.40±175.08 IU/ml and 355.47±464.36 IU/mL in mild and severe asthmatics respectively (normal < 94 IU/ml). The MCh challenge test was performed in MSA (n=13) and C groups (13). The mean PC_{20} MCh levels were in MSA 4.35±5.19 mg/ml, and in control group PC_{20} MCh >16 mg/ml.

Total and differential cell counts in induced sputum

Sputum induction was successfully performed in 42 subjects without any complication including bronchoconstruction. The selected sputum was mucoid and was minimally contaminated with saliva. It was sampled from lower respiratory tract as confirmed by the presence of high percentage of

Table 1. Group characteristics

	1-Healthy Control (C)	2-Mild Stable Asthma (MSA)	3-Severe Stable Asthma (SSA)	p values
Number*	13	15	14	NS
Gender*	9 F-4 M	10 F-5 M	9 F- 5 M	NS
Age*	36.6±11.9	36.8±11.4	44.4±12.2	NS
Asthma (yr)*	———	10.2±8.2	15.5±10.0	NS
FEV_1 (%pred.)	102.7±9.0[a,d]	90.0±12.5[c]	46.6±12.4	0.00001
FEV_1/FVC (%)	104.4±7.2[a,e]	91.0±9.5[c]	54.9±6.9	0.00001
FEF_{25-75} (%pred.)	102.3±12.6[a,b]	61.7±23.8[c]	19.9±8.6	0.00001
BD response (%)	———	13.75±12.59	22.88±9.99	0.002
MCh (PC20) mg/ml	>16	4.35±5.19	———	
IgE (IU/L)	38.46±22.68	191.40±175.08	355.47±464.36[f]	

NS*: Not significant.
a: p<0.0001 when compared C with SSA.
b: p<0.0001 when compared C with MSA.
c: p<0.0001 when compared MSA with SSA.
d: p<0.01 when compared C with MSA.
e: p<0.0002 when compared C with MSA.
f: p<0.001 when compared C with SSA.

Table 2. Sputum total and absolute cell counts (x10⁶cells/g).

	C	MSA	SSA	p values
Total cell	3.1±3.4	1.58±1.36	3.0±2.4	NS
Macrophage	2.2±2.6	1.0±0.9	1.9±1.4	NS
Neutrophil	0.8±0.9	0.4±0.4	0.78±0.77	NS
Eosinophil	0.005±0.013[a]	0.14±0.15	0.299±0.322	0.000001
Lymphocyte	0.05±0.06	0.03±0.04	0.03±0.04	NS

NS: Not significant.
a: p<0.00001 when compared C with MSA and SSA.

macrophages. The mean total cell counts (per ml and per gram) of MSA and SSA were not significantly different from the mean cell count of C (1.97±1.91x10⁶ cells/ml, 3.29±2.63x10⁶ cells/ml, 2.80±3.12x10⁶ cells/ml, respectively (p >0.05); 1.58±1.36x10⁶ cells/g, 3.01±2.42x10⁶ cells/g, 3.08±3.49x10⁶ cells/g, respectively (p >0.05) (Table 2.). The sputum cell profiles are shown in Table 3, Figure 1. Induced sputum cell counts in all subjects are characterized by a predominance of macrophages. In asthma groups, absolute number and percentage of eosinophils were higher than those of healthy subjects (absolute counts: 0.299x10⁶ cells/g(SSA), 0.146x10⁶ cells/g (MSA), 0.005x10⁶ cells/g (C) (p<0.00001), percentages: 9.017% (SSA), 8.533% (MSA), 0.230% (C) (p <0.00001).

Blood cell counts

Patients with mild and severe asthma only had higher percentages of blood eosinophils 5.6±3.4%, 8.2±3.6% in comparison with healthy control subjects 2.3±1.0% respectively (p<0.0002).

Discussion

The results of this study showed that the majority of the cells were macrophages whereas eosinophils were significantly higher in asthmatic subjects than those in healthy control group (p <0.00001). We also demonstrated that the patients with asthma in comparison with control subjects had higher portions of blood eosinophils (p<0.0002). However, the portions of eosinophils in induced sputum and blood showed no difference between mild and severe asthmatic patients.

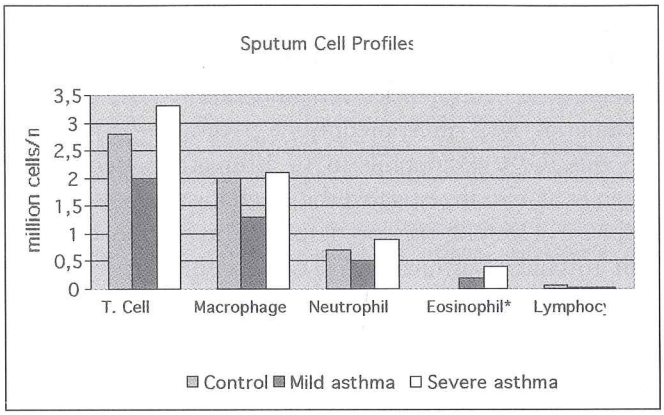

Figure 1. *Sputum cell profiles among three groups.*
**p<0.000001 the eosinophil counts were significantly more in asthma groups than healthy control subjects*

Many studies have included asthmatic and healthy nonsmoking subjects within their study samples (3,4,22,23), defining the small sample sizes ranging between 6 and 20; but only Belda et al., recently reported a larger sample size of healthy nonsmoking adults' induced sputum cell counts (n=118). However, they didn't study any asthmatic patient (24). That study can be a reference value for only total and differential cell counts in induced sputum of healthy adults. They found total cell counts (x10⁶ cells/g) and sputum differential cell percentages as follows 4.12±4.81x10⁶ cells/g, macrophages 58.8±21.0%, neutrophils 37.5±20.1%, eosinophils 0.4±0.9%, lymphocytes 1.0±1.1%. In present study, total cell counts were 3.1±3.4x10⁶ cells/g and 2.8±3.2x10⁶ cells/ml and sputum differential cell percentages were 71.8±9.35% macrophages, 26.3±9.1% neutrophils, 0.2±0.4% eosinophils, 1.0±1.1% lymphocytes. And the induced sputum cell profiles of the other two studies as percentages in healthy adults (both cases n=10) were macrophages 83%, 62.9%, neutrophils 10.3%, 24.1%, eosinophils 0%, 0.5%, lymphocytes 0%, 1.3%, total cell counts as x10⁶ cells/ml 1.5 and 3.1 respectively (25,26). The results of these studies are similar to our results. Other studies searched the sputum of asthmatics, generally emphasized only the percentages of neutrophils and eosinophils and they investigated mechanisms of exacerbations and treatments (27-28). Pizzichinni and et al. reported that examination of spontaneous or induced sputum selected from saliva can be used successfully to examine and follow the effects of treatment in severe exacerbation of

Table 3. Sputum total and absolute cell counts and percentages

	C	MSA	SSA	p values
Total cell (x10⁶cells/ml)	2.80±3.12	1.97±1.91	3.29±2.63	NS
Macrophage (x10⁶cells/ml) (%)	2.0±2.3	1.3±1.2	2.1±1.5	NS
	(71.8±9.35)	(67.9±8.9)	(66.5±8.9)	NS
Neutrophil (x10⁶cells/ml) (%)	0.7±0.7	0.456±0.454	0.841±0.787	NS
	(26.3±9.1)	(20.8±5.8)	(24.0±7.1)	NS
Eosinophil (x10⁶cells/ml) (%)	0.004±0.01[a]	0.178±0.192[b]	0.319±0.348	0.000001
	(0.2±0.4)[a]	(8.5±5.1)[b]	(9.0±4.5)	0.000001
Lymphocyte (x10⁶cells/ml) (%)	0.06±0.07	0.03±0.05	0.03±0.4	NS
	(1.5±0.9)	(2.2±1.5)	(0.7±0.9)	NS

NS: Not significant.
a: p<0.00001 when compared C with MSA and SSA.
b: NS when compared MSA with SSA.

asthma. They showed neutrophils and eosinophils as percentages before and after 21-day-treatment with prednisone; 45% neutrophils, 3% eosinophils in acute attack of asthmatics and after prednisone treatment 55% neutrophils, 2.7% eosinophils in 10 asthmatic patients (27). Veen et al. also investigated the mechanism of asthma and response of steroid withdrawal in patients with severe-difficult to control asthma (n=13) and stable-severe asthma (n=15) by examining induced sputum. In difficult to control asthma group, the neutrophils (29.3%) and eosinophils (6.7%) were treated with steroids and following the withdrawal of steroids, the percentages of sputum neutrophils were 28% and the percentages of eosinophils were 14.5%. In severe asthma group treated with steroids, the neutrophil and eosinophil percentages were as follows; 39.5% neutrophils, 3.1% eosinophils and after tapering of steroids, the percentages of sputum neutrophils and eosinophils were 46.8% and 12.5% respectively (28). Both studies had similar results, however, in the present the study, neutrophil percentage of stable-severe asthmatics (24%) was lesser than those, but eosinophil percentage (9%) was similar to these studies.

The method of processing sputum may have an influence on both the total and differential counts. We have examined sputum after selected from expectorate (sputum plus saliva). The other examination method of sputum is to process the whole expectorate. The whole cell count will be higher when it is expressed per gram and per mililitre of selected sputum, but the count will be reduced by the process of selection and filtering the suspension of dithiothreitol and PBS-treated sputum before counting. Although some studies found that, the differential cell count should not be altered by whether sputum or expectorate is examined or not (10). Magnussen et al. reported that the methods are not interchangeable and they emphasized that whatever method has been chosen, it must be used consistently (29). We used the same method during the study and the total cell counts were similar to other studies that used the same method. We also found similar total and differential cell counts between two stable asthmatic groups, this may be argued that the total and differential cell counts can imply the exacerbation of the disease rather than the severity of the stable asthma.

It is known that the usefulness of induced sputum as a marker of airway inflammation in asthma has been thoroughly evaluated by various groups, with regard to both reliability and validity (2,4,5). Both cell fraction and the fluid phase of induced sputum clearly differ between healthy subjects, asthmatics and smokers with chronic obstructive pulmonary disease (COPD) (3,11). Airway eosinophilic inflammation is a characteristic feature of asthma. Overall, sputum from asthmatic subjects contains an increased proportion of eosinophils (>3%), whereas sputum of COPD clearly characterized by an increased proportion of neutrophils (75-85%) (5,11,19). Similarly, we found significantly higher portions of eosinophils in the asthmatic patients than healthy subjects ($p<0.00001$).

Pizzichini et al. found that patients with stable asthma (n=19) in comparison with healthy nonsmokers (n=10) had a higher portion of sputum eosinophils (5.2%, 0.3% respectively $p<0.001$) and higher numbers of blood eosinophils ($350x10^6$/L, $155x10^6$/l $p<0.003$), but they concluded that " the portions of eosinophils in sputum is more accurate marker of asthmatic airway inflammation than the portion of blood eosinophils" (29). We also showed the similar results, but all our asthmatics patients (n=29) were atopic whereas the control group (n=13) was not. This could be a significant positive influence of atopy on the eosinophil counts. Other studies have also identified an influence of atopy on blood eosinophilia (30, 31).

In conclusion, we have determined the cell profile of induced sputum and blood sample for nonsmoking healthy adult populations and atopic, stable-mild and severe asthmatic patients to provide a normal range for similar populations in our country using similar methods of induced sputum examination. Sputum and blood eosinophil counts are significantly higher in atopic asthmatic patients than healthy control group, but there is no significant difference between stable-mild and stable-severe asthmatic patients.

Acknowledgement: The authors thank to Yeşim Elbir for her helps to process the sputum samples.

References

1. Gibson PG, Dolovich J, Girgis-Gabardo A, et al. The inflammatory response in asthma exacerbation: changes in circulating eosinophils, basophils and their progenitors. Clin. Exp. Allergy 1990;20:661-668.
2. Pin I, Gibson PG, Kolendowicz R, et al.Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992;47:25-29.
3. Fahy JV, Liu J, Wong H, et al. Cellular and biochemical analysis of induced sputum from asthmatic and from healthy subjects. Am. Rev. Respir. Dis. 1993;147:1126-1131
4. Pizzichini E, Pizzichini MMM, Efthimiadis A, et al. Indices of airway inflammation in induced sputum: reproducelibility and validty of cell and fluid phase measurements. Am. J. Respir. Crit. Care Med. 1996;154:308-317
5. Hargreave F. Induced sputum for the investigation of airway inflammation: evidence for clinical application. Can. Respir. J. 1999;6:169-174
6. Maestrelli P, Sactta M, Di Stefano A, et al. Comparison of leucocyte counts in sputum, bronchial biopsies and bronchoalveolar lavage. Am. J. Respir. Crit. Care Med. 1995;152:1926-1931.
7. Keatings VM, Evans DJ, O'Connor BJ, Barnes PJ. Cellular profiles in asthmatic airways: a comparison of induced sputum, bronchial washings, and bronchoalveolar lavage fluid. Thorax 1997;52:372-374
8. Grootendorst DC, Sont JK, Willems LNA, et al. Comparison of inflammatory cell counts in asthma. Induced sputum versus bronchoalveolar lavage and bronchial biopsies. Clin. Exp. Allergy 1997;27:769-779.

9. Fahy JV, Wong H, Liu J, et al. Comparison of samples collected by sputum induction and bronchoscopy from asthmatics and healthy subjects. Am. J. Respir. Crit. Care Med. 1995;152.53-58.
10. Kips JC, Pauwels RA. Noninvasive indicators of airway inflamation: induced sputum in allergic disease. Eur. Respir. Rev. 1998;8:64, 1095-1097.
11. Ronchi MC, Piragino C, Rosi E, et al. Role of sputum differential cell count in detecting airway inflammation in patients with chronic bronchial asthma asthma or COPD. Thorax 1996;51:1000-1004.
12. Turner MO, Hussack PA, Efthimiadis A, et al. Sputum yo investigate the anti-inflammatory effect of salmeterol versus beclomethasone. Am. J. Respir. Crit. Care Med. 1996;153: A293
13. Leff JA, Pizzichini E, Efthimiadis A, et al. Effect of Montelukast (MK-0476) on airway eosinophilic inflammation in mildly uncontrooled asthma: a randomized placebo-controlled trial. Am. J. Respir. Crit. Care Med.1997;155:A977.
14. Taylor DA, Evans DJ, Keller A, et al. Theophylline plus low dose inhaled steroid is as effective as high dose inhaled steroid in the control of asthma. Eur. Respir. J. 1997;10, Suppl.25,2s
15. Kips JC, O'Connor BJ, Svensson K, et al. Low dose budesonide plus formeterol versus high high dose budesonide in asthma therapy: effects on markers of airway inflammation. Eur. Respir. J.1997; 10,Suppl. 25,1s
16. Pizzichini MMM, Pizzichini E, Cleland L, et al. Sputum in severe exacerbations of asthma. Kinetics of inflammatory indices after prednisone treatment. Am. J. Respir. Crit. Care Med. 1997;155:1501-1058.
17. Fahy JV, Kim KW, Liu J, et al. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. J. Allergy Clin. Immunol 1995;95: 843-852.
18. Turner MD, Hussack P, Sears MR, et al. Exacerbations of asthma without sputum eosinophilia. Thorax 1995;50:1057-1061.
19. Guidelines for the diagnosis and management of asthma National Institutes of Health, National Heart, Lung and Blood Institude Bethestda MD Publications;1997 No 97-4015.
20. Sterk PJ, Quanjer PH, Fabbri LM, et al. Airway responsiveness: standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Eur. Respir. J. 1993;6(suppl 16):53-83.
21. Pepys J. Skin test in diagnosis. In: Gell PGH, Coombs RRA and Lachmann PJ, editors. Clinical aspects of immunology. 3rd Ed. London:Blackwell Scientific Publications;1975: 55-80.
22. Ceyhan BB, Enc FY, Demiralp E.Transforming growth factor beta-1 levels in induced sputum samples of asthmatic subjects. Turkish Respir. J. 2000;1:46-50
23. Enc FY. Astımda indüklenmiş balgam örneklerinde ve serum de interleukin-2, 10 ve soluble vaskuler hücre yapışma molekülünün rolü. Uzmanlık Tezi. Istanbul. 1997; 37-57.
24. Belda J., Leigh R, Parameswaran K, et al.Induced sputum cell counts in healthy adults. Am. J. Respir. Crit. Care Med. 2000;161:475-478.
25. Vignola AM, Riccobono L, Mirabella A, et al. Sputum metalloproteinase-9/tissue inhibitor of metalloproteinase-1 ratio correlates with airflow obstruction in asthma and chronic bronchitis. Am. J. Respir. Crit. Care Med. 1998 ;158:1945-1950.
26. Paword D, Pizzichini MMM, Pizzichini E, et al.The use of induced sputum to investigate airway inflammation. Thorax 1997;52:498-501.
27. Pizzichini MMM, Pizzichini E, Clelland L, et al. Sputum in severe exacerbations of asthma: kinetics of inflammatory indices after prednisone treatment. Am. J. Respir. Crit. Care Med. 1997;155:1501-1508.
28. in't Veen JCCM, Smits HH, Hiemstra PS, et al. Lung function and sputum characteristics of patients with severe asthma during an induced exacerbation by double-blind steroid withdrawal. Am. J. Respir. Crit. Care Med. 1999;160:93-99.
29. Magnussen H, Holz O. Monitoring airway inflammation in asthma by induced sputum. Eur. Respir. J. 1999;13:5-7.
30. Mensinga TT, Schouten JP, Rijcken B, et al. Host factors and environmental determinants associated with skin test reactivity and eosinophilia in a community-based population. Ann. Epidemiol. 1994;4:382-392.
31. Mensinga TT, Schouten JP, Weis ST, et al. Relationship of skin reactivity and eosinophilia to level of pulmonary function in a community-based population study. Ann. Rev. Respir. Dis. 1992;146:638-643.