Original Article

The Eosinophilic and Neutrophilic Counts in Sputum of Asthmatic Iraqi Patients and its Correlation with Asthma Control

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Abstract

Background: Asthma control is very critical guide for therapy according to the current asthma guidelines. The asthma control test (ACT) was successfully used to assess the control level. However, variation in clinical features which composite the items of the test does not always associate with the extent of the structural changes in pulmonary airways. Thus, there is a necessity for the surrogate marker to assess the control level. Eosinophilic and neutrophilic cells have an important role in the pathological process of asthma. **Aims:** The aim of this study is to assess the sputum eosinophil and neutrophils as a discriminative marker between different levels of asthma control. **Patients and Methods:** This cross-sectional study included 88 asthmatic patients. Only 50 patients produced sputum. Demographic data, ACT parameters took from patients. Spirometer test has been done. Sputum study includes processing of sputum, counting of sputum total white cell were done. **Results:** According to sputum cells count and differentiation, 64% eosinophils, 10% neutrophils, 6% mixed and 20% was paucigranulocytic (non). Eosinophilic phenotype patients were poorly control with a statistically significant difference. While neutrophilic phenotype was nonstatistically significant difference. **Conclusions:** The most common type of asthma phenotyping is eosinophil which in turn correlated with poor asthma control while other types like neutrophil are not correlated.

Keywords: Asthma control test, eosinophil, neutrophil, pulmonary function test

Introduction

Asthma is a common and potentially serious disease, which is characterized by chronic airway inflammation,[1] the World Health Organization Global Burden of Disease Study estimates that 13.8 million disability-adjusted life years are lost annually due to asthma, representing 1.8% of the total global burden of disease.[2] Asthma has different clinical phenotypes that vary in expression depending on the interaction between many environmental and genetic.[3] The prevalence of asthma has been rising in recent years; more than 300 million people worldwide have the disease.[4] Specific populations of asthmatic patients having higher morbidity and mortality rates. In US, the death rate from asthma is about 30%, more in female, 75% more in the African–Americans than in other nationalities white people.[5]

Inflammation plays an important role in the pathogenesis and progression of asthma.[6] Existence of inflammation is not limited to severe asthma, but even mild and moderate asthma are also associated with inflammation of the airway wall with abnormal accumulation of inflammatory cells and airway hyperresponsiveness.[7]

There are many inflammatory cells concerned in the pathogenesis of asthma; however, the exact role of each cell is not yet confirmed. Obviously, there is no single inflammatory cell is able to descript for a complex pathophysiology of asthma, but some of the cells are predominate in the inflammation of the disease.[8]

Eosinophilic inflammation has considered as one of the most characteristic pathological hallmarks of the asthma. The hypothesis of that is the eosinophil cell considered to be the key effector cell which involved in the pathogenesis

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of asthma has run into difficulty for many causes.[9] It is well established now that the relationship between the sputum eosinophilic inflammation and rate of asthma exacerbations exists.[10] This phenotype patients show a persistent eosinophilic airway inflammation in spite of the treatment with inhaled corticosteroids, which associated with a more disease severity and inferior prognosis.[11,12]

There is an increase evidence that the contribution of neutrophil cells in allergic diseases, especially in asthma patients with symptomatic disease who have high levels of the peripheral neutrophils which show the signs of activation. Both the number and the activation levels of these cells are lower in the absence of symptoms or after treatment and resolution of the allergic process.[13] The chemotactic activity of the neutrophil cells induced by platelet-activating factor is greater in the asthma patients than in those healthy people, and inversely related to the production of the 5-hydroxyeicosatetraenoic acid, which is a derivative of the arachidonic acid which produced by neutrophil cells.[13]

Patients and Methods

Eighty-eight patients was enrolled in this cross-sectional study and only 50 patients were successfully produce sputum, in the respiratory outpatient of Imamain Kadhimain Medical City from March 2017 to April 2018. According to history, physical examination, pulmonary function test (PFT), and chest X-ray, we diagnosed asthma and then we used asthma control test (ACT), to classify asthma adult patients to well, partially, and poorly controlled. Then, sputum eosinophil and neutrophil were measured for the asthmatic patient with successful sputum induction. The threshold values which defined the eosinophilic phenotype and neutrophilic phenotype were considered as sputum eosinophil count ≥3% and as sputum neutrophil count ≥76%.[14] Mixed granulocytic phenotype defined as high in both sputum eosinophil count and neutrophil count, while the paucigranulocytic phenotype defined as sputum eosinophil count and neutrophil count less than thresholds.

Inclusion criteria

All asthmatic patients, except who could not do the sputum induction, after getting patients' consent. The patients were in their treatments.

Exclusion criteria

One patient in respiratory distress who could not do the sputum induction was excluded Two patients with signs and symptoms of chest infection as fever, increase in cough severity, sputum, with signs of consultation. Three patients with active smoking to exclude other diseases which may be caused by smoking. Four patients with cardiac cause of respiratory distress. Five patients could not produce sputum.

Spirometry

Spirometry performed by using spirometer with the best of the three readings, according to the American Thoracic Society criteria before and 10 min after inhalation of salbutamol.[15]

Induction of sputum

The induced sputum was according to Pizzichini *et al*.[16] method, before the induction of sputum, all patients did spirometry. Hypertonic saline 4.5% used in nebulizer. The participants were inhaled the hypertonic saline for 7 min duration, and participants may be asked stopping inhalation with regular intervals to cough a sputum (e.g., every 5 min), or to stop the inhalation when they feel there is the urge to cough and asked to clean their mouth by water before the coughing and to blow their nose t o avoid contamination of induced sputum samples with the saliva. Then, the sputum samples analyzed as described by the method of Pizzichini *et al*.[16]

Statistical analysis

In this study, the statistical analysis was c with the Statistical Package for the Social Sciences 20.0 (analysis was performed with IBM SPSS Statistics 20 (SPSS, Chicago, IL, USA)) and Microsoft Excel 2013. Categories data formulated as a count and the percentage. The Chi-square test was used to describe the association of the data. The numerical data with a normal distribution were described as the mean and standard deviation, and independent sample *t*-test was used in comparison between the two groups. The lower accepted statistical significant difference is ≤ 0.05.

Results

In this study, 50 patients underwent a successful sputum induction, 21 (42%) was male and 29 (58%) female, as shown in Figure 1.

The age of patient ranged 16–80 years with a mean age 43.2, the duration of asthma from 3 months to 50 years with a mean age 12.1. Body mass index ranges from 21.2 to 39.2 with mean 34.45 years. Moreover, ACT ranges from 5 to 22 with a mean age 12.98 years, also forced expiratory volume1 (FEV1) ranges from 26.8 to 97.9 with a mean age 68.09 years, forced volume vital capacity (FVC) ranged from 33 to 91.4 with a mean age 70.42, and FEV/FVC ratio ranged from 46.3 to 100 with mean age 77.26, as shown in Table 1.

According to ACT, it shows 28 (56%) poor control, 21 (42%) was partial control and only 1 (2%) well control, as shown in Figure 2.

While according to sputum cells type, asthma subtype who had eosinophilic inflammation in 32 (64%), neutrophilic inflammation 5 (10%), mixed granulocytic 3 (6%), and with paucigranulocytic (non) 10 (20%), as shown in Figure 3.

According to the asthma phenotype and ACT, poorly controlled eosinophilic asthma Group 23 (82%), while partial control eosinophilic asthma Group 9 (41%). Neutrophilic inflammation and ACT the (23%) partial and good control group, while poorly control zero. Mixed asthma phenotype has 1 (4%) poor controlled and 2 (9%) partial controlled, paucigranulocytic

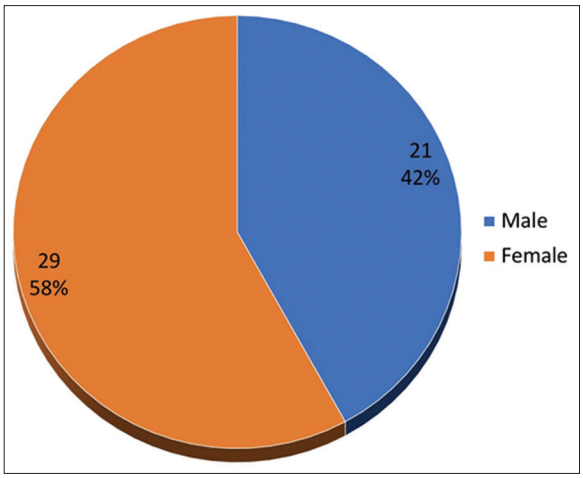

Figure 1: Asthmatic patients according to gender

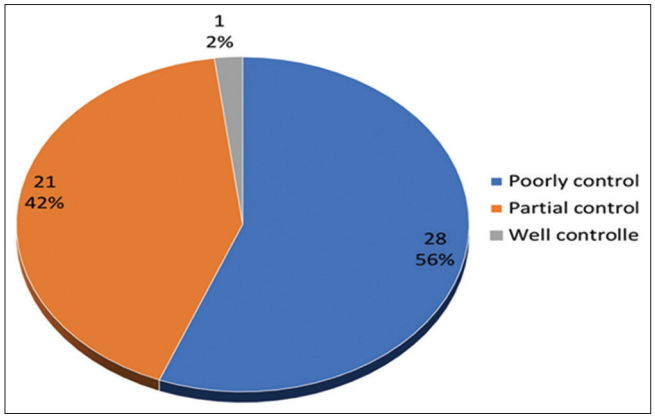

Figure 2: Asthma control test in asthmatic patients

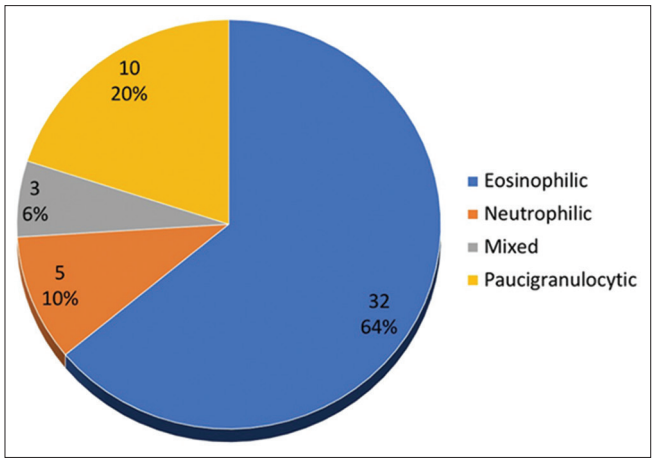

Figure 3: Asthma phenotype

asthma phenotype has 4 (14%) poorly controlled and 6 (27%) with partial controlled. The correlation between ACT and all asthma phenotypes shows statistically significant with $P = 0.010$, as shown in Table 2.

The mean of poorly controlled neutrophilic was 40.18 ± 14.16, and the mean of partially and well-controlled neutrophilic (42.86 ± 25.72), which shows no statistical significance (the $P = 0.640$). The mean of poorly controlled eosinophilic was 15.07 ± 13.63, while the mean of partially and well-controlled eosinophilic was 6.50 ± 8.44, which showed statically significant with a $P = 0.013$, as shown in Table 3.

According to asthma phenotype (eosinophil and neutrophil) in correlation with PFT which show a strong relationship between eosinophilic asthma and FEV1 with statistically significant *P* value (0.025). Moreover, eosinophilic asthma and FVC showed statistically significant with *P* value = 0.013. Furthermore, eosinophilic asthma and FEV1\FVC ratio showed statistically significant with *P* value = 0.028. While neutrophilic type shows no correlation with (FEV1, FVC. FEV1%) and statistically nonsignificant with $P = 0.478$, 0147, and 0.708, respectively, as shown in Table 4.

Table 1: The demographic data of asthmatic patients

	Mean	Minimum	Maximum	SD	SEM
Age	43.24	16	80	16.35	2.31
Duration	12.1	0.25	50	11.24	1.59
BMI	34.45	21.2	38.8	37.43	5.29
Act	12.98	5	22	4.41	0.62
FEV_1	68.09	26.8	97.9	17.05	2.41
FVC	70.42	33	91.4	13.07	1.85
FEV/FVC ratio	77.26	46.3	100	14.62	2.07

SEM: Standard error of the mean, BMI: Body mass index, FEV_1: Forced expiratory volume in 1 s, FVC: Forced vital capacity, SD: Standard deviation

Table 2: Asthma phenotype percentage according to asthma control test

Asthma phenotypes	ACT	
	Poorly controlled	Partially controlled
Eosinophilic (%)	23 (82)	9 (41)
Neutrophilic (%)	0 (0)	5 (23)
Mixed (%)	1 (4)	2 (9)
Paucigranulocytic (%)	4 (14)	6 (27)
Total	28	22
P value	0.010*	

ACT: Asthma control test, *Statistical significant difference

Table 3: Correlation between (eosinophilic and neutrophilic asthmatic patients) with asthma control test

	n	Mean±SD	*P*
Neutrophil (%)			
Poorly controlled	28	40.18±14.16	0.640 (NS)
Partially + well controlled	22	42.86±25.72	
Eosinophil (%)			
Poorly controlled	28	15.07±13.63	0.013*
Partially + well controlled	22	6.50±8.44	

*Statistical significant difference (0.05). NS: None statistical significant difference (>0.05), SD: Standard deviation

Table 4: Asthma phenotype (eosinophil and neutrophil) in correlation with pulmonary function test

	FEV_1	FVC	FEV_1 (%)
Neutrophil (%)			
R	0.103	0.208	−0.054
P	0.478	0.147	0.708
Eosinophil (%)			
R	−0.316*	−0.348*	−0.233*
P	0.025	0.013	0.028

*Statistical valid correlation (P=0.05). FEV_1: Forced expiratory volume in 1 s, FVC: Forced vital capacity

Discussion

The sputum eosinophil and neutrophil cells are characteristics of the bronchial airway inflammation in asthma, which reflect the pathologic process, and the usefulness to anticipate the treatment response.[17]

This study showed that there are four phenotypes of asthma, 64% had eosinophilic inflammation, 10% neutrophilic inflammation, 6% mixed granulocytic, and 20% paucigranulocytic. Gibson *et al*.'s study showed that 41% of the nonsmoking asthmatics has eosinophilic inflammation with a sputum eosinophil counts >2.5%.[18] In a Simpson study that conducted on 93 patients, 41% eosinophilic asthma, 20% neutrophilic, 31% paucigranulocytic, and 8% mixed granulocytic[19] who used thresholds of 1% for eosinophilic and 61% for neutrophilic inflammation.

Furthermore, the study of Schleich *et al*.[20] on 508 patients who underwent the successful sputum induction, showed that (42%) had eosinophilic inflammation, (16%) neutrophilic inflammation, (3%) mixed granulocytic, and (40%) paucigranulocytic who used thresholds eosinophilic (≥3%) and neutrophilic (≥76%) airway inflammation.

The difference in the results of the studies may related to the number of the patients included in the study, or may be due to the threshold of cells in each study.

For correlation between (eosinophilic and neutrophilic asthmatic patients) with asthma control, there was statistically significant relationship between eosinophilic asthma and poorly controlled asthma with $P = 0.013$. In neutrophilic type showed no statically significant correlation with poorly controlled asthma. These results in agreement with that of Romagnoli *et al*.'s study, in which eosinophils is higher in poorly controlled asthma than in controlled asthma with $P = 0.01$. Furthermore, Louis *et al*.'s study[21] found the eosinophil number in sputum is higher in the patients with more severe asthma. While for neutrophils, there was no statistically significant between poorly controlled asthma and controlled asthma, and it agrees with Demarche *et al*.[22] which shows no clinical significant in asthma control and sputum neutrophils. In Fahy[23] showed that the neutrophil numbers were not associated with more severe in airway reactivity to the methacholine test.

Shaw *et al*.'s study[24] showed that airway neutrophil is a characteristic of disease severity. The difference of this study may due to the large number 1197 patients with asthma, and small number in our study.

According to asthma phenotype (eosinophil and neutrophil) in correlation with PFT, this study showed a statistical significant relationship between eosinophilic asthma and FEV1, FVC, and FEV1\FVC ratio. While neutrophilic type shows statically nonsignificant correlation with FEV1, FVC, and FEV1\FVC ratio. In Schleich *et al*.'s[20] study of the 508 patients who underwent a successful sputum induction showed statistically significant relationship between both eosinophilic and neutrophilic asthma and FEV1, FEV1\FVC ratio. The disagreement with our study may also due to the difference in number of patients. Pizzichini *et al*.'s[25] study showed the FEV1/FVC ratio, an index of the airway narrowing, correlated to the sputum eosinophil.

Conclusions

Eosinophilic asthma is the most common phenotype. The patients with eosinophilic asthma have poor asthma control. Mixed granulocytic asthma is the least common phenotype. Neutrophilic asthma not associated with poor asthma control in this study.

However, the number of patients in this study was small.

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Conflicts of interest

There are no conflicts of interest.

References

1. Global Initiative for Asthma (GINA). The Global Strategy for Asthma Management and Prevention. June. 2019. Available from: http://www.Ginasthma.org (2019).
2. Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, *et al*. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2163-96.
3. Murphy DM, O'Byrne PM. Recent advances in the pathophysiology of asthma. Chest 2010;137:1417-26.
4. Edwards MR, Bartlett NW, Hussell T, Openshaw P, Johnston SL. The microbiology of asthma. Nat Rev Microbiol 2012;10:459-71.
5. Akinbami LJ, Moorman JE, Bailey C, Zahran HS, King M, Johnson CA, *et al*. Trends in asthma prevalence, health care use, and mortality in the United States, 2001-2010. NCHS Data Brief 2012;(94):1-8.
6. Popov TA, Petrova D, Kralimarkova TZ, Ivanov Y, Popova T, Peneva M, *et al*. Real life clinical study design supporting the effectiveness of extra-fine inhaled beclomethasone/formoterol at the level of small airways of asthmatics. Pulm Pharmacol Ther 2013;26:624-9.
7. Kasayama S, Tanemura M, Koga M, Fujita K, Yamamoto H, Miyatake A. Asthma is an independent risk for elevation of plasma C-reactive protein levels. Clin Chim Acta 2009;399:79-82.
8. Busse WW, Lemanske RF Jr. Asthma. N Engl J Med 2001;344:350-62.
9. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. J Allergy Clin Immunol 1995;95:843-52.
10. Tillie-Leblond I, Montani D, Crestani B, de Blic J, Humbert M, Tunon-de-Lara M, *et al*. Relation between inflammation and symptoms

in asthma. Allergy 2009;64:354-67.
11. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, *et al*. Severe adult-onset asthma: A distinct phenotype. J Allergy Clin Immunol 2013;132:336-41.
12. Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. Ann Allergy Asthma Immunol 2014;113:19-24.
13. Rabier M, Damon M, Chanez P, Mencia Huerta JM, Braquet P, Bousquet J, *et al*. Neutrophil chemotactic activity of PAF, histamine and neuromediators in bronchial asthma. J Lipid Mediat 1991;4:265-75.
14. Louis R, Godinas L, Schleich F. Induced sputum – Towards normal values. Non Invasive Assessment of Airways Inflammation in Asthma and COPD. Athens: Paschalidis Medical Publications; 2011. p. 113-23.
15. Global Strategy for Asthma Management and Prevention. NIH publication no. 02-3659. Updated from: NHLBI/WHO Workshop Report: Global Strategy for Asthma Management and Prevention Issued January, 1995; 2002.
16. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, *et al*. Indices of airway inflammation in induced sputum: Reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med 1996;154:308-17.
17. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: Identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. Thorax 2002;57:875-9.
18. Gibson PG, Simpson JL, Saltos N. Heterogeneity of airway inflammation in persistent asthma: Evidence of neutrophilic inflammation and increased sputum interleukin-8. Chest 2001;119:1329-36.
19. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: Assessment and identification using induced sputum. Respirology 2006;11:54-61.
20. Schleich FN, Manise M, Sele J, Henket M, Seidel L, Louis R. Distribution of sputum cellular phenotype in a large asthma cohort: Predicting factors for eosinophilic vs. neutrophilic inflammation. BMC Pulm Med 2013;13:11.
21. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanović R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000;161:9-16.
22. Demarche SF, Schleich FN, Paulus VA, Henket MA, Van Hees TJ, Louis RE. Asthma control and sputum eosinophils: A longitudinal study in daily practice. J Allergy Clin Immunol Pract 2017;5:1335-43.
23. Fahy JV. Eosinophilic and neutrophilic inflammation in asthma: Insights from clinical studies. Proc Am Thorac Soc 2009;6:256-9.
24. Shaw DE, Berry MA, Hargadon B, McKenna S, Shelley MJ, Green RH, *et al*. Association between neutrophilic airway inflammation and airflow limitation in adults with asthma. Chest 2007;132:1871-5.
25. Pizzichini E, Pizzichini MM, Efthimiadis A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: Eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. J Allergy Clin Immunol 1997;99:539-44.