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Role of Induced Sputum to Assess Airway Inflammation in Asthma - Study from a Tertiary Care Centre

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Abstract

Asthma is a serious global health issue affecting all age groups. Asthmatics are usually assessed by spirometry, which means the physiologic impairment. But their hall mark is airway inflammation, which is rarely assessed. Airway inflammation has been studied widely in patients with chronic asthma, and it is seen that it correlates with the severity of the disease. The objective of this study was to assess the use of induced sputum cytology to classify asthmatics as neutrophil & eosinophil predominant, and to study the relationship of level of control, severity of obstruction and their response to inhaled steroids in asthmatics with the inflammatory subtypes in stable asthmatic patients.

Methods: *A total of 80 asthmatics who attend the outpatient clinic at the department of respiratory medicine, govt medical college Kannur, who met the inclusion & exclusion criteria were taken for the study. Initially history was taken and spirometry done and graded according to severity and then sputum induction done with 3% saline. Sputum processed and diffentiated into neutrophil, eosinophil or mixed/ paucigranulocyte type. A comparative study was done and patients were treated with inhaled corticosteroid for 2 weeks and their response were evaluated.*

Result: *Out of total 80 patients 60% were females. 18.8% were uncontrolled & 40% were well controlled. 21 patients had a neutrophil predominant picture, 44 had eosinophil predominant&6 had mixed picture. There was significant association between sputum phenotype & level of control, showing predominant neutrophil in uncontrolled. The response to inhaled steroid showed a significant improvement with eosinophilic phenotype.*

Conclusion: *Sputum induction can be used as an important noninvasive measurement of airway inflammation, in the diagnosis and management of asthma. It can also be used to assess the type of airway inflammation in asthmatic patients who were not controlled by asthma management.*

Keywords: *Sputum Induction, Airway Inflammation, Sputum Cytology.*

Introduction

Asthma is a serious global health issue affecting all age groups, with increasing prevalence in many developing countries, causing an increased treatment costs, and a rising burden for patients and the community[1]. Normally there is a fine balance between immune cells, the epithelium, and the host immune response. Airway inflammation in asthma reflects a distortion of this balance and is orchestrated through a complex interplay between multiple effectors and target components[2]. The relationship between severity of asthma and the intensity of inflammation is not yet clearly established[3]. In stable asthmatics invasive methods like bronchial biopsy and Bronchoalveolar lavage have been used to study airway inflammation[4,5,]. But discomfort, inconvenience, and risks limit their use. Examination of sputum is a less invasive alternative[6], but sputum cannot always be produced spontaneously. When sputum is not otherwise available, induced samples may allow secretions from the lower airways to be sampled. In asthma, the analysis of cells and mediators in induced sputum has been applied for studying bronchial inflammation. Correlation between type of airway inflammation and asthma control and effect on sputum cellularity can help to guide asthma treatment. Sputum induction is a non invasive method to assess the airway inflammation. The analysis of induced sputum is a reproducible means to evaluate airway inflammation in asthma[7]. Quality of cells and their viability are significantly higher in induced sputum samples than in spontaneous sputum samples and there is no significant differences in total or differential cell counts[8,9]. Although sputum eosinophilia is a typical feature of asthma, the application of induced sputum analysis has led to the recognition that inflammation in asthma is more heterogeneous than previously believed with the identification of non eosinophilic asthma[10,11]. Non-eosinophilic asthma is common, accounting for 25–55% of corticosteroid-naive asthmatics.

Asthmatics with sputum eosinophilia have a favourable response to corticosteroids[12]. Non-eosinophilic asthma is associated with a poor response to inhaled steroids. Thus sputum differential count isan important factor in determining the response to inhaled steroids in asthma[13].

The aim of our study was to assess the use of induced sputum cytology to classify them as neutrophil predominant and eosinophil predominant in stable asthmatic patients. And also to assess the relationship of severity of asthma with the inflammatory subtypes and study their response to inhaled steroids.

Materials and Methods

Hospital Based Descriptive study was conducted in subjects with asthma who attended the outpatient clinic atthe department of Respiratory Medicine of Pariyaram Medical College, Kannur for a period of one year from March 1ST 2015 to February 29TH 2016. Consecutive sampling method was used.

Study Sample: 80 Patients with Asthma.

Sample Size Justification: Audited no. of patients with newly detected cases of asthma, who attended last year in our OP was 150. Hence the sample population of 80 patients who satisfied the criteria described below were selected.

Inclusion Criteria

A. All asthmatic patients except patients with contra-indication for sputum induction.

B. Asthma is diagnosed according to the criteria recommended by the GlNA 2016[1].

Exclusion Criteria

- Haemoptysis of unknown origin.
- Acute respiratory distress.
- Unstable cardiovascular status, (arrhythmias, angina).[14]
- Thoracic, abdominal or cerebral aneurysms.
- Hypoxia (SaO2 less than 90% on room air).
- Lung function impairment (FEV1 less than 1.0 L).

- Pneumothorax.
- Pulmonary emboli
- Fractured ribs or other chest trauma.
- Recent eye surgery.
- Patients who are unable to follow instructions

Study Design

Patients visiting the outpatient clinic at the department of respiratory medicine were recruited for the study. Initially a detailed history which includes exposure to risk factors, past history, addictions and family history were taken .Then the patients were classified according to history into well controlled, partially controlled and uncontrolled. Then the patient was clinically assessed and a spirometry was done. It was performed using a spirometer and the best of three consecutive readings according to the American Thoracic Standards, before and 10 min after the inhalation of 200 mcg salbutamol is taken. Those patients who were diagnosed as stable asthmatics who met the inclusion criteria were then registered in the asthma clinic with their consent. They were treated symptomatically with bronchodilators (steroids are avoided) and advised to reviewafter 1 wks for sputum induction.

Sputum Induction: Sputum is induced according to the method of Pizzichini et al[15] Induction is performed in the spirometry room equipped with pulse oxymeter, nebulizer and central O2supply. Before sputum induction, the procedure is explained to the patient. The patient is then examined clinically and vitals are recorded. Then he/she will undergo a screening spirometry, with FEV1 and vital capacity measurements, before and 10 min after the inhalation of 200 mcg salbutamol by a meter dose inhaler. Hypertonic saline 3% is then used to nebulize the patient using an ultrasonic nebulizer with output set at 1.5 ml/min at room temperature. The subjects are asked to inhale hypertonic saline for a 5 min duration. Subjects are asked to stop inhalation at regular intervals in order to cough up sputum, or to stop only when they feel the urge to cough and are asked to rinse their mouth with water before coughing and to blow their nose to avoid salivary contamination of induced sputum samples. Sputum induction is discontinued if the FEV1 declined by 20%. Selected sputum plugs from saliva are then analyzed.

Sputum Processing: Sputum samples are processed within 2 hours after the collection, according to "Pick and Smear" Technique of Sputum Processing. The sputum is first carefully inspected. Select any bloody, discoloured, or solid particles, if present, and place a small portion of each particle, not larger than the size of a small pea, on each of four plain slides. With a clean glass slide, crush the particle of sputum on each of the four slides, using a rotary motion. Then, with overlapping horizontal strokes, spread the material evenly over the slide so that the final preparation is only slightly thicker than a blood smear. Place the prepared slides immediately in a kopliks jar with 95% ethyl alcohol fixative making sure that the smeared surfaces remain separated. The slides are then air-dried and stained by Giemsa and 200-400 non squamous cells are counted.

If > 80% of the cells consisted of squamous cells, the quality of the sputum sample is judged unsatisfactory and excluded from the analysis. Then sputum differential count is done. Subjects are then started on inhaler steroids (budesonide 200mcg) MDI/DPI based on patients acceptance and supportive inhaled broncho dilators. This is based on the GINA 2016 guidelines. They were then reviewed after 2weeks and their symptomatology were reassessed and a spirometry was done to measure FEV1.

Statistical Procedure: All the data collected will be entered and analyzed using SPSS version software. Descriptive statistical methods like mean, standard deviation, frequency and proportions will be used .Inferential statistical methods like chi square test, Wilcoxon signed rank test and one way Anova tests were used. P value was measures and significance evaluated.

Outcome Measures: The induced sputum cytology was assessed to classify them as

neutrophil predominant and eosinophil predominant in stable asthmatic patients. The relationship of severity of asthma with the inflammatory. The relationship of severity of asthma with the inflammatory subtypes andtheir response to inhaled steroids were also assessed.

Results

80 patients were included in the study group, out of which males constituted 60 % and rest of them were females (40%).Most common age group was 21 – 30 years (42.5%). The mean age of the study population was 30.7years with a standard deviation of 8.2.
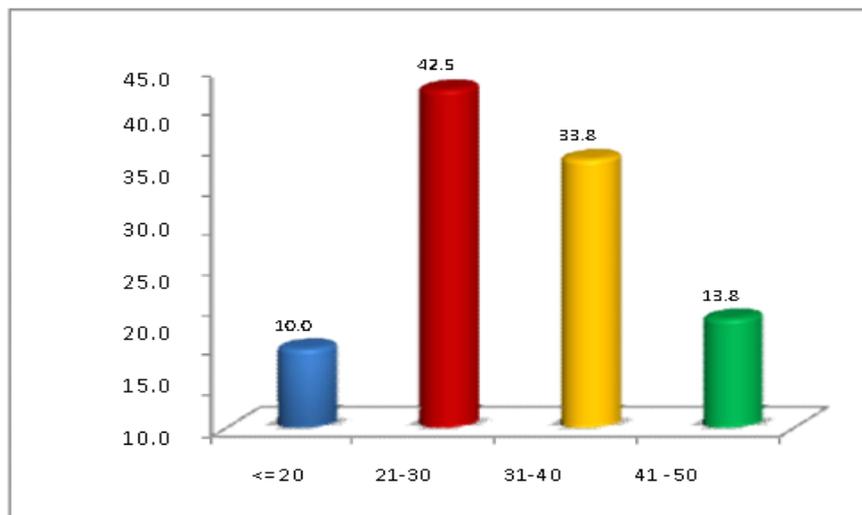
Figure: 1

48 patients (60%) had daytime symptoms for more than 2wks, 15 patients (18%) had night time symptoms and 48 patients (60%) needed relievers more than twice weekly.

Table 1: Percentage distribution of the sample according to symptoms

Symptoms	Count	Percent
Daytime symptoms more than 2/wk	48	60.0
Night symptoms	15	18.8
Need relievers more than twice / wk	48	60.0

Out of 80 patients, 33 patients (41%) had history of allergic rhinitis, 20 patients (25%) had history of eczema and 8 patients (10%) had history of food and drug allergy. Out of 80 patients 40 patients had family history of asthma and 10 patients (12.5) had family history of allergy.

Table 2 Percentage distribution of the sample according to family history

Family history	Count	Percent
Asthma	40	50.0
Allergy	10	12.5

13 patients (16.3%) were smokers Out of the 80 patients, 15 patients (18.8%) were classified as uncontrolled, 33 patients (41.3%) were classified as partially controlled and rest 32 patients (40%) were classified as well controlled.

Table 3 Percentage distribution of the sample accordin

| Treatment history	Count	Percent
IBD	69	86.3
ICS	27	33.8

69 patients were on IBD and 27 patients were on ICS.

The mean pre FEV1 was 66.5 with a standard deviation of 11.0

Table 4: Descriptive statistics for spirometry

|	Mean	SD	Median	Minimum	Maximum
Pre FEV1 %	66.5	11.0	69.0	43.0	87.0
FVC%	81.7	5.1	81.0	72.0	91.0
FEV1/FVC	69.0	5.7	69.5	58.0	80.0

The patients were then classified according to degree of obstruction into mild, moderate and severe
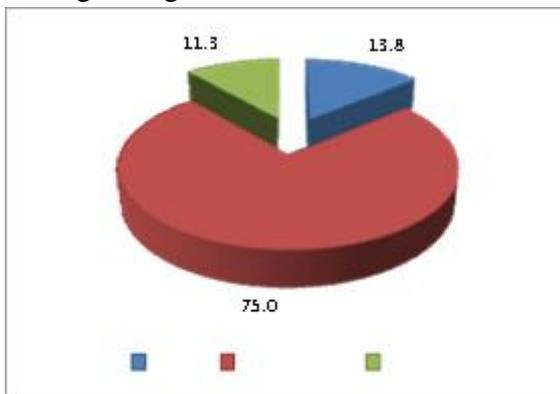
Figure 2: Percentage distribution of the sample according to Degree of Obstruction

71 patients slides met the criteria of selection for adequate sample. These 71 slides were then classified into neutrophil predominant, eosinophil predominant, mixed and pauci granulocytic.21 patients (29.6%) had neutrophil predominant , 44 patients (62.0%) had eosinophil predominant and 6 patients (8.5%) had mixed/pauci granulocytic picture.
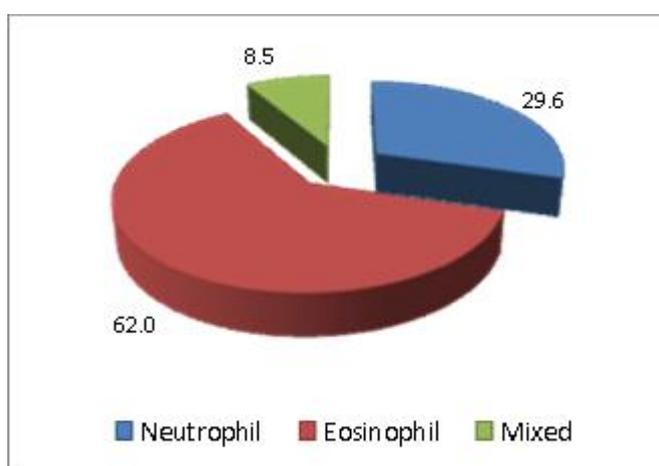

Figure 3: Percentage distribution of the sample according to sputum induction response

Association of sputum induction results and level of control

Comparison of level of control and sputum induction reports was done using chi square test and the p value was calculated. Results showed that in uncontrolled patients 78.6% were neutrophil predominant and 21.4% were eosinophil predominant. In partially controlled patients 34.5% were neutrophil predominant, 44.8% were eosinophil predominant. And 20.7 % were mixed. All the well-controlled patients had eosinophil predominant sputum. The chi square value was 40.19 with a p value <0.01 which was found to be statistically significant.
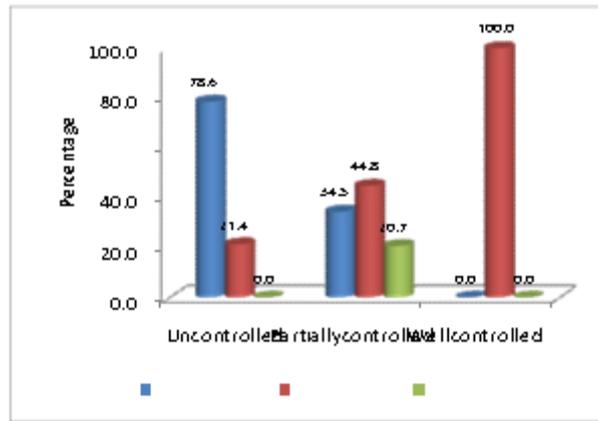
Figure 4: Comparison of level of control based on sputum induction

Association of history with sputum induction reports

Comparison study was done using chi square test which showed that there is no statistically significant association between types of sputum response and the family history of asthma and allergy. There was eosinophil predominance in patients with history of allergic rhinitis and food and drug allergy and this was found to be statistically significant.

Table 5: Comparison of history based on sputum induction

		Neutrophil		Eosinophil		Mixed		χ^2	p
		Count	Percent	Count	Percent	Count	Percent		
Family history	Asthma	12	33.3	19	52.8	5	13.9	3.9	0.142
	Allergy	3	37.5	3	37.5	2	25.0	3.98	0.136
Smoking history		5	38.5	5	38.5	3	23.1	5.87	0.053
Past History	Allergic rhinitis	10	34.5	14	48.3	5	17.2	6.37*	0.041
	Eczema	11	61.1	2	11.1	5	27.8	28.83**	p<0.01
	Food & drug allergy	1	14.3	1	14.3	5	71.4	39.91**	p<0.01

**: - Significant at 0.01 level, *: - Significant at 0.05 level

Effect of treatment on the level ofcontrol:

The response to ICS treatment for 2 wks were assessed by clinical assessment and spirometry. Clinically in the neutrophil predominant patients, number of uncontrolled patients decreased from 52.4 % to 33.3% and was found to be statistically significant using Wilcoxon signed rank test with a p value < 0.01. There were also significant increase in the number of well controlled asthmatics from 63.1% to 84.1% in eosinophil predominant patients after 2 wks ICS treatment.

Table 6: Effectiveness of treatment in level of control

		Pre		Post		Z#	p
		Count	Percent	Count	Percent		
Neutrophil	Uncontrolled	11	52.4	7	33.3	2.83 **	0.005
	Partially controlled	10	47.6	10	47.6		
	Well controlled	0	0.0	4	19.0		
Eosinophil	Uncontrolled	3	6.8	0	0.0	3.46 **	0.001
	Partially controlled	13	29.5	7	15.9		
	Well controlled	28	63.6	37	84.1		
Mixed	Uncontrolled	0	0.0	0	0.0	1.41	0.157
	Partially controlled	6	100.0	4	66.7		
	Well controlled	0	0.0	2	33.3		

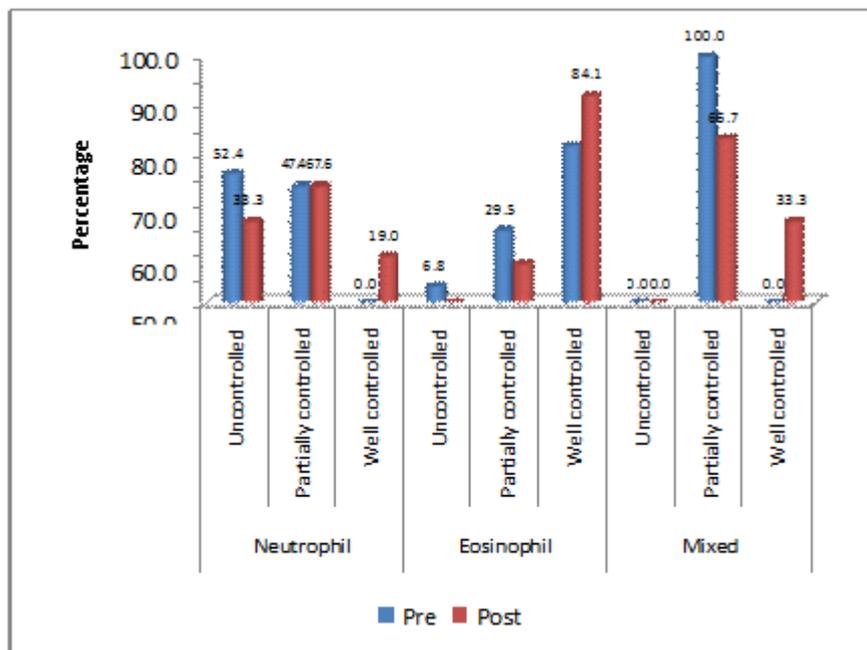#Wilcoxon Signed Rank Test   **: - Significant at 0.01level

Figure 5: Effectiveness of treatment in level of control

Effect of treatment on FEV1

The pre FEV1 was compared with the type of induced sputum which showed that the mean FEV1 was high (71.4) in eosinophil predominant with a standard deviation SD of 8.6.this was found to be statistically significant using One way anova test with a p value<0.01

Table 7: Comparison of pre FEV% based on sputum induction

Sputum induction	Mean	SD	N	F	p
Neutrophil	56.3	9.3	21		
Eosinophil	71.4	8.6	44	21.83**	p<0.01
Mixed	61.8	7.8	6		

**:- Significant at 0.01 level One way anova test

The post treatment increased FEV1% was compared with the level of control which showed that the mean increase in FEV1 in uncontrolled is 8.6 %, partially controlled is 10.7% and a maximum increased in well controlled of 11.2 %. But was not found to be statistically significant when assessed by one way anova test (p value0.061)

Table 8 Comparison of increase in FEV% based on level of control

Level of control	Mean	SD	N	F	p
Uncontrolled	8.6	5.0	14		
Partially controlled	10.7	2.8	29		
Well controlled	11.2	2.9	28	2.92	0.061

Discussion

In our study 80 patients were included, out of which most common age group was 21- 30years. JMajority of them were males (60%). Out of the 80 patients, 71 (89%) patients slides met the criteria of selection for adequate sample. There were no major adverse effects noted. Al Zahrani et al have reported an overall success of 93%[16]. He has also shown that the procedure is safe even amongst patients with an FEV1 less than 60%of predicted value or less than one L .Our study also agreed to these results.

Hunter et al and jones et al have proved that when sputum induction is performed with b2-agonist

pretreatment using ultrasonic nebulizer with 4.5% hypertonic saline, it gave 98% procedure completion rate. 4% of patients demonstrated 15% decrease in FEV1. An adequate sputum sample could be obtained in 92% of the children. Distressing cough was noticed in 13%. In 1% mucosal irritation was noticed[17,18]. Sputum was then processed and analyzed with "pick and mix technique" and stained with Giemsa stain. Out of the 80 patients, 71 (89%) patients slides met the criteria of selection for adequate sample. There were no major adverse effects noted. Al Zahrani et al have reported an overall success of 93%[16]. He has also shown that the procedure is safe even amongst patients with an FEV1 less than 60% of predicted value or less than one L. Our study also agreed to these results. Thus carefully standardized sputum induction can be safe and successful procedure in patients with asthma and COPD in clinical practice, even in presence of moderate to severe airflow limitation. Hunter et al and jones et al have proved that when sputum induction is performed with b2-agonist pretreatment using ultrasonic nebulizer with 4.5% hypertonic saline, it gave 98% procedure completion rate. 4% of patients demonstrated 15% decrease in FEV1. An adequate sputum sample could be obtained in 92% of the children. Distressing cough was noticed in 13%. In 1% mucosal irritation was noticed (17,18).The accepted 71 patients slides were classified into neutrophil predominant, eosinophil predominant, mixed and pauci granulocytic .

Our results showed that asthma is a heterogeneous disease having various subtypes of airway inflammation and this is in agreement with previous studies that identified different inflammatory phenotypes in subjects with asthma. Wenzel et al and Simpson et al. showed that asthmatic patients had four subtypes of airway inflammation neutrophilic, eosinophilic, mixed type and paucigranulocytic (non eosinophilic non neutrophilic)[19,20]. Comparison of level of control and sputum induction reports was done using chi square test and the p value was calculated .Results

showed that in uncontrolled patients 78.6% were neutrophil predominant and 21.4% were eosinophil predominant.

In partially controlled patients 34.5% were neutrophil predominant, 44.8% were eosinophil predominant and 20.7 % were mixed. All the well-controlled patients had eosinophil predominant sputum. The chi square value was 40.19 with a p value <0.01 which was found to be statistically significant. i.e. .subjects with neutrophilic inflammation had poor level of control. These results agree with results reported by Shaw and colleagues showing that airway neutrophilia is a characteristic of more severe asthma and suggest a possible mechanistic link between airway neutrophils and chronic airway narrowing in asthma[21]. Douwes et al. also found that only around 50% of asthma cases was associated with eosinophilic inflammation, and that in most other cases asthma who had poor control was accompanied by an increase in airway neutrophils and interleukin 8 (IL-8)[22]. Li et al. reported that a significant proportion of asthma and wheezing illness in both adults and children is associated with neutrophilic airway inflammation and that this pattern is not limited to individuals with severe symptoms. This raises important and interesting questions regarding the mechanisms and consequences of neutrophilic inflammation, as well as presenting a novel and inviting therapeutic target[23].

Comparison study was done about the association of history with sputum induction reports, using chi square test, which showed that there is no statistically significant association between types of sputum response and the family history of asthma and allergy Also smoking history had no statistically significant association with the sputum response. But Stapleton et al. has showed that there is association between the sputum phenotype and asthma[24]. There was eosinophil predominance in patients with history of allergic rhinitis and food and drug allergy and this was found to be statistically significant.ThepreFEV1 was compared with the type of induced sputum

which showed that the mean FEV1 was high (71.4) in eosinophil predominant with a standard deviation SD of 8.6. This was found to be statistically significant using Onewayanova test with a p value <0.01. Woodruff et al. also demonstrated low FEV1 in asthmatic patients with neutrophilic bronchitis[25]. Results reported by Elbehidy and colleague showing that there was significant negative correlation between changes in FEV1 and change in eosinophils[26]. There was also negative correlation between neutrophilic asthma and FEV1 which also was significant (p-value <0.01) and these results agree with results reported by Peleman et al.[27]. Negative correlations between the neutrophil count in induced sputum of asthmatic patients vs. FEV1, and the reduction in annual FEV1 values suggested a significant effect of neutrophils on pulmonary function and the influence on the severity of the disease. The response to ICS treatment was assessed by clinical assessment and spirometry. Clinically in the neutrophil predominant patients, number of uncontrolled patients decreased from 52.4 % to 33.3% and was found to be statistically significant using Wilcoxon signed rank test with a p value < 0.01.There were also significant increase in the number of well controlled asthmatics from 63.1% to 84.1% in eosinophil predominant patients after ICS treatment.

This is in agreement with Bacci et al. who investigated adults, before and after 2 and 4 weeks of treatment with beclomethasone 500 mcg twice daily who founded that Sputum eosinophilia was associated with improvement in symptoms, peak expiratory flow and methacholine airway responsiveness[28]. Also Elbehidy and colleague showed that sputum eosinophilia is the best predictor of steroid response in asthmatics and support the use of sputum cell counts to guide steroid treatment[26]. This is in agreement with Brightling who founded strong evidence that sputum eosinophilia (>3%) is a predictor of clinical improvement with corticosteroid treatment[29]. The post treatment increased

FEV1% was compared with the level of control which showed that the mean increase in FEV1 in uncontrolled is 8.6 %, partially controlled is 10.7% and a maximum increased in well controlled of 11.2 %. But was not found to be statistically significant when assessed by one way anova test (p value 0.061).Green et al. also demonstrated heterogeneity in induced sputum cell counts of patients with mild to moderate asthma who have a predominantly neutrophilic airway inflammation and who respond less well to treatment with inhaled corticosteroids[30]. Our results are in agreement with Fahy et al. who described the fact that ICSs are regarded the most effective anti-inflammatory therapy for asthma, and significantly improve symptoms, inflammation, and airway function, but according to our results this improvement is significant in asthmatic patients with eosinophilic inflammatory subtype and to a minimal level in neutrophilic inflammation[31] Our results are in agreement with Elbehidy and colleagues who described the fact that ICSs are regarded as the most effective anti-inflammatory therapy for asthma, and significantly improve symptoms, inflammation, and airway function[26]

Conclusion

Standardized sputum induction is a non-invasive, safe and well tolerated procedure in patients with asthma in clinical practice, even in presence of moderate to severe airflow limitation. Induced sputum is a reproducible noninvasive method to assess airway inflammation. Categorization of patients according to different inflammatory phenotypes in induced sputum is important for every patients who are diagnosed with asthma, even before starting treatment. Inflammatory phenotype guided treatment helps in better understanding of the treatment responses.

Limitations of the Study
- It is a single centre study.
- More specific methods of sputum processing like Saccomanno's Technique

could not be used to due to limited facility.

• Follow up duration of 2wks could only be assessed. Longer period of follow up and a repeat induced sputum response could have had a better view of the response to treatment.

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