INTRODUCTION

Asthma has an estimated prevalence of 300 million people and is responsible for one in every 250 deaths worldwide. Although, only 5 - 10% of the asthma population has severe disease, it remains difficult to control despite optimal management which thus is associated with substantial morbidity and mortality, along with an increased health-care resource utilization. The Real-world Evaluation of Asthma Control and Treatment (REACT) study on patients with moderate to severe asthma found that 55% patients had uncontrolled asthma.

Among factors to account for poor control in patients with severe persistent asthma, airway remodelling and inflammatory / cytokine profiles have gained pivotal role. In a study by Gibson et al. sputum neutrophilia was identified in 59% of patients with persistent asthma. Although cause of neutrophilic inflammation in asthma is unknown; external factors, such as current or previous tobacco smoke exposure, occupational exposure to irritants or viral infections could be involved. Additionally, treatment with high doses of corticosteroids can lead to sputum neutrophilia by steroids induced reduction in apoptosis of neutrophils.

More recently, interest has concentrated on the identification of asthma phenotypes / endotypes based on the underlying pathogenetic mechanisms for the disease thus providing a window to individualize the asthma management. The application of induced sputum as a non-invasive ‘inflammometer’ could potentially lead to more-specific phenotypic identification, and thus more individualized asthma management.

The present study was conducted to determine the frequency of neutrophilia in induced sputum samples in cases of severe persistent bronchial asthma patients on standard therapy.

METHODOLOGY

This cross-sectional study was carried out at the Out Patient Department of Medicine, Military Hospital, Rawalpindi, from November 2009 to November 2010. Consecutive (non-probability) sampling was used, with sample size of 195 calculated by WHO calculator. Patients of severe persistent bronchial asthma, between 12 - 40 years of age, of either gender, were recruited till 195 sputum samples of good quality were obtained for the study. Severe persistent bronchial asthma was defined as asthma symptoms throughout the day in the previous 04 weeks despite of use of high dose inhaled corticosteroids (i.e. beclomethasone dipropionate > 1000 µg per day or equivalent) along with montelukast, salbutamol, and ipratropium inhalers. All patients underwent a 1-month run-in period and only patients with correct technique of use of inhaler with or without spacer and compliance with prescribed standard therapy were included. Smokers, patients with aspirin...
sputum neutrophilia). COPD, GERD, obesity (BMI > 30 kg/m²) history of pulmonary tuberculosis, current respiratory tract infection, oral candidiasis and those who had an asthma exacerbation in the last 4 weeks were excluded.

All subjects were premedicated with salbutamol inhalation (200 µg), 10 minutes before saline nebulizations and whole sputum-induction procedure was explained to them. Sputum induction was done with normal saline (0.9%) and then if tolerated by serially higher concentrations of hypertonic saline (3%, 4% and 5%) nebulizations through a nebulizer (Nidek Medical pulmo-mist compressor). Each nebulization was done for a period of 7 minutes. All subjects were asked to blow their nose, rinse their mouth, and swallow the water to minimize contamination with postnasal drip and saliva; afterwards they were encouraged to cough deeply. Induced sputum was collected after 10 - 15 minutes of saline nebulizations into a sterile sputum container until an adequate sample containing > 0.5 mL visible mucocellular material was obtained. Sputum plugs were separated from contaminating saliva using sterile forceps and were processed within 2 hours of induction.

The induced sputum samples were centrifuged on Cytospin 2 centrifuge (manufactured by Shand) at 200-300 revolution per minute for 5 minutes. The sediment was prepared on slide and stained with Leishman’s stain for differential cell counts by pathologist. Sputum samples containing > 20% of squamous cells and with cell viability < 70% were excluded from analysis as an indication of poor cytospin quality. Neutrophils were counted out of 200 cells and percentage calculated by manual differential leucocyte count. Sputum neutrophilia was defined as neutrophil percentages at more than 65% (130 x 10⁶/ml) out of 200 cells of induced and processed sputum samples. One consultant pathologist, well trained in cytology studies, recorded his findings on all induced processed sputum samples. The patients were well briefed about the nature of the study.

The collected data was processed in Statistical Package for Social Sciences (SPSS) version 11. Mean ± standard deviation was calculated for quantitative variables (age, sputum neutrophilic count) and frequency / percentage for qualitative variables (gender, presence or absence of sputum neutrophilia).

RESULTS

A total of 210 cases of severe persistent bronchial asthma were recruited into the study, out of which 195 patients were studied. Fifteen (7.14%) patients were excluded due to suboptimal sputum samples, either containing > 20% of squamous cells or had cell viability < 70%. Out of 195 study cases, 129 (66.2%) were males and 66 (33.8%) females. The mean age of the patients was 27.01 ± 6.92 years with minimum age of 13 and maximum age of 39 years. Mean neutrophilic count was 126.47 x 10⁹/ml with minimum 93 x 10⁶/ml, and maximum of 165 x 10⁶/ml ± 16.52 x 10⁶/ml. Mean neutrophilic percentage in all the patients was 63.18 ± 8.3363% with minimum 45.50% and maximum of 82.50%. Sputum neutrophil percentage of 65% (130 x 10⁶/ml) out of 200 cells of induced sputum sample was considered as positive. Sputum neutrophilia was present in 84 (43.10%) and was absent in 111 (56.90%) of patients. In patients with sputum neutrophilia, mean neutrophilic count was 142.40 ± 8.49 x 10⁹/ml with minimum of 130 and maximum of 165 cells and the mean neutrophilic percentage was 71.20 ± 4.2441%.

Four (2.25%) cases developed bronchospasm with isotonic saline, which was managed with repeated salbutamol and ipratropium nebulizations.

DISCUSSION

As reported to be safer by Vieira et al., isotonic saline was first used followed by hypertonic saline nebulizations (if required) to obtain lower airway lining fluid for assessment of airway inflammation. Sputum induction allows assessment of airway inflammation in subjects with chronic airway diseases like asthma and COPD with comparable results to bronchoscopic airway biopsy and BAL fluid analysis with good reproducibility. Different studies showed that monitoring inflammation by induced sputum analysis or the measurement of exhaled nitric oxide (ENO), led to a lower frequency of exacerbations in asthma and even allowed to reduce the dose of inhaled corticosteroids.

Sputum induction is also a useful tool to assess levels of inflammatory mediators which correlate with airway remodelling severity and thus to enhance current biological therapies options for asthma. Although ENO also provides a non-invasive assessment of airway inflammation but is costly and provides only evidence for eosinophilic airway inflammation, which can be difficult to interpret, when e.g. high NO values persist, despite increases in steroid dose. A study by Holz and colleagues concluded that even spontaneous sputum cytology can be helpful in the diagnosis of inflammatory airway diseases.

Seven point fourteen percent cases were excluded due to suboptimal sputum sample which is comparable to other studies. The present study has also demonstrated that sputum induction is a safe procedure and can be safely used as an outdoor procedure. Sixty-six percent of patients were males, which could be attributable to our social setup where males have more access to medical facilities and sampling was based on non-probability convenience technique, as most of the individuals were selected from male outpatient department.

This study like other studies on the subject has confirmed that there is heterogeneity of airway inflammation in poorly controlled asthma and identified...
evidence of neutrophilic inflammation in severe persistent asthma. In this study, there was increase in both relative and absolute neutrophil numbers which is comparable to other studies.\(^6\) Whereas stable asthma has no evidence of sputum neutrophilia; severe persistent / symptomatic cases have neutrophilic inflammation as in those with viral-induced exacerbations.\(^6\) Neutrophil products can cause airway narrowing, increased mucus secretion, and increased airway smooth-muscle responsiveness.\(^6\) Levels of transforming growth factor β (TGF-β) and matrix metalloproteinases-9 (MMPs) are higher in BAL fluid, sputum, and bronchial mucosal biopsies in patients with severe bronchial asthma and has been implicated in angiogenesis and smooth muscle hyperplasia which contributes towards airway remodelling and could explain poor response of neutrophilic patients to bronchodilators and corticosteroids.\(^20\)

The relationship between airway neutrophilia and airflow obstruction in asthma has been studied by Shaw and colleagues. They concluded that patients with predominant neutrophilic inflammation not only show poor response to bronchodilator therapy but are associated with low post-bronchodilator FEV\(_1\) and calculated that a 10-fold increase in neutrophil count is associated with a 92 ml reduction in post-bronchodilator FEV\(_1\).\(^{21}\) In a study by Maria et al. analysis of induced sputum samples of 37 atopic asthmatic patients taking inhaled corticosteroids (400 mg/day fluticasone) and inhaled bronchodilators concluded that 46% of patients had neutrophilic asthma with predominance in older age (mean age of 45 ± 18 years).\(^{22}\) Their results are comparable to this study (neutrophilic percentage of 43.10%) but in this study, patients with atopy were excluded because neutrophilic airway inflammation is also seen after an allergen challenge. Sputum neutrophilia was found in younger age group where mean age was 26.93 ± 7.29 years as upper limit of age in the inclusive criteria was set to be 40 years to rule out age related reduction in FEV\(_1\). Together, these studies show 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.

Recent work by Simpson and colleagues has further clarified the understanding of the neutrophilic phenotype of asthma.\(^{23}\) They assessed the inflammatory profile of 93 patients with asthma of varying severity and identified four distinct inflammation phenotypes. The largest group (41% of patients) had eosinophilic inflammation, 20% had sputum neutrophilia, 8% had increased levels of both eosinophils and neutrophils and 31% of patients were said to have 'paucigranulocytic asthma' with sputum cell count within the normal range. Their findings of neutrophilic phenotype of 20% are less than the present result of 43.10%. Higher incidence of neutrophilic phenotype in this study is probably due to inclusion of only patients with severe persistent asthma as compared to asthma patients of varying severity by Simpson et al.

The management of neutrophilic phenotype of asthma is increasingly being noted in severe / refractory asthma and therapies targeting it are also under investigation. Macrolide antibiotics, such as clarithromycin, have shown in vitro efficacy against IL-8 and neutrophils. In another study carried out by Simpson and colleagues,\(^{24}\) 45 patients with severe refractory asthma were randomized to receive clarithromycin (500 mg twice daily) for 8 weeks. Clarithromycin therapy significantly reduced airway concentration of IL-8, neutrophil numbers, neutrophil elastase and MMP-9 concentrations. These reductions in inflammation were most marked in those with refractory neutrophilic asthma. In a recent study by Wood et al.,\(^{25}\) the incidence of systemic inflammation was noted to be increased in neutrophilic asthma. They found that the proportion of subjects with elevated IL-6 and CRP levels was higher in the neutrophilic asthma group than both the non-neutrophilic asthma and the healthy control groups. Thus, control of systemic inflammation in neutrophilic asthma may provide a useful therapeutic target.

**CONCLUSION**

Sputum neutrophilia is a frequent finding in cases with severe persistent bronchial asthma. Thus, sputum neutrophilia can be used to identify predominant neutrophilic phenotype of severe persistent bronchial asthma. This recognition will lessen the inappropriate use of corticosteroids with their associated long-term side effects.

**REFERENCES**


