

## ORIGINAL ARTICLE

# ASTHMA INFLAMMATORY SUBTYPE SPECIFIC TREATMENT; A RANDOMIZED CLINICAL STUDY

By

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**Introduction:** *Macrolides antibiotics, such as clarithromycine express immunomodulatory and tissue reparative effects that are distinct from their anti-infective properties, and have in vitro efficacy against neutrophils.*

**Aim of study:** *To determine the efficacy of add-on therapies that target eosinophilic and noneosinophilic airway inflammation and their effects on asthma control test, pulmonary function and asthma symptoms.*

**Methods:** *single blind randomized clinical trial; asthmatic children with persistent symptoms undergoing treatment with fluticasone 100 mg bid and  $\beta_2$  agonist as required were studied. Group A (23 males/17 females, aged  $11.5 \pm 1.8$  years) received fluticasone 200mg bid, and group B (21males/19females, aged  $11.5 \pm 1.8$  yrs) clarithromycine 15mg/kg bid, in addition to fluticasone 100 mg bid for 8 weeks. (FEV1%, C-CAT, SABA use, sputum induced % of eosinophils and neutrophils) were compared before and after treatment in each group.*

**Results:** *In group A there is significant reduction of eosinophils percentage after treatment, and non significant increase in neutrophils percentage. There was significant improvement in FEV1% predicted. While in group B there was non significant decrease in eosinophils, and significant decrease in neutrophils. In group A there was significant negative correlation between changes in FEV1% and change in eosinophils and week positive correlation between changes in FEV1% and changes in neutrophils. In group B there was significant positive correlation between basal eosinophils and change in FEV1% and significant negative correlation between basal neutrophils and change in FEV1%.*

**Conclusion:** *Steroids were effective in targeting eosinophilic inflammation and clarithromycine target neutrophilic inflammation. High eosinophils and neutrophils percentage in sputum are best predictors of response to steroids or clarithromycine treatment respectively.*

**Keywords:** *Asthma, eosinophil, neutrophils, inhaled steroid, clarithromycine, children.*

## INTRODUCTION

Asthma is characterized by variable airflow limitation, which is validated by spirometry or measurements of airway responsiveness and is treated by bronchodilators.<sup>(1)</sup> It is also associated with airway inflammation, which is traditionally considered to be eosinophilic and is treated

by avoidance of any causes<sup>(2)</sup> and by anti-inflammatory medications of which corticosteroids are the most effective.<sup>(3)</sup>

Spontaneous or induced sputum cell counts is a noninvasive test or relatively non invasive and has

excellent reliability, validity and responsiveness.<sup>(4)</sup>

This phenotype is common, accounting for 25 to 55% of patients with corticosteroid-naive asthma, and is repeatable.<sup>(2)</sup> This asthma phenotype is not reserved to patients with severe asthma, nor is it a consequence of asthma therapy, but it is present across the range of asthma severity.<sup>(5)</sup>

Such phenotyping has therapeutic implications as patients with noneosinophilic inflammation respond poorly, if at all, to treatment with inhaled corticosteroids.<sup>(6)</sup> Airway neutrophilia in patients with asthma is likely to be multifactorial, and is dependent on a complex interplay of lipid mediators and chemokines, from both resident airway cells and inflammatory cells in addition to enhanced adhesion molecule expression. Interleukin (IL)-8, and is a potent neutrophil activator and chemoattractants.<sup>(7)</sup>

The non-ribosomal effects of macrolides include Immunomodulation, decreasing bacterial virulence and biofilm formation and decreasing mucus hyper secretion. These effects are unrelated to antimicrobial effects, take several weeks to manifest.<sup>(8)</sup>

Macrolides appear to modulate inflammatory activity in airway epithelial cells by inhibiting NF- $\kappa$ B activation that leads to IL-8 production and enhanced neutrophil accumulation. Additionally, *in vitro* studies have suggested that macrolides inhibit the expression of ICAM, thereby also modulating the recruitment of neutrophils to inflamed sites.<sup>(9)</sup> The extraribosomal effects of macrolides reduce the number of neutrophils in the BAL fluid from patients with neutrophilic, inflammatory airway diseases. Newer macrolides have rare side effects if any and are less likely to interact with the cytochrome P-450 and thus don't affect steroid and theophylline metabolism.<sup>(8)</sup>

**Aim of the study:** The purpose of our study is to introduce add-on therapies that target eosinophilic and noneosinophilic airway inflammation in uncontrolled asthmatics and the first outcome is the effect of specific treatment on inflammatory cells and the second outcome is changes in clinical parameters in response to treatment.

## PATIENTS AND METHOD

The present study was designed as a randomized single blind clinical trial, in total, 90 nonsmoking asthmatic children aged 9-14 years were selected for this study. The asthmatic children were recruited from our outpatient clinic.

Inclusion criteria were: 1) doctor-diagnosed asthma for at least 1 year, with a history of at least two of the following:

recurrent wheeze, chest tightness, shortness of breath, and/or cough; and 2) mild or moderate persistent asthma requiring, for at least 6 months, daily treatment with ICS in the form of 100ug fluticasone twice daily [10].

Exclusion criteria were: 1) clinical evidence of a respiratory infection in the 4 weeks prior to the study; and 2) co morbidities such as severe mental retardation, congenital anomalies of the respiratory tract, or congenital cardiac defects. 3) If they had received any systemic corticosteroids during the previous 4 weeks, or had a recent (past 4 weeks) asthma exacerbation,

All parents gave written informed consent. The study was approved by the Medical Ethics Committee of the Zagazig University.

Each patient was randomized to one of the study groups (A-B) by a research nurse who played no further role in the study

Recruitment was discontinued when 90 patients were randomized (45 per arm), and investigators were blinded with regard to the type of treatment received.

Patients in group A of the study received inhaled fluticasone 200ug twice daily, In group B, in addition to inhaled fluticasone 100ug twice daily received clarithromycin 15mg/kg bid for 8 weeks and All patients were receiving inhaled, short-acting,  $\beta$  2-agonist rescue medication on an as-needed basis for the relief of asthma symptoms

Six of them were excluded due to failure to trace them, and four were excluded due to asthma exacerbation during treatment so statistical analysis was done on 80 patients.

**Lung Function Tests:** Bronchodilator medication was stopped prior to lung function testing; short-acting bronchodilators at least 8 hr before the test. Dynamic spirometry was performed by means of a pneumotachograph (Masterlab Jaeger, Wu'rzberg, Germany), with measurement of forced expiratory volume in 1 sec (FEV1)  $\bar{n}$  predicted, according to the standards of the European Respirator Society. The highest values of FEV1 of three forced expiratory maneuvers were used for data analysis.

**Sputum Induction and Processing:** Sputum was collected either spontaneously or induced with hypertonic saline nebulization from all subjects. Prior to sputum induction, children inhaled 200  $\mu$ g of salbutamol to minimize broncho-constriction during the induction procedure.

Sputum was induced by inhalation of 3% hypertonic saline solution for 5 min (DeVilbiss 65 ultrasonic

nebulizer; DeVilbiss, Somerset, PA, USA), and the subjects were encouraged to cough and expectorate sputum into sterile containers. FEV1 was measured after nebulization. Nebulization was stopped if a fall in FEV1 of >20% compared to baseline values occurred or if troublesome symptoms appeared.<sup>(11)</sup>

The volume of the selected sputum is measured and 0.1% dithiothreitol (Sigma Chemicals, Poole, United Kingdom) added to the sputum in a 4:1 ratio to break up the disulphide bonds and disperse the cells. The cell suspension is aspirated until homogenized and filtered to remove any remaining debris. Phosphate-buffered saline is then added to the cell suspension. The non-squamous cell count and cell viability (with trypan blue) are determined in a haemocytometer. The cell suspension is centrifuged at 400 g for 10 minutes and cytopins made and stained by May-Griunwald Giemsa stain (Bio Optica Milano s.p.a). Four hundred non-squamous cells are counted: an adequate sample is defined as less than 50% squamous cells. The eosinophils and neutrophils count are then expressed as a percentage of the total cell count as it

is more accurate than absolute count.<sup>(12)</sup>

**Childhood asthma control test (C-ACT):** The C-ACT is a seven-item child- and caregiver-completed tool with a scoring range of 0–27; higher scores indicate better control. A score of 19 or less indicates that the asthma may not be well controlled. The C-ACT is intended for use in children up to the age of 14 year.<sup>(13)</sup>

**Statistics:** Descriptive statistics are presented as mean-SD. In each of the treatment groups (A, B), neutrophilic percentage, eosinophilic percentage, FEV<sub>1</sub> predicted, SABA use and CACT score before and after treatment with the study medication were compared using a Wilcoxon signed-rank test (for non-normally distributed variables) or a two-tailed paired t-test (for normally distributed variables). correlation between Changes in neutrophilic and eosinophilic percentage and changes in FEV1 after treatment were done by spearman test.

## RESULTS

**Table 1. Demographic and other parameters of both studied groups before treatment.**

	Group A n=40 Mean ±SD Range	Group B n=40 Mean ±SD Range	P
Age (years)	11.5±1.8 (9-14)	11.4±1.6 (9-14)	>0.05†
Sex male/female	23/17	21/19	>0.05†
Duration of disease (months)	71±6 (60-83)	72±8 (60-83)	>0.05†
SABA	2.1±2 (0-7)	2.5±1.6 (0-7)	>0.05†
C-CAT	12.4±1.9 9-16	13.1±2.2 10-18	>0.005
FEV1% predicted	69.5±6.5 (60-80)	71.3±7.1 (60-80)	>0.05†
Sputum Eosinophile	4.6±4.6 (0-18)	5.3±4.8 (0-16)	>0.05†
Sputum Neutrophile	36±25 (12-88)	36.7±23.8 (12-87)	>0.05†

† Non significant.

**Table 2. Comparison between different parameters before and after treatment in group A and B.**

	Group A Before treatment	Group A After treatment	T	P	Group B Before treatment	Group B After treatment	T	P	P Between group A and group B
SABA Mean ±SD range	2.1±2 (0.0-7)	1.78 ±2.17 (0.0-7)	1.24	>0.05†	2.25±1.7 (0.0-7)	0.55±0.8 (0.0-3)	9.276	<0.001**	<0.001**
FEV1 Mean ±SD range	69.5±6.5 (60-80)	86.5±16.98 (60-110)	6.52	<0.001**	71.4±7.1 (60-83)	94.1±12 (70-110)	11.22	<0.001**	<0.001**
C- CAT	12.4±1.9 9-16	24.3±2.3 20-27	29.39	<0.001**	13.1±2.2 10-18	24.1±2.6 19-27	20.16	<0.001	>0.05†
Sputum Eosinophile Mean ±SD range	4.7±4.5 (0.0-18) 3	1.75±2.2 (0.0-9) 1	6.82	<0.001**	5.2±4.4 (0.0-16) 4	3.6±3.6 (0.0-11) 2.5	7.08	>0.05†	<0.001**
Sputum Neutrophile Mean ±SD range	40.8±27.4 (12-88) 29	52.87±28.2 (16-92) 50	8.44	>0.05†	42.5±27.5 (12-88) 28	18.3±16.7 (1-59) 12	8.26	<0.001**	<0.001**

\*\* Highly Significant.

† Non significant.

**Table 3. Correlation between change of FEV1 and cells in groups.**

	R	P	R	P
Change in FEV1 and basal sputum Eosinophile	0.43	<0.001**	-0.61	<0.001**
Change in FEV1 and change in sputum Eosinophile	-0.45	<0.001**	0.37	>0.05†
	<b>Group A</b>		<b>Group B</b>	
Change in FEV1 and basal sputum Neutrophile	-0.73	<0.001**	0.82	<0.001**
Change in FEV1 and change sputum Neutrophile	0.28	<0.001*	-0.67	<0.001**

\*\* Highly Significant.

† Non significant.

We studied 80 patients with asthma they were divided into group A received inhaled fluticasone 200ug twice daily, and group B, in addition to inhaled fluticasone 100ug twice daily received clarithromycine 15mg/kg bid for 8 weeks, our patients in group A were 23 males to 17 females, in group B were 21 males and 19 females, the mean age, duration of having asthma, frequency of using short acting  $\beta_2$  stimulant, basal FEV1% predicted were matched in both groups, also sputum Eosinophile and Neutrophile percentage were matched at base line of starting therapy.

After 8 weeks of therapy we found SABA usage was decreased in group A and B but it was of statistical

significance only in group B; this makes a significant improvement in group B over group A.

As regard FEV1% predicted there was significant improvement in both groups after 8 weeks of therapy, and there was significant better improvement in group B than group A.

Eosinophile in sputum was significantly decreases after therapy in group A, and it was decreased in group B but not to a significant level, so eosinophile in sputum was significantly lower in group A than group B.

Neutrophile in sputum showed non significant statistical

increase in group A, while it was significantly lower in group B after therapy. Thus Neutrophile was significantly lower in group B with clarithromycine therapy than group A with increasing steroid dose.

By studying the correlation between changes of FEV1% predicted after therapy in group A with different parameters we found; a significant positive correlation with basal sputum eosinophile and change in sputum

Neutrophils, and a significant negative correlation with change in sputum Eosinophile and basal neutrophile.

In group B correlation between changes in FEV1% and different parameters showed that; there was a positive correlation with change in basal Neutrophile, while there was a negative correlation with basal sputum Eosinophile and change in sputum Neutrophile, and there was no correlation with basal eosinophile.

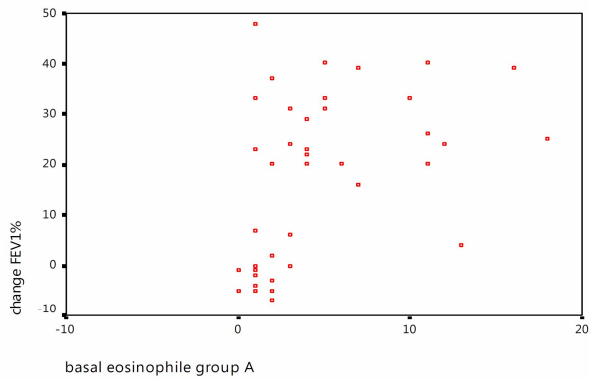


Figure 1: Correlation between change in FEV1% and basal eosinophile in group A.

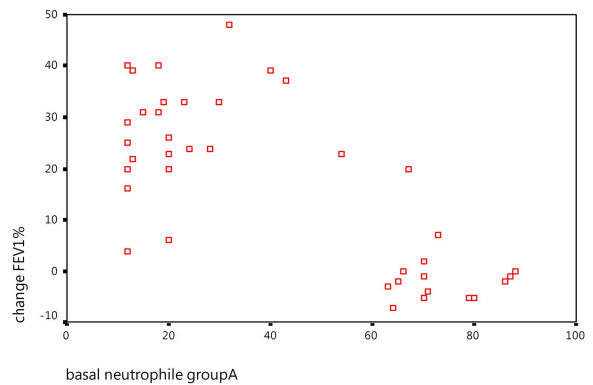


Figure 2: Correlation between FEV1% and basal neutrophile in group A.

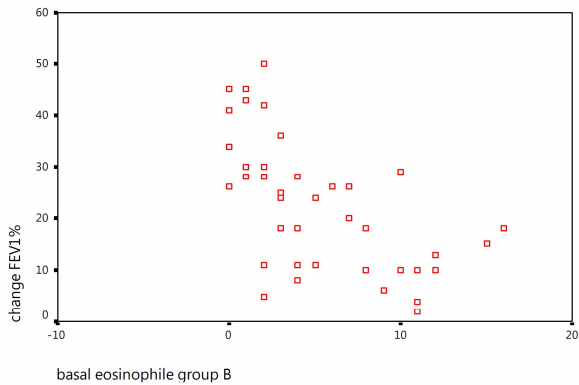


Figure 3: Correlation between FEV1% and basal eosinophile in group B.

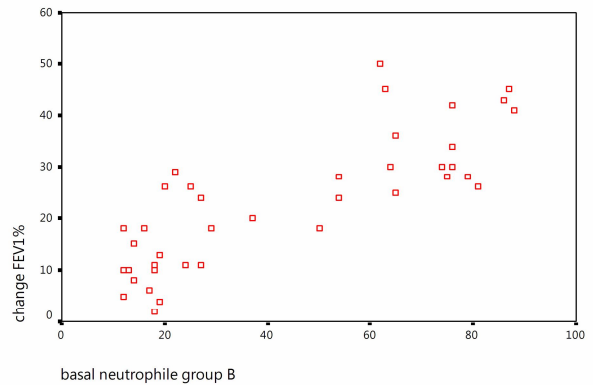
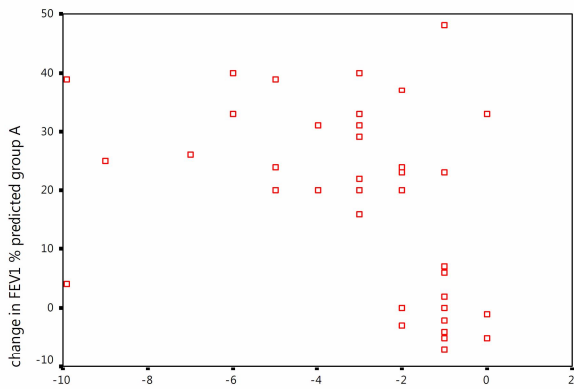
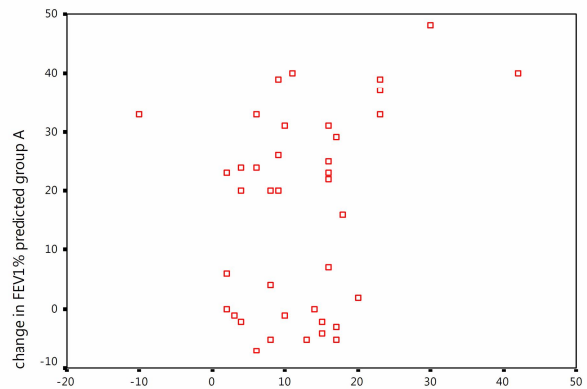


Figure 4: Correlation between FEV1% and basal neutrophile in group B.



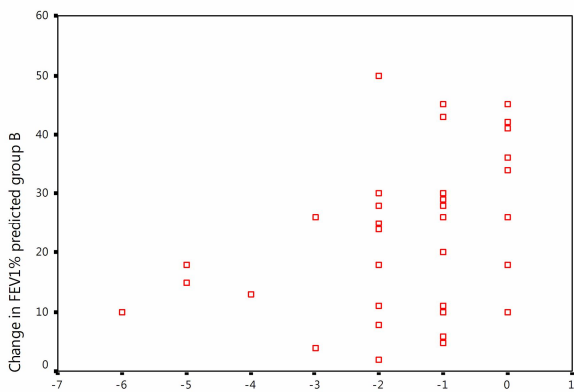
change in eosinophiles group A

Figure 5: Correlation between FEV1% and change in eosinophile in group A.



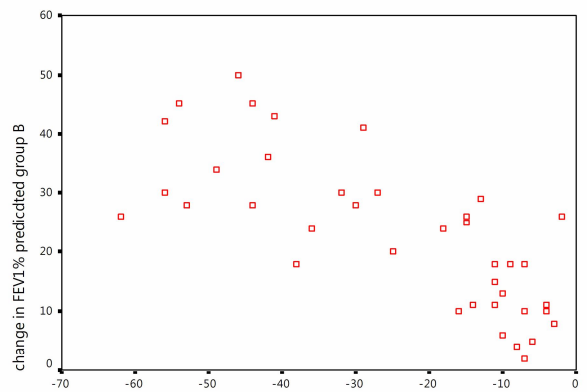
change in neutrophiles group A

Figure 6: Correlation between change in FEV1% and change in neutrophile in group A.



change in eosinophiles Group B

Figure 7: Correlation between change in FEV1% and change in eosinophiles in group B.



change in neutrophile group B

Figure 8: Correlation between change in FEV1% and change in neutrophiles in group B.

## DISCUSSION

The population at risk of asthma because of eosinophilic inflammation is about 50%, and conversely, this means that up to 50% of asthma cannot be attributed to eosinophilic inflammation and represents asthma associated with non-eosinophilic processes.<sup>(14)</sup>

Non-eosinophilic asthma represents a pathologically distinct disease phenotype which is characterized by the absence of airway eosinophilia, normal subepithelial layer thickness and a poor short-term response to treatment with inhaled corticosteroids.<sup>(15)</sup> Knowledge of inflammatory phenotype is useful because it relates to

treatment response, mechanistic pathways involved in disease pathogenesis and future disease risk. Because macrolides modulate the neutrophils activity, they may have a potential role in the treatment of patients with noneosinophilic asthma.<sup>(16)</sup>

In our single blind randomized clinical trial, in both groups A and B, higher percentages of either neutrophils or eosinophils in induced sputum at baseline were associated with lower FEV1, increase in use of rescue medication and increase in asthma symptom score. This is in favor of asthma recognition as a heterogeneous disease with different pattern of airway inflammation. The severity of disease is related to degree of airway inflammation, as both eosinophilic and neutrophilic types,

independently contribute to abnormalities of FEV1 in asthma.<sup>(12)</sup>

An important finding in our study was that, children with high sputum eosinophils at baseline, in group A (treated with increasing steroid dose) responded best to treatment with inhaled fluticasone. As there is positive correlation between improvement in FEV1% and eosinophilic percentage in sputum at baseline. This is in agreement with Brightling who founded strong evidence that sputum eosinophilia (>3%) is a predictor of clinical improvement with corticosteroid treatment,<sup>(17)</sup> and. Bacci et al. who investigated adults, before and after 2 and 4 weeks of treatment with beclomethasone 500mg twice daily who founded that Sputum eosinophilia was associated with improvement in symptoms, peak expiratory flow and methacholine airway responsiveness.<sup>(18)</sup> These studies and our study supported that sputum eosinophilia is the best predictor of steroid response in asthmatics and support the use of sputum cell counts to guide steroid treatment.

Eight weeks of stepping up inhaled fluticasone treatment in group A decreased eosinophils percentage in sputum with significant improvement in clinical status. There is significant improvement in FEV1% predicted, asthma symptom score and decrease in use of rescue medication. The change in FEV1 was correlated with change reduction in eosinophilic percentage in sputum, this was previously found by Kim, et al.<sup>(19)</sup> There was slight none statistically significant increase in neutrophils percentage in sputum and no correlation between changes in neutrophils percentage and changes in FEV1%. The increase in neutrophils percentage may be due to the effect of steroids in delaying neutrophils apoptosis.

Thus in our study the improvement in clinical status in group A patients was owing to the effect of the increased dose of steroids in controlling eosinophilic inflammation. Zacharasiewicz et al. in a study of children with stable asthma, in whom the Inhaled corticosteroids (ICS) dose was halved at intervals of 8 weeks, observed that steroid reduction was successful in all children with no eosinophils in induced sputum before the attempted reduction.<sup>(20)</sup> Our results are in agreement with Kelly 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 valuable only in asthmatic patients with eosinophilic inflammatory subtype and even may be detrimental in neutrophilic inflammation as proved by Fahy.<sup>(21)</sup>

Because sputum neutrophilia was not decreased in subjects using inhaled corticosteroids (in group A), other anti-inflammatory therapies directed specifically at control

of neutrophilic inflammation might be useful in improving airway caliber and symptoms.

In our study in clarithromycin treated patient (group B), the eosinophilic percentage in induced sputum decreased slightly with no statistical significance and there was no significant correlation between reduction in eosinophils and improvement in clinical status. The slight decrease in eosinophilic percentage may be explained by the decrease in neutrophilic inflammatory mediators which activate eosinophils. These data are in concordance with Liu et al who demonstrated the effect of neutrophils mediators on eosinophilic activation.<sup>(22)</sup> There was highly significant decrease in neutrophilic percentage in sputum and this may be due to increased (phagocytic capacity of alveolar macrophages,<sup>(23)</sup> for apoptotic neutrophils,<sup>(24)</sup> or acceleration of neutrophils apoptosis due to increased cyclic adenosine monophosphate (AMP) levels in neutrophils treated by macrolides. There is significant increase in FEV1% predicted and significant decrease in asthma symptom score and use of rescue medication after macrolides therapy. Hence in our study the improvement in air flow and clinical status is due to resolution of neutrophilic inflammation, and this is supported by the correlation between change reduction in neutrophils percentage and changes in FEV1%.

Other studies supported the usage of macrolides in controlling asthma as Garey and his colleagues who conducted a placebo-controlled trial of CAM 500 mg twice daily in 21 adults with steroid-dependent asthma. Over the course of 6 weeks subjects on CAM had better pulmonary function and fewer symptoms,<sup>(25)</sup> and Kostadima et al in a randomized double-blind placebo-controlled study, CAM 750 mg/day for 8 weeks increased the provocative dose of methacholine.<sup>(26)</sup>

In our study the improvement in FEV1% is positively correlated with neutrophils percentage at baseline. Patients with high neutrophilic percentage at baseline had the best improvement; this also agreed with Shaw et al<sup>(27)</sup> and Little et al.<sup>(28)</sup>

We suggest that as high eosinophils percentage is a predictor of response to steroid, high neutrophils percentage may be a predictor of response to clarithromycine.

This is supported by a study carried by Jodie and his associates who studied the effect of clarithromycine on neutrophils and demonstrated that the reduction in inflammation and improvement in quality of life scores were most marked in those with refractory noneosinophilic asthma.<sup>(29)</sup>

Kobayashi et al also founded that Neutrophils migrated by IL-8 may lead eosinophils to accumulate in the airways of patients with severe asthma. On the other hand, it is unlikely that eosinophils migrated by chemoattractants such as CC chemokines regulate neutrophilic inflammation.<sup>(30)</sup>

From these data we can conclude that clarithromycine can be the drug of choice in addition to steroid in mixed inflammatory asthma subtype (where both eosinophils and neutrophils percentages are elevated). Further studies are needed to evaluate this possibility. However; limitations of our study were as it is single blind study, it is uncontrolled study; as no cases managed by placebo and we didn't classify study population from the start for specific inflammatory subtype.

## CONCLUSION

This study demonstrates that asthma care can be improved when indicators of airway inflammation are targeted for treatment along with the usual goals of therapy. Steroids were effective in targeting eosinophilic inflammation and clarithromycine target neutrophilic inflammation. High eosinophils and neutrophils percentage in sputum are best predictors of response to steroids or clarithromycine treatment respectively.

Measuring airway inflammation by quantitative sputum cell counts gives the most comprehensive information and is of most clinical value in initiating early effective treatment in order to avoid irreversible airway remodeling.

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