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EVALUATION OF THE ROLE OF VITAMIN C IN CHRONIC BRONCHIAL ASTHMA

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Keywords:

Vitamin C,chronic bronchial asthma, oxidative stress, serum malondialdehyde, lung function tests

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ABSTRACT

Objective: To determine the pathophysiological role of oxidative stress and the usefulness of antioxidant therapy using Vitamin C in patients with chronic bronchial asthma.

Methods: An open randomized, prospective study was performed to study the beneficial effect of vitamin c as add on therapy to the standard drug therapy in chronic bronchial asthma patients. After clinical examination, 80 patients who fulfilled the inclusion criteria were randomly divided into 2 groups of 40 each. The control group received standard drugs for asthma (Tab. Salbutamol 4mg bid and Tab. Aminophylline 100mg tid) while the study group received Tab.Vitamin C 500mg bid in addition to standard drugs. Patients were assessed once in two weeks for a period of 8 weeks. All the patients were evaluated using ACQ score (Juniper asthma control questionnaire) for asthma control, Lung function tests (PEFR & FEV1) for improvement in lung function, Serum Malondialdehyde (a marker of oxidative stress), and Serum C-reactive protein(a marker for inflammation). Other hematological and biochemical parameters were measured before and after the study.

Results: It was observed that the study group showed improved clinical response and decreased disease activity to a greater extent than control group as evidenced from ACQ score and Lung function tests ($p< 0.05$). Mean serum MDA which was higher initially in both the groups showed statistically significant decline in the study group ($p = .001$) while it was increased in control group ($p =0.41$).

Conclusion: Vitamin C as add on therapy showed better asthma control, reduced complications and improved quality of life.

INTRODUCTION

Asthma is one of the major public health problems affecting 5% of the world population[1]. It is a universal disease affecting people of all ages resulting in variable restriction to the physical, emotional and social aspects of an individual's life. According to GINA (Global Initiative for Asthma), asthma is defined as "a chronic inflammatory disorder of the airways characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli"[2]. The major symptoms of asthma are paroxysms of dyspnea, wheeze and cough, which may be mild and almost undetectable to severe and unremitting. Asthma is a highly complex inflammatory disorder with many potential therapeutic approaches.Treatment with a combination of drugs which contain a corticosteroid and a long acting β2 adrenoreceptor agonist is the most effective therapy. Despite major advances in therapy, patient's symptoms are not adequately controlled.Recent evidence on the role of oxidative stress and inflammatory mediators has fostered considerable interest in new approaches for the treatment of bronchial asthma. Epidemiological and observational studies suggest that increased oxidative stress or defective antioxidant status may be associated with an increased risk of asthma or faster disease progression.Oxidative stress is caused by a large variety of free oxygen radicals known as the Reactive Oxygen Species. The generation of oxygen free radicals by activated airway inflammatory cells produces many of the pathophysiological changes associated with asthma[4] such as airway smooth muscle contraction[5], airway hyperresponsiveness[6,7], mucus hypersecretion[8,9], epithelial shedding[10] and vascular exudation[11,12] .Recent studies have also suggested that consuming antioxidants such as Vitamin C, Vitamin E, β carotene, flavonoids, selenium and other nutrients reduces the risk of bronchoconstriction associated with asthma.This suggests that antioxidants have a significant role in decreasing the incidence and severity of asthma[13]. Ascorbic acid or Vitamin C as it is commonly called is the major antioxidant present in the airway surface of the lungs suggesting a protective role of this vitamin against oxidative stress.Studies have shown that Vitamin C intake in the general population correlates negatively with asthma.Patients with asthma may have low supplies of Vitamin C or increased demand for Vitamin C in the face of an oxidant load resulting in depletion of this vitamin.With increasing evidence on the role of oxidative stress in the pathogenesis and severity of asthma, it is imperative to study whether supplementation with antioxidant Vitamin C could offer benefit in the prevention and management of asthma.

OBJECTIVE

To study the beneficial effects of Vitamin C as an add on therapy to the standard drug therapy in patients with chronic bronchial asthma using

1. Asthma Control Questionnaire (ACQ) score for asthma control
2. Lung Function Tests (a) PEFR (b) FEV1
3. Serum Malondialdehyde (MDA), a marker of oxidative stress
4. Serum C - reactive protein, a marker of inflammation.

METHODOLOGY

STUDY DESIGN: Phase III prospective, open, two arm, parallel group, out patient randomized, active controlled study.

STUDY CENTRE: Asthma clinic, Department of Internal Medicine, Stanley Medical College Hospital, Chennai.

STUDY PERIOD: January 2008 to December 2008

STUDY DURATION: Active drug therapy-2 months

Follow up-1 month

STUDY POPULATION: Patients attending the Outpatient Asthma clinic, Department of Internal Medicine, Stanley Medical College Hospital, Chennai.

SAMPLE SIZE:

80 patients

40 patients in each group

STUDY PROCEDURE

The study was started after obtaining the approval and clearance from the Institutional ethics committee.

INCLUSION CRITERIA

» Age group 18 – 60 years

» Both sexes

» Patients with bronchial asthma

» Duration of disease >5 years

EXCLUSION CRITERIA

» Age group < 18 years and > 60 years

» Patients with COPD, TB and Cardiac disease

» Smokers

» Pregnant and lactating women

ENROLLMENT VISIT

Patients who attended the Outpatient Department of Asthma clinic, Stanley Medical College Hospital were explained in detail about the study procedure, purpose and its benefits. Written informed consent was obtained from the patients willing to participate in the study, in the prescribed format in the regional language prior to the commencement of the study procedure. If the patient was illiterate, the left thumb impression was sought. This was done in the presence of an impartial witness. Patients were advised to come the next day at 8.00 AM for the screening procedure.

SCREENING

Patients who had given the written informed consent for participation in the study were screened by detailed medical history, objective measurement of lung function and physical and systemic examination. Baseline demographic characteristics were recorded. Blood was drawn for determining the haematological and biochemical parameters.

RECRUITMENT

80 patients who fulfilled the inclusion were recruited for the study.5 more patients, than the required sample size were recruited to compensate for the dropouts.

RANDOMISATION

Among the 85 patients, all the odd number patients were given Vitamin C in addition to the regular medications (study group) and even number patients were given only the regular medications (control group)..

DOSAGE AND ADMINISTRATION

Control group: Tab. Salbutamol 4mg b.i.d. + Tab. Aminophylline 100mg t.i.d. for 8 weeks.

Study group: Tab.Vitamin C 500mg b.i.d. + Tab.Salbutamol 4mg b.i.d. + Tab.Aminophylline 100mg t.i.d. for a period of 8 weeks.

STUDY VISITS

Patients were assessed once in two weeks for a period of 8 weeks for the following outcomes.

General medical history and physical examination, Asthma Control Questionnaire (ACQ), Lung function tests-PEFR, FEV1, Serum Malondialdehyde, Serum C – reactive protein and other routine haematological and biochemical parameters.

EVALUATION

I. ASTHMA CONTROL SCORE

Asthma control was assessed using the Juniper asthma control questionnaire (ACQ)[3] on each visit. For the standard clinical version of the ACQ score, there are seven questions, each scored on a seven point scale (0 = good control, 6 = poor control).The overall score is the mean of the seven responses which include the following.

1. Nocturnal symptoms
2. Severity of asthma attacks
3. Limitation of activities
4. Shortness of breath
5. Frequency of asthma attack
6. Short-acting bronchodilator use
7. Forced expiratory volume in one second (FEV1) / Peak expiratory flow rate (PEFR)

II. LAB INVESTIGATIONS

Haemoglobin, Total count and differential count, Bleeding time and clotting time, Random blood sugar, Blood urea, Serum creatinine, Urine analysis, Serum C - reactive protein and Serum Malondialdehyde

A. ESTIMATION OF SERUM MALONDIALDEHYDE BY DRAPER AND HADLEY METHOD

Reagents required: Phosphotungstic acid (PTA) 10%, Thiobarbituric acid (TBA) 0.67%, n-Butanol, Sulphuric acid 4M, MDA Standard (tetra methoxy propane in distilled water)

Procedure: 0.1 ml of serum was mixed with 0.5 ml of sulphuric acid, 0.4 ml PTA and 1 ml of distilled water. The tube was centrifuged for 10 minutes at room temperature. The supernatant was aspirated and the remaining pellet was mixed with 1 ml sulphuric acid and 0.15 ml PTA. This was centrifuged for 10 minutes, supernatant was discarded and the pellet was resuspended in 2 ml of water.0.5 ml TBA was added and the contents heated in a boiling water bath for 60 minutes. The tubes were cooled and 2.5 ml butanol was added. The tubes were centrifuged, the supernatant was added to the cuvette and the absorbance was measured at 533 nm.A standard calibration curve was prepared by taking various concentrations of MDA standard, treated similarly with TBA.The values are expressed in nM/mL.Normal value of serum MDA is 12-15 nM/mL.

B. MEASUREMENT OF PEFR

The severity of bronchial asthma depends on the degree of bronchial obstruction and bronchospasm and is measured as Peak expiratory flow rate (PEFR).Thus PEFR by determining the degree of obstruction of airways aids in the diagnosis, assessing the severity and also monitoring the response to therapy. Peak expiratory flow meter (pulmopeak) measures the peak expiratory flow rate (PEFR) which is a valuable indicator of lung function and meets the new technical standards established by the National Asthma Education Programme.

C. SPIROMETRY

Spirometry testing was conducted with MIR SPIROLAB II spirometer with an attached microprocessor. All testing was performed at the Department of Physiology, Stanley Medical College, using the spirometry standards of the American Thoracic Society.

PROCEDURE

- Before performing the test, spirometer calibration was checked.
- The procedure was explained in detail to the patients.
- Height and weight of the patients were noted
- Patients were instructed to assume the correct posture with the head slightly elevated
- Patients were instructed to inhale rapidly and completely, closing their lips around the mouthpiece forming a tight seal.
- Patients were then asked to exhale maximally until no air can be expelled while maintaining an upright posture.
- This maneuver was done thrice and tested for repeatability.

COMPLIANCE

Compliance was recorded by 1. Daily drug reminder chart 2. Examining the number of unutilized capsules in each medication pack

FOLLOW UP

At the end of two months of active drug therapy, both the control and study groups were followed up for a further period of one month. The results were subjected to statistical analysis.

RESULTS

- The data obtained at the end of this study was analyzed using SPSS software.
- The following tests were used,

Student independent 't' test, Student paired 't' test, Chi square test ,Oneway ANOVA F-test, Repeated measures of ANOVA test.

- P value ≤ 0.05 was considered significant

ASTHMA CONTROL QUESTIONNAIRE SCORE (ACQ)

Table 1 :

	ACS (Baseline)		ACS (2 weeks)		ACS (4 weeks)		ACS (6 weeks)		ACS (8 weeks)		ACS (12 weeks)		Oneway ANOVA F-test	Repeated Measures of ANOVA
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Control	21.30	5.18	21.08	5.26	20.90	5.38	20.40	5.26	19.83	5.08	19.70	5.38	t=1.66 P=0.20	Between groups F=3.06P=0.05*
Study	20.90	6.13	20.58	6.26	19.50	6.40	18.02	6.35	16.68	6.42	17.00	6.49	t=15.92. P=0.001*	Within group F=169.17* P=0.001
Student independent t-test	t=0.31 P=0.75		t=0.38 P=0.70		t=1.06 P=0.29		t=2.01* P=0.05		t=2.43* P=0.02		t=2.03* P=0.05			

*Significant

There was no statistically significant difference among the groups at the baseline, and at the end of the 2nd and 4th week, while there was a statistically significant difference in the study group when compared with the control group at the end of the 6th, 8th and 12th week.

Figure 1 :

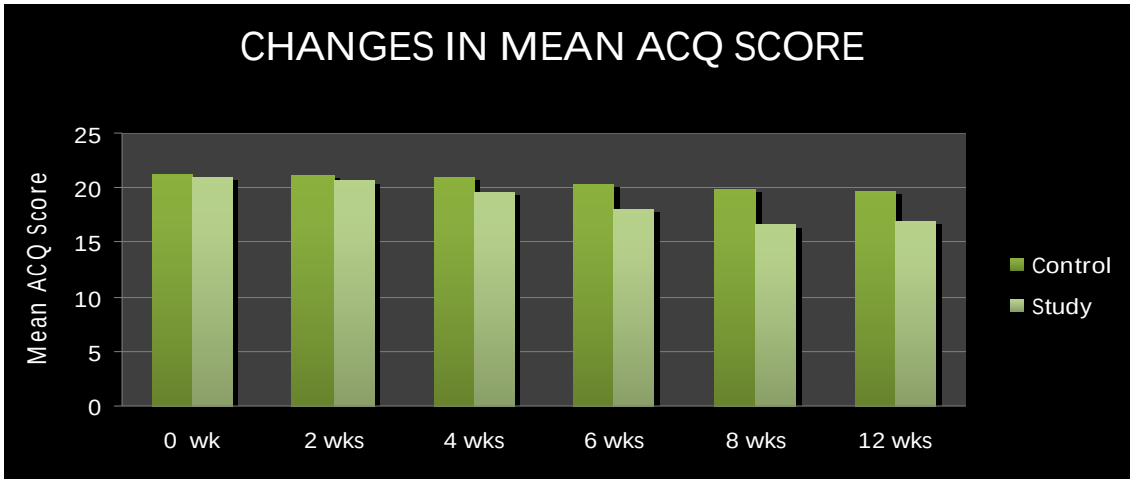
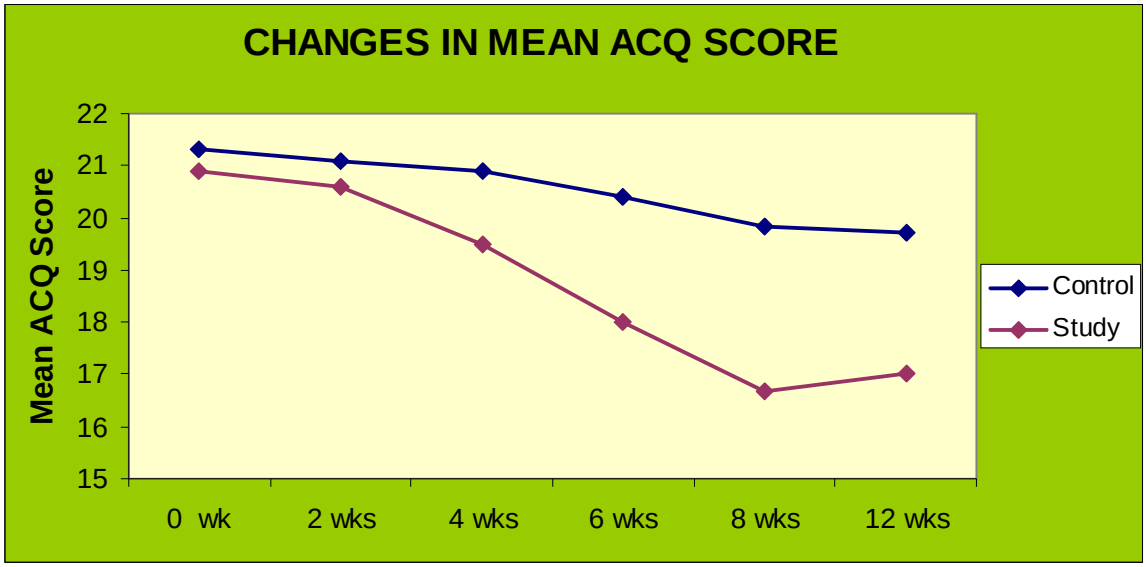


Figure 2 :



Figures 1 and 2 show the diagrammatic representation of the Asthma Control Questionnaire score in the study and the control groups at the baseline and at the end of the 2nd, 4th, 6th, 8th and 12th week.

PEAK EXPIRATORY FLOW RATE

Table 2:

	PEFR (Baseline)		PEFR (2 weeks)		PEFR (4 weeks)		PEFR (6 weeks)		PEFR (8 weeks)		PEFR (12 weeks)		Oneway ANOVA F-test	Repeated Measures of ANOVA
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Control	278.00	77.96	283.25	77.74	288.00	76.60	293.00	76.60	298.25	75.78	296.50	79.28	t=0.82 P=0.36	Between groups F=4.08P=0.05*
Study	279.50	84.52	295.75	84.00	305.00	87.47	330.00	80.19	334.50	84.55	325.00	93.42	t=13.46. P=0.001*	Within group F=17.34* P=0.01
Student independent t-test	t=1.54 P=0.13		t=0.90 P=0.36		t=0.30 P=0.76		t=2.11* P=0.04		t=2.86* P=0.01		t=1.47 P=0.14			

*Significant

There was no statistically significant difference among the groups at the baseline, and at the end of the 2nd and 4th week, while there was a statistically significant difference in the study group when compared with the control group at the end of the 6th, 8th and 12th week.

Figure 3 :

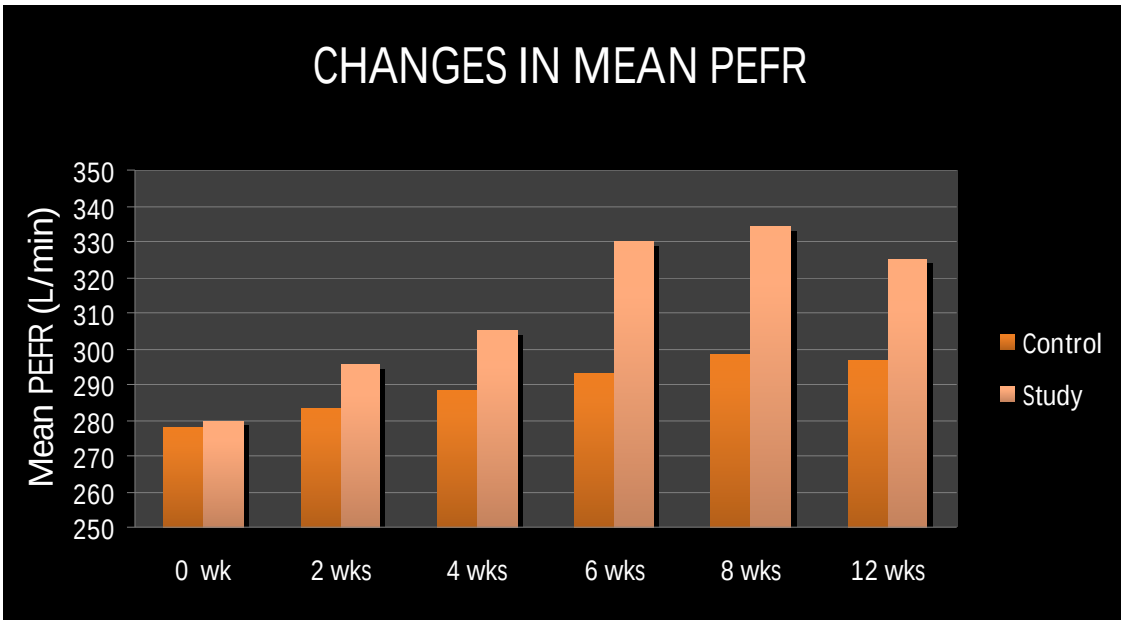
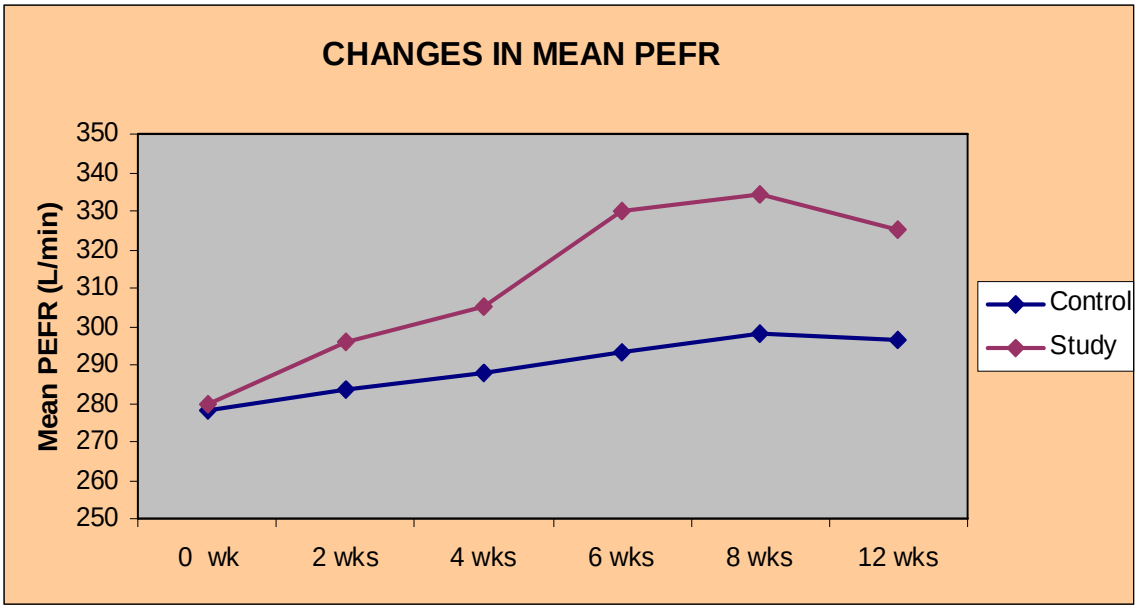


Figure 4:



Figures 3 and 4 show the diagrammatic representation of Peak expiratory flow rate at the baseline and at the end of the 2nd, 4th, 6th, 8th and 12th week among the study and the control groups.

FORCED EXPIRATORY VOLUME IN ONE SECOND (FEV_1)

Table 3:

	FEV_1 (Baseline)		FEV_1 (4 weeks)		FEV_1 (8 weeks)		FEV_1 (12 weeks)		Oneway ANOVA F- test	Repeated Measures of ANOVA
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Control	67.5	14.67	68.55	14.44	70.13	14.25	69.95	14.47	F=1.17 P=0.56	Between groups F=6.02 P=0.01*
Study	68.43	15.96	72.53	15.47	77.88	14.47	76.25	14.38	F=14.56 P=0.001*	Within group F=22.27* P=0.001
Student Independent t-test	t=1.05 P=0.74		t=1.18 P=0.23		t=2.22 P=0.02*		t=1.96 P=0.05*			

*Significant

There was no statistically significant difference among the groups at the baseline and at the end of the 4th week, while there was a statistically significant difference in the study group when compared with the control group at the end of the 8th and 12th week.

Figure 5 :

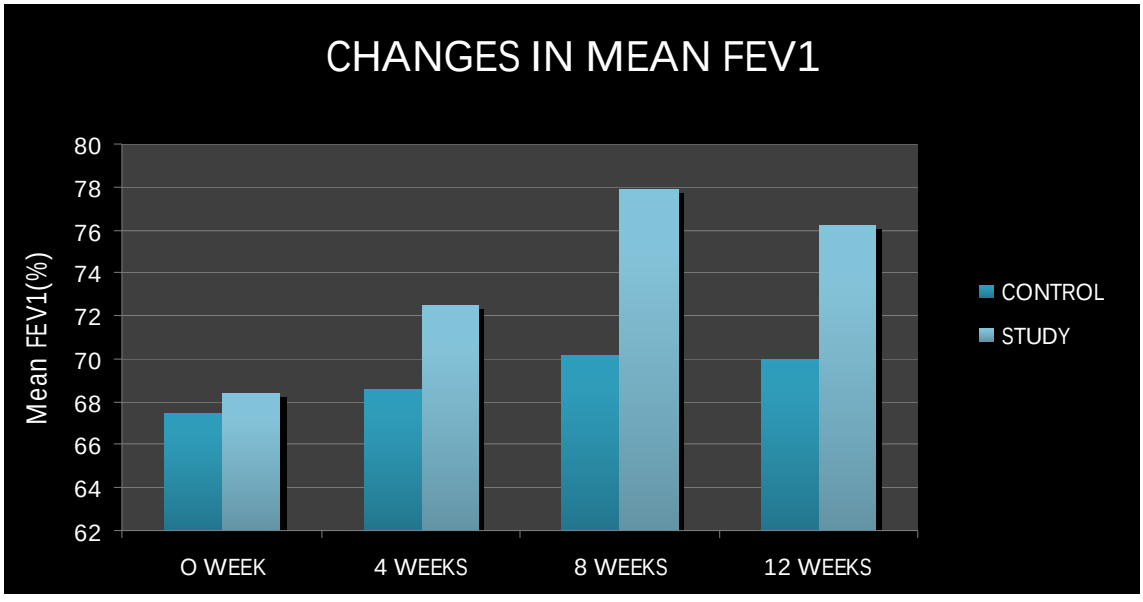
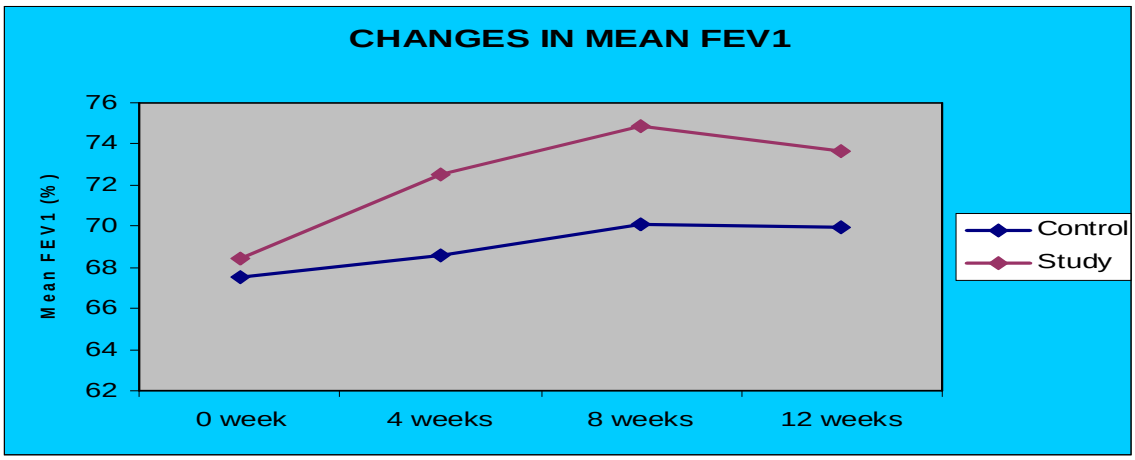


Figure 6 :



Figures 5 and 6 show the diagrammatic representation of the changes in the mean FEV_1 of the study and the control groups at the baseline and at the end of the 4^{th}, 8^{th} and 12^{th} week.

Changes in mean serum Malondialdehyde (MDA) level

Table 4:

Group		Mean	Std. Deviation	Student paired t-test
Control	MDA 1 (Baseline)	23.18	4.242	t=0.84
	MDA 2 (8 Weeks)	23.38	3.946	P=0.41
Study	MDA 1 (Baseline)	24.70	6.981	t=4.46
	MDA 2 (8 Weeks)	23.13	5.923	P=0.001*

There was a statistically significant decrease in the mean serum MDA level in the study group when compared to the control group.

Figure 7 :

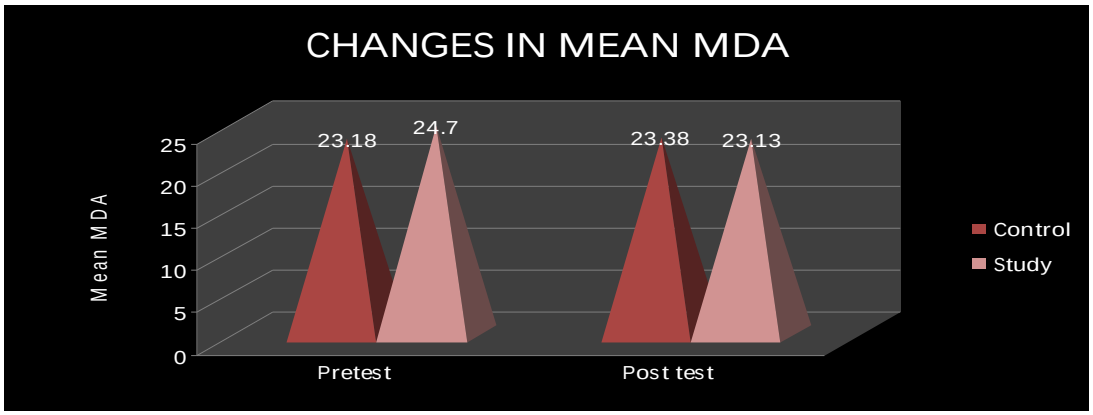
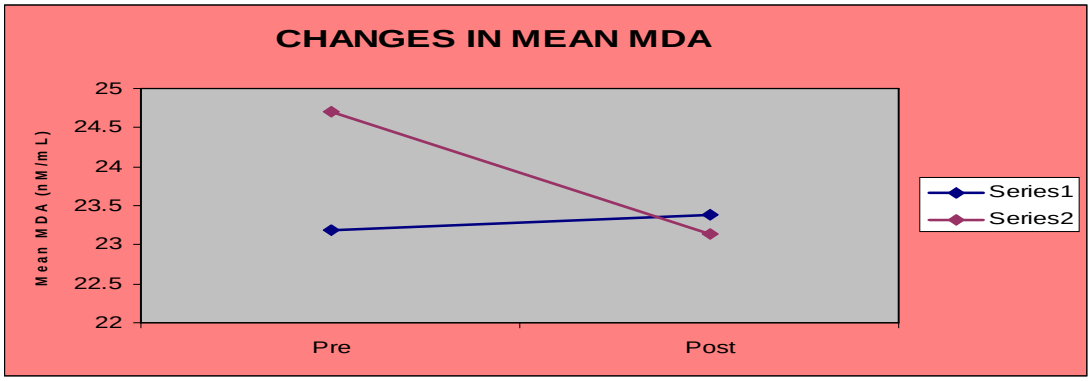


Figure 8 :



Series1-control, series 2-study

Figures 7 and 18 show the diagrammatic representation of the changes in the mean serum MDA level in the study and the control groups.

Percentage of reduction/increase in ACS, PEFR and FEV_1

Table 5 :

S.No	Parameters	% of reduction /increase in scores	
		Control Group	Study Group
1	ACS	- 7.5%	- 20.2%
2	PEFR	7.59%	19.68%
3	FEV_1	3.59%	13.81%

Figure 9:

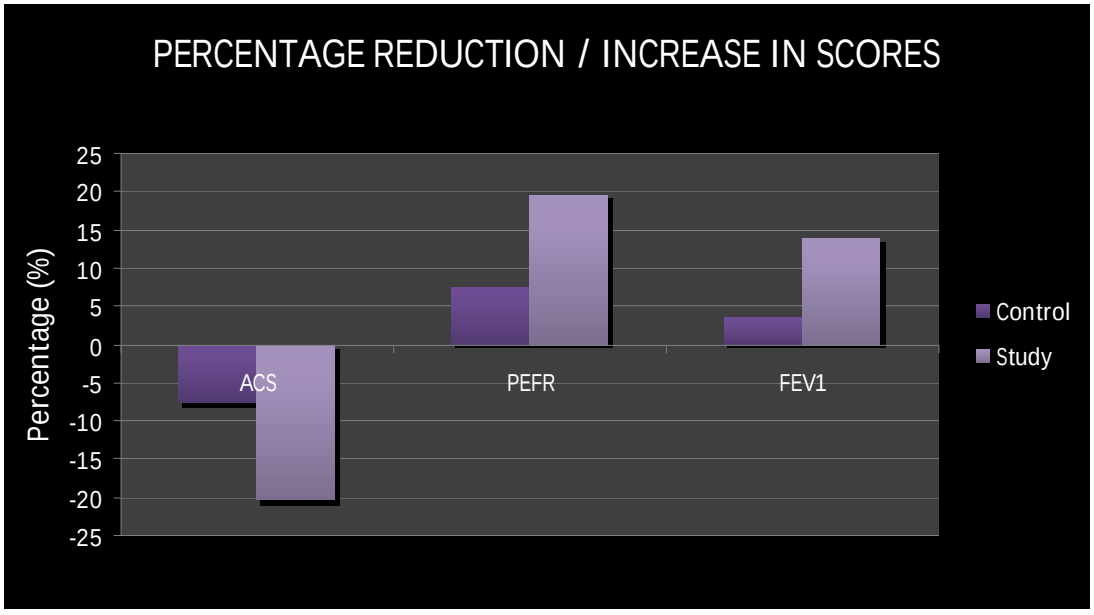
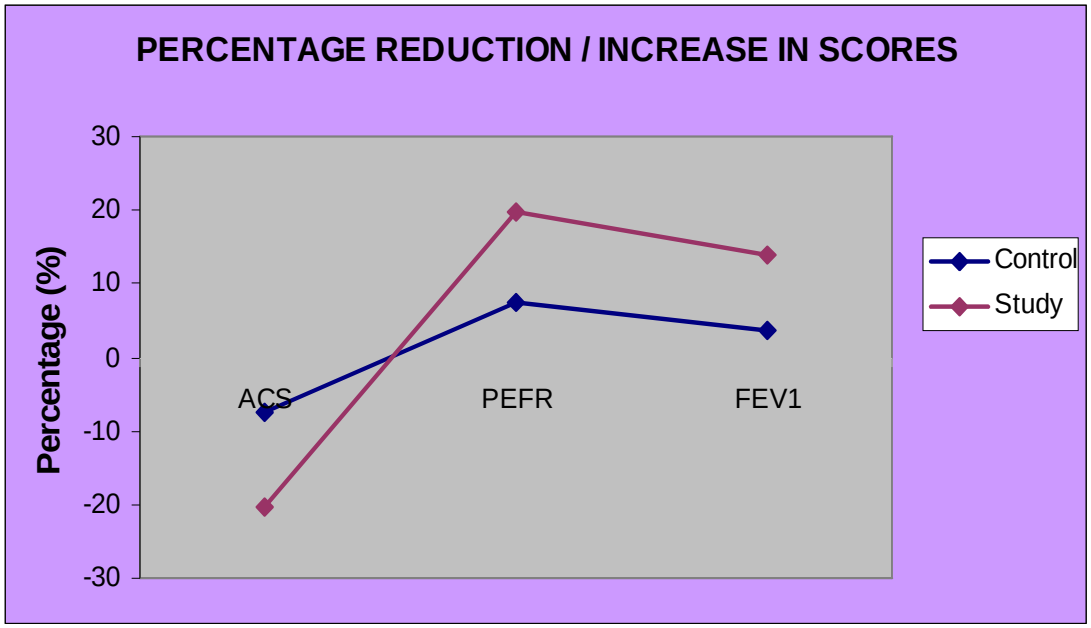


Figure 10 :



DISCUSSION

This 12-week randomized, active controlled, prospective study examined the beneficial effects of Vitamin C supplementation in patients with chronic bronchial asthma. The results of our study showed that administration of Vitamin C as add on therapy had a significant effect in reducing the morbidity, improving the lung function, and the quality of life in asthmatics.

Out of the 112 patients screened, 85 patients who fulfilled the inclusion criteria were recruited for the study. They were randomized into control and study groups, 42 and 43 in number respectively. There were 5 dropouts, 2 from the control group and 3 from the study group.40 patients from the control group and 40 patients from the study group completed the study and were included in the statistical analysis. None of the dropouts were due to adverse effects.

The majority of patients in this study were in the age group of 30-50 years with high prevalence among females. This was in correlation with the established demographic reports (Sally E Wenzel et al, 2000)[14]. Efficacy variables such as ACQ score and PEFR were measured at the baseline and at the end of the 2nd, 4th, 6th, 8th, and 12th week, while FEV1 was measured at the baseline and at the end of the 4th, 8th and 12th week. Serum Malondialdehyde (MDA-a marker of oxidant injury) and Serum C – reactive protein (CRP- a marker of inflammation) were measured at the baseline and at the end of the 8th week. Other routine hematological and biochemical parameters were done before and after the study.

ACQ SCORE

Clinically, asthma control was assessed using the Juniper Asthma Control Questionnaire (ACQ) score.ACQ was developed to optimize asthma control and thus reduce the risk of life threatening exacerbations and long term morbidity.It measures both the adequacy of asthma control and change in asthma control, which occurs either spontaneously or as a result of treatment.

From our study, it was evident that Vitamin C was effective in lowering the ACQ score as shown by a decline in nocturnal symptoms, severity of asthma attack, limitation of activities, shortness of breath and frequency of asthma attack (vide table 1, figure 1 and 2).This was in correlation with the results of Sandra L.Tecklenburg et al, 2007 who showed that ascorbic acid supplementation significantly improved the ACQ score[15].

LUNG FUNCTION TESTS

Lung function parameters like Peak expiratory flow rate (PEFR) and Forced expiratory volume in one second (FEV1) were significantly increased in the study group when compared to the control group (vide tables 2 and 3, figure 3, 4, 5 and 6).These results were consistent with the data obtained from the studies of AR Ness and colleagues et al, 1996 at the Institute of Public Health,Cambridge,UK[16] who reported that Vitamin C is protective for lung function as evidenced by a positive correlation between plasma Vitamin C and FEV1.This was supported by Schatcher and Schlesinger et al, 2001 who studied the effects of ascorbic acid in exercise induced asthma and concluded that ascorbic acid supplementation has a protective effect on Peak expiratory flow rate (PEFR), Forced expiratory volume in one second (FEV1) and Forced vital capacity (FVC) when compared to placebo[17].

SERUM MALONDIALDEHYDE

It is an established fact that the etiopathogenesis of asthma is related to excessive free radical formation.Free radicals are continuously produced in the body mostly by biochemical redox reactions involving oxygen, which occurs as a part of normal cell metabolism.

Oxidative stress occurs when there is an imbalance between the production and scavenging of free radicals, leading to oxidative deterioration of polyunsaturated fatty acids or lipids, thus compromising cellular function and the antioxidant status of the body.With excessive free radical production, there is an increase in lipid peroxidation as indicated by an increase in the levels of Serum Malondialdehyde (MDA), an aldehydic end product of lipid peroxidation.

Vitamin C exerts its antioxidant effect by curbing the excessive free radical formation and inhibiting the initiation of lipid peroxidation, thereby controlling the MDA levels. In the present study, MDA was found to have higher values in both the groups at the start of the study (vide table 4, figure 7 and 8).This was in correlation with the research done by NadeemAChhaba et al 2003 who reported increased lipid peroxidation products and an increase in MDA levels, in patients with bronchial asthma [18].

There was a statistically significant decrease in the MDA levels in the study group at the end of the study which was not seen in the control group (vide table 4, figure 7 and 8).This decrease is probably due to supplementation with Vitamin C which is able to counter the excess free radical production and thus inhibits lipid peroxidation .The results obtained in this study were comparable with the results of A Olayaki and Sabhu M Ajao et al, 2008 and Jaswal S Mehta HC et al, 2003 who showed a decrease in the concentration of MDA levels after antioxidant therapy[19,20].

SERUM C-REACTIVE PROTEIN

Chronic inflammation of the airway plays a major role in the pathogenesis of asthma which is associated with an increased plasma CRP level.CRP is used mainly as a marker of inflammation. Measuring and charting C – reactive protein values can prove useful in determining the disease progression or the effectiveness of treatment. This was supported by the research work done by Ahmed A Arif and George L Deluos et al, 2002 who showed that adults with asthma and asthma symptoms have higher levels of CRP[21].In our study, there was a decrease in the CRP levels in the study group when compared to the control group which was not statistically significant. This may be due to the fact that the CRP levels in our study were estimated using the ELISA technique whereas a significant decrease could probably be appreciated only when measured using high sensitivity technique as reported by M Takemura and M Matsumoto et al, 2006[22].

The other hematological and biochemical parameters measured before and after the study were found to have no statistical difference among both the groups. Mild adverse effects such as nausea, dyspepsia, diarrhea and headache occurred in both the groups.

FOLLOW UP

At the end of active drug therapy, patients were asked to report to the asthma clinic after 1 month for follow up. All patients, both in the control and study group were assessed clinically by using subjective and objective criteria. There was no change observed in the control group while there

was a slight decline in the improvement observed in the study group which was not statistically significant (vide tables 1, 2 and 3, figure 1,2,3,4,5 and 6).

Vitamin C is an important antioxidant in the extracellular respiratory lining fluid that protects proteases, antiproteases, epithelial and immune cells from oxidant attack and low levels of this antioxidant may leave the lungs relatively unprotected from oxidant stress. As an antioxidant, Vitamin C's primary role is to neutralize free radicals. Since it is water soluble, Vitamin C can work both inside and outside the cells to combat free radical damage. As explained earlier, free radicals will seek out an electron to regain their stability. Vitamin C is an excellent source of electrons, therefore it can donate electrons to free radicals such as hydroxyl and superoxide radicals and quench their reactivity. In addition to its work as a direct scavenger of free radicals in fluids and lipids, Vitamin C also contributes to the antioxidant activity of Vitamin E and glutathione. Thus, from this study it is evident that oral administration of Vitamin C as add on therapy produces significant improvement in asthma control and severity.

CONCLUSION

In conclusion, this study proves the role of Vitamin C in reducing the oxidant injury associated with chronic airway inflammation in asthma. This study also shows that Vitamin C as add on therapy to the existing standard therapy improves the clinical response and decreases the disease activity to a greater extent than with routine standard drug therapy alone. In view of its low cost, safety and efficacy, the routine use of Vitamin C in chronic bronchial asthma warrants consideration. Further, the combination of Vitamin C with other known antioxidants like Vitamin A, Vitamin E, and β carotene in achieving better asthma control can also be evaluated.

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