Indian J Physiol Pharmacol 2011; 55 (3) : 281–287

PLASMA OXIDANT-ANTIOXIDANTS STATUS IN ASTHMA AND ITS CORRELATION WITH PULMONARY FUNCTION TESTS

REENA KAUR RUPRAI*

Department of Physiology,
Indira Gandhi Govt. Medical College,
Nagpur – 440 010

(Received on October 27, 2009)

Abstract : The present study was planned to asses the levels of oxidant and antioxidant in asthmatic patients and to evaluate the existence of correlation between oxidant-antioxidant levels and pulmonary function test. Asthma is a well know chronic inflammatory disorder where disturbances in the oxidative system have been observed. To investigate the oxidative stress plasma malondialdehyde and ascorbic acid (Vitamin C) were measured. FEV_1, FEV_1% and PEFR values were taken with computerized spirometry. Results revealed statistically significantly reduced PFT values (***P<0.001) in asthmatics. Increase serum MDA (***P<0.001) and decrease vitamin C (***P<0.001) in patients suggestive of oxidative imbalance in asthma was revealed. However no correlation was found between PFT and oxidative stress indicators. The study thus supported the emerging concept of free radical injury in asthma and therefore a thought can be given to whether antioxidant supply could have a beneficial impact on the free radical induced injury and improvement of respiratory reserve in Asthmatics.

Key words : asthma, oxidants, antioxidants, PFT, MDA, ascorbic acid

INTRODUCTION

The earth, fire, water, space and wind are the unparalleled forces of nature. When the season changes, the mist and fog play a symphony with the five elements. One can walk on the clouds and breathe-in the greens. But, life is not the same for everyone. There are people who can't even breathe-in the necessary air to live with changing seasons and then, there are few unfortunate ones who have difficulty in breathing perennially. These people are destined to live with an incurable, though treatable episodic airway disease called "bronchial asthma". Each inhalation taken seems like a gift of life for a patient of asthma.

Another important aspect associated with

*Corresponding Author : Reena Kaur Ruprai, Plot No. 20, Near Medical College, Wanjari Nagar, Ajni, Nagpur – 440 003, Mob. : 9922466044; Email: reeenakaur.ruprai@rediffmail.com

Asthma is the role of free radicals. Disturbances in the oxidative system in the asthmatic patients have been observed (1). The lungs are directly exposed to very high amount of oxygen, it is imperative for the organ to possess the defense against possible oxidative challenge. The lungs are therefore endowed with an armamentarium of a battery of endogenous agents called antioxidants. The antioxidants helps the lungs ward off the deleterious consequences of a wide variety of oxidants/reactive oxygen species. The major non enzymatic antioxidants of the lungs are glutathione, vitamin C, vitamin E, beta-carotene, uric acid and the enzymatic antioxidants are super oxide dismutase, catalase, peroxidase. Aberrations in oxidants: antioxidants balance can lead to asthma (2).

Free radical injury specifically lipid peroxidation is believe to contribute to pathphysiology of asthma (3). Free radicals by their unstable and transient nature are difficult to measure directly. Their tendency to cause lipid peroxidation has been used as an indirect measure. Increase lipid peroxidation is correlated with increase free radical activity and MDA is a widely used marker of lipid peroxidation. Its level is known to be elevated in asthma (4). The antioxidant defense systems are often examined as an indirect marker of oxidative injury. Ascorbic Acid is the first line of defense in plasma against lipid peroxidation and scavenges the free radicals (5). It is found to be decreased in asthma. Ochs Balcom (6) in a cross-sectional study explored the association of antioxidant nutrients and markers of oxidative stress with forced expiratory volume in the first second (FEV_1%) and forced vital capacity (FVC %) and observed that imbalance in antioxidant/ oxidant status is associated with chronic airflow limitation, and that dietary habits and/or oxidative stress play contributing roles. The pulmonary functions are decreased in asthma, in the present work PFT showing the ventilation status are performed as a tool for testing.

The present study is attempted to find out the existence of oxidant antioxidant imbalance in asthma and to evaluate if there is any correlation between this oxidative stress and magnitude of dysfunction in pulmonary function in asthmatics.

MATERIAL AND METHODS

The study work was carried out on 40 male asthmatic patients attending the asthma clinic in the CHEST& TB OPD of IGGMC, Nagpur, in the age group of 30-45 after the institutional ethical committee clearance. Only diagnosed cases, criteria being bronchodilator reversibility test (post bronchodilator FEV_1 > 200 ml or 12% of baseline) (7), were enrolled in the study after informed consent.

Age, height, weight matched 40 healthy males were taken for comparison.

Exclusion criteria:

1. Subjects who have chronic respiratory illness other than asthma like bronchiactesis, emphysema, pulmonary tuberculosis, tropical eosinophilia etc.

2. Subjects having cardiovascular disease.

3. Subjects with altered plasma vitamin c levels for reasons other than asthma such

as sickle cell disease, diabetes mellitus, cancer where free radical injury may alter vitamin C level were excluded.

4. Smokers, alcoholics.

Inclusion criteria :

1. Age group between 30 and 45 males were taken for study.

2. Known case of asthmatic patients on treatment (A positive bronchodilator reversibility test showing an increase in FEV_1 by more than 12% and at least more than 200ml in comparison to base line value).

Pulmonary function test were done by computerized spirometer (SIROLAB-2) in sitting position with nose clip. FEV_1, FEV_1%, & PEFR were taken for study.

Serum samples were analyzed for ascorbic acid using bakemans spectrophotometer (8) and MDA based on colorimetric method (9).

Statistical analysis of data

Mean and standard deviation were calculated and significance of difference was tested statistically by the unpaired students 't' test (9). $P<0.05$ was taken to be significant. Linear correlation between Vitamin C, MDA and PFT variables were analyzed using pearson coefficient of correlation.

RESULTS

As seen in TABLE I both the groups are comparable without statistically significant difference at base line. TABLE II depicts significantly decreased level of vitamin C while, consistently high level of MDA in study group in comparison to control group. Significantly decreased pulmonary function parameters are found in the study group (Table III).

TABLE I : Age & anthropometric parameters of subjects of control & study groups.

Parameters	*Control group*	*Study group*	*P value*
Age (years)	37.55±5.84	38.38±6.10	0.54
Body weight (kg)	56.30±4.30	56.05±5.12	0.81
BMI (kg/m^2)	20.05±1.79	20.76±5.36	0.43

Date presented are Mean±SD.
BMI : Body Mass Index.

TABLE II : Oxidative stress indicators in control and study group.

Parameters	*Control group*	*Study group*	*P value*
Vitamin C (mg/l)	0.93±0.08	0.55±0.07	<0.001***
MDA (µmol/l)	1.66±0.26	3.15±0.53	<0.001***

Date presented are Mean±SD.

TABLE III : Pulmonary function test in control and study group.

Parameters	*Control group*	*Study group*	*P value*
FEV1 (lit)	3.30±0.44	1.87±0.39	<0.001***
FEV1 (%)	87.76±6.79	62.31±5.21	<0.001***
PEFR (L/s)	8.48±0.89	4.36±1.23	<0.001***

Date presented are Mean±SD.
FEV_1 : Forced expiratory volume in 1 second in litre,
PEFR : Peak expiratory flow rate in litre/second.

The results of the study are as follows :

No significant correlation was found between Vitamin C and PFT variables as well

as among MDA and PFT. Also no correlation was found between Vitamin C and MDA (Tables IV & V, Scatter diagram as in Fig. 1).

TABLE IV: Showing correlation of vitamin c with pulmonary function test.

PFT Parameters		*Study Group (n=40)*
FEV_1	r	0.12
	p	0.16
FEV_1 %	r	0.23
	p	0.15
PEFR	r	–0.01
	p	0.16

TABLE V: Showing correlation of MDA with pulmonary function test

PFT Parameters		*Study Group (n=40)*
FEV_1	r	–0.48
	p	0.14
FEV_1 %	r	–0.34
	p	0.15
PEFR	r	–0.01
	p	0.16

r: correlation coefficient, p: probability, n: numbers of observations.

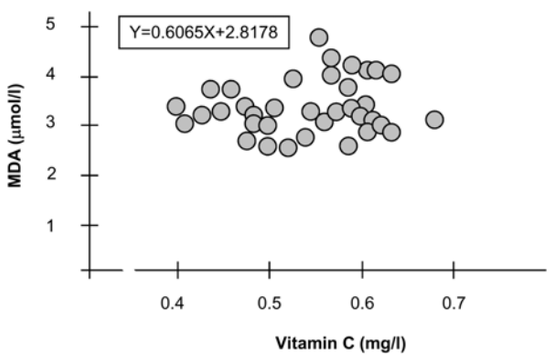

Fig. 1: Scatter plot showing lack of association (r=0.08; P=0.16) between Vitamin c and MDA.

DISCUSSION

Asthma is a disease characterized by wide variation over short period of time in resistance to flow of air in intrapulmonary airways. Another aspect of asthma is the role of free radicals. Chronic immuno-allergic inflammatory reaction plays a key role in the pathogenesis of asthma.

The study revealed raised MDA level in asthmatic patients thus supporting the view of oxidative stress involved in asthma. Are study maintains fair correlation with the findings of work done in the past. Shanmusgasundaram (10) concluded in his studies that lipid per oxidation products is higher and serum levels of antioxidant vitamins are significantly decreased in Bronchial Asthma. Wood (3) also supported the conclusion of oxidative stress in asthma along with disturbed antioxidant status. Nadeem (11) investigated range of oxidants and antioxidants in patients with bronchial asthma to create a comprehensive picture of oxidant-antioxidants imbalance. Asthmatic patients showed increased superoxide generation from leucocytes, increased nitrites and lipid peroxidation products indicating increased oxidative stress and decreased glutathione peroxidase activity in red blood cells, thus concluding that there are alterations in a wide array of oxidants and antioxidants with balance towards increased oxidative stress in asthma. Sharma (4) studied the levels of free oxygen radicals in children with bronchial asthma during attack and symptom free period by estimating serum MDA levels and concluded that serum MDA levels were highest at the time of admission and decreased 24-48 hours with treatment but MDA levels continued to remain higher than controls indicating thereby the relation of level of serum MDA with that of the underlying inflammatory process in bronchial asthma. Vural (12) estimated lipid peroxidation (LPO),

Glutathione peroxide (GSH-Px), Superoxide dismutase and Vitamin C levels in patients with bronchial asthma and controls and suggested that alterations in a wide array of oxidants and antioxidants with balances shifting towards increased oxidative stress in bronchial asthma.

The extent of lipid per oxidation denotes the amount of free radicals generated which have not been probably scavenged by defense mechanism. Lipid per oxidation may not be related to primary tissue injury but may amplify the original injury. Joel Schwartz (13) assessed the relationship between dietary Vitamin C intake and level of pulmonary function (Forced expiratory volume in 1sec FEV_1) in 2526 adults and found there were positive and significant association of dietary Vitamin C intake and the level of FEV_1. These data are consistent with the hypothesis that Vitamin C intake has a protective effect on pulmonary function. Kongerud (14) performed induced sputum (IS) in a group of mild asthmatics (n=16) and healthy controls (n=18) in order to compare constitutive levels of antioxidants in the airways of these two groups. They reported that asthmatics had significantly decreased AA [ascorbic acid] in both the cellular (17±3 ng/10(6) cells vs. 40±4 ng/10(6) cells) and fluid-phase fraction (616±152 ng/ml vs. 937±161 ng/ml) of the IS sample compared to normal's. No differences were found with glutathione (GSH) and alpha-tocopherol. These results suggest that a deficiency may be either an underlying factor in the pathophysiology of asthma or a response to asthmatic airways inflammation.

Vitamin C scavenges the free radicals by forming ascorbyl radial which is stable and fairly non reactive and also is a regenerating agent for Vitamin E. It is also a part Glutathione peroxidase path way for repairing oxidative damage to the lipid membrane (5).

Another reason for decrease in the vitamin is, being water soluble not stored in the body as well as no additional dietary or medicinal supplementation is provided to the patient. Thus conclusively even with normal dietary intake patient have low level due to increase expenditure in asthmatics like acting as a strong reducing agent providing protection from free radical injury, helping in regeneration of Vitamin E, immunological functions, prevention in chronic diseases. Ascorbic acid stimulates phagocytic action of leucocytes and helps formation of antibodies. Study done by Sagun (15) showed that Vitamin C enters mitochondria via facilitative glucose transporter 1 (Glut 1) in the oxidized form, dehydro ascorbic acid (DHA) and accumulates inside the mitochondria as mitochondrial ascorbic acid (mtAA) and protects mitochondria from oxidative injury.

Burns (16) examined the association of dietary factors (fruit, vegetables, Vitamins C and E, beta-carotene, retinol, n-3 fatty acids) with respiratory health in a cohort of 2,112 students assessed the associations between dietary factors and pulmonary function with linear mixed models, and respiratory symptoms with logistic regression using generalized estimating equations (GEE), adjusted for individual and group level covariates and observed low dietary fruit intake was associated with lower forced expiratory flow in one second (–1.3%; 95% CI: –2.4%, –0.2%) and increased odds of

chronic bronchitic symptoms (OR=1.36; 95% CI: 1.03, 1.73) compared with higher intake Ford ES (17) also studied the serum concentration of antioxidants in asthma and reported that Vitamin C concentration were low among people with current or former asthma than among people who never had asthma (P=0.014). Ochs Balcom (6) in a cross-sectional study explored the association of antioxidant nutrients and markers of oxidative stress with forced expiratory volume in the first second (FEV_1%) and forced vital capacity (FVC %). The study data included 218 persons with chronic airflow limitation recruited randomly from the general population, after adjustment for covariates, multiple linear regression analysis showed that serum beta-cryptoxanthin, lutein/zeaxanthin, and retinol, and dietary beta-carotene, beta-cryptoxanthin, lutein/zeaxanthin, Vitamin C and lycopene were positively associated with FEV_1% ($P<0.05$, all associations). Serum vitamins beta-cryptoxanthin, lutein/zeaxanthin, and lycopene, and dietary beta-cryptoxanthin, beta-carotene, Vitamin C and lutein/zeaxanthin were positively associated with FVC% ($P<0.05$, all associations). Erythrocytic glutathione was negatively associated with FEV_1%, while plasma thiobarbituric acid-reactive substances (TBARS) were negatively associated with FVC% ($P<0.05$). These results support the hypothesis that an imbalance in antioxidant/oxidant status is associated with chronic airflow limitation, and that dietary habits and/or oxidative stress play contributing roles, thereby supporting our present study.

No correlation was found between oxidative stress indicators and PFT. This could be due to the following reasons.

1. Age
2. Duration/Length of the disease
3. Susceptibility of the individual
4. Rate of deterioration

The response given to the allergen also varies from person to person. Also importantly the treatment that is given to the patient is providing symptomatic relief and to some extent controlling inflammation. But no definite radical cure is given to the injury, which means that underlying disease process is on going. Thus considering the above facts their need not be correlation between PFT parameters and oxidative stress indicators.

Conclusion

This finding of raised level of oxidants and simultaneously decrease level of antioxidants suggest the presence of oxidative injury. The respiratory reserve of the patient gradually goes on decreasing as reflected in PFT. Exposure to allergen and broncho constriction is one side, but even if there is no exposure to allergen i.e. the disease is in the sub clinical or non attack state still the body immunity is providing a protective front to repair the already existing injuries. This leads to remodeling which is a feature of asthma. The deterioration in PFT is due to the process of airway remodeling in asthma (18). From the work done we can hypothesize that a corresponding supply of Vitamin C may protect the lungs tissue against reactive oxygen species induced injury, adverse respiratory effect and improve the prognosis for bronchial asthma. Consumption of fresh fruits have beneficial

impact on lung function among children ,fruits rich in Vitamin C has been related to decrease in the prevalence of asthma and high lung function. It is also related to decrease in airway hyper reactivity and improve lung function in adults as well. Forastiere (19) also supported that consumption of fruits rich in vitamin c may reduce wheezing symptoms in children. Thought can also be given whether periodical MDA estimation can be qualified as a good marker of parenchymal damage. Finally further study is needed to prove that antioxidant supplementation could have beneficial impact on pulmonary function and may improve the prognosis in bronchial asthma and other respiratory diseases involving free radical injury.

REFERENCES

1. Rogala B, Polanikr, Grzanka A, Ciesielska-Kopacz N, Birkner E. Assessment of Antioxidative enzymes activity and intensification of lipid peroxidation in asthma subjects. *Pneumonal Alergol Pol* 2004; 72: 472–476.
2. Rahman I. Oxidative stress and gene transcription in asthma and chronic obstructive pulmonary disease: Antioxidants Therapeutic Targets. *Curr Drug Targets Inflamm Allergy* 2002; 1: 291–315.
3. Wood LG, Gibson GP, Garg LM. Biomarkers of lipid peroxidation, airway inflammation and asthma. *Eur Respir J* 2003; 21: 177–186.
4. Sharma Anita, Bansal Satish, Nagpal RK. Lipid peroxidation in bronchial asthma. *Ind J Pediatr* 2003; 70: 715–717.
5. Johnson FC, Sinclair HM. The antioxidant Vitamins. *Crit Rev Food Sci Nutr* 1970; 11: 217.
6. Ochs-Balcom HM, Grant BJ, Muti P. Antioxidants, oxidative stress, and pulmonary unction in individuals diagnosed with asthma or COPD. *Eur J Clin Nutr* 2006; 60: 991–999.
7. Carpo B. James, Glassroth Jeffrey. Baum's Textbook of Pulmonary Diseases 7th Edition, A&C Black Publishers Ltd, Philadelphia: Lippincott Williams and Wilkins 2004; 181–187.
8. Kyaw A. A simple calorimetric method for ascorbic acid determination in blood plasma. *Clin Chimica Acta* 1978; 86: 153–157.
9. Motghare KS, Bhutey A, Murrhar BB, Gupta M. Lipid Peroxidation and Glutathione peroxidase in ischemic heart diseases. *Ind J Clin Biochem* 2001; 16: 213–215.
10. Shanmusgasundaram KR, Kumar SS, Rajajee S. Excessive free radical generation in the blood of children suffering from bronchial asthma. *Clin Chim Acta* 2001; 305: 107–114.
11. Nadeem A, Chabra SK, Masood A, Rai HG. Increased oxidative stress and altered levels of antioxidants in asthma. *J Allergy Clin Immunol* 2003; 111: 72–77.
12. Vural H, Aksoy N, Ceylan E, Gencer M, Ozguner F. Leukocyte oxidant and antioxidant status in asthmatic patients. *Arch Med Res* 2005; 36: 502–506.
13. Joel Schwartz, Weiss Scott T. Relationship between dietary Vitamin C intake and Pulmonary Function in the First National Health and Nutrition Examination Survey (NHANES). *Am J Clin Nutr* 1994; 59: 110.
14. Kongerud J, Crissman K, Hatch G, Alexis N. Ascorbic acid is decreased in induced sputum of mild asthmatics. *Inhal Toxicol* 2003; 15: 101–109.
15. Sagun KC, Juan M. Carcamo, David W. Golde Vitamin C enters mitochondria via facilitative glucose transporter (Glut1) an confirms mitochondrial protection against oxidative injury. *The FASED Journal* 2005; 19: 1657–1667.
16. Burns JS, Dockery DW, Neas UN, Schwartz J, Coull BA, Raizenne M, Speizer FE. Low dietary nutrients intakes and respiratory health in adolescents. *Chest* 2007; 132: 238–245.
17. Ford ES, Mannino DM, Redd SC. Serum Antioxidant concentration among US adults with self reported asthma. *J Asthma* 2004; 41: 179–187.
18. Lloyd CM, Robinoson DS. Allergen-induced airway remodeling. *Eur Respir J* 2007; 29: 1020–1032.
19. Forastiere F, Pistelli R, Sestine P, Fortes C, Rezoni E, Dellorco Vet al. Consumption of fresh fruits rich in Vitamin C and wheezing symptoms in children. *Thorax* 2000; 55: 238–288.