Salivary Total Antioxidant Activity as a Non Invasive Biomarker for Oxidative Stress In Asthmatic Patients

K Sayedda, Q S Ahmed

Associate Professors, department of Pharmacology, Shri Ram Murti Smarak Institute Of Medical Sciences, Bareilly (U.P.) - India

Abstract: Background: Bronchial Asthma is a chronic inflammatory disease along with hyperresponsiveness of bronchi. The present study was designed to assess the redox status of patients suffering from bronchial asthma and to compare it with that of normal healthy controls, by determining the total antioxidant activity (AOA) of the serum and saliva and correlating it with the disease status. Method: Total AOA was assayed spectrophotometrically in saliva and serum of two groups; asthmatic patients attending OPD of pulmonary Medicine and healthy controls. The patients were followed for a period of three months after start of therapy and total AOA was measured post therapy. Results: Asthmatic patients exhibited significantly($p<0.05$) decreased serum and salivary total AOA as compared to healthy controls. Decreased contents of total AOA in serum and saliva was positively correlated with the severity of disease process. Total AOA in serum was significantly ($p<0.0001$) higher than salivary AOA in all the categories of asthmatic patients. Total AOA in serum was significantly ($p<0.0001$) higher than that in saliva of the control subjects. The depressed total AOA returned to near normal values post treatment. In Conclusion: Total AOA in serum and saliva is a good indicator for assessing the severity and progress of bronchial asthma. Salivary total AOA can be taken as a potential biomarker for oxidative stress in asthmatic patients. [Sayedda K et al NJIRM 2012; 3(1) : 8-12]

Key Words: Bronchial asthma, oxidative stress, saliva, total antioxidant activity

Author for correspondence: Dr. Kauser Sayedda ,Associate Professor. Department of Pharmacology, SRMSIMS, Bareilly (U.P.), Pin- 243001 INDIA E-mail : quazi800@yahoo.com

Introduction: Bronchial asthma is considered as a chronic inflammatory illness with bronchial hyper–reactivity resulting in bronchospasm[1,2]. It is a heterogeneous disorder grouped into (1) atopic or extrinsic and (2) non atopic or intrinsic. The former is often associated with a personal and or family history of allergic disease such as rhinitis, urticaria and eczema accompanied with production of abnormal amounts of IgE antibodies in response to contact with environmental allergens[3]. In contrast, in the intrinsic asthma group, no such relation to allergic disorder has been seen. Oxidative stress has been a subject of intense study in recent times and has directly or indirectly been implicated in the causation and progression of many disease conditions[2,4-7]. Its role as a potential contributor to the pathophysiology of bronchial asthma has been less explored. There is evidence of an oxidant–antioxidant imbalance in asthma[8] . An increased oxidative stress in patients with asthma is derived from increased burden of inhaled oxidants, and from the increased amounts of reactive oxygen species (ROS) like superoxide radical, hydroxyl radical and hydrogen peroxide generated by several inflammatory, immune and structural cells of the airways[6-8].

Total AOA of saliva and serum is documented as a potential biomarker for redox state of body[9-11]. In the saliva, uric acid is the predominant antioxidant, albumin and ascorbate providing minor contributions, the total AOA of saliva correlates well with the salivary concentration of uric acid[12.13] .

The present study was designed to assess the redox status of patients suffering from asthma and to compare it with that of normal healthy subjects, by determining the total AOA of their serum and saliva and correlating it with their disease state.

Material and Methods: The subjects belonging to the age group of 20-50 years of both the sex are divided into two groups normal healthy volunteers (n=25) served as control group , bronchial asthma patients (n=45). Out of these, 36 patients could be followed up after 3 months of treatment for asthma. All the subjects were explained the procedure and purpose of the study prior to their inclusion in this study. All procedures were approved and were as per the guidelines by the Institutional Ethical Committee for human studies. Bronchial asthma patients, are selected from the outpatient department (OPD) of Pulmonary

Medicine, of Shri Ram Murti Smarak Institute Of Medical Sciences, Bareilly (U.P.)

Evaluation of bronchial asthma: The severity of bronchial asthma are categorized as per criteria laid down by Global Initiative for Asthma (GINA).

1. Intermittent
2. Mild persistent
3. Moderate persistent
4. Severe persistent

Patients received one or more of the following drugs for treatment of asthma or added infection of bronchopulmonary tree: Salbutamol, salmeterol, theophylline, fluticasone, prednisolone and chemotherapeutic agents.

Patients were followed up for a minimum period of three months from the initiation of drug therapy to assess the success/failure of drug treatment. Samples of saliva and serum were taken in sterile sample vials and analyzed for antioxidant activity. Patients were asked to refrain from oral intake of any kind 20 minutes prior to sample collection. All the samples were stored at 4- 8° C.

Determination of Antioxidant activity: The method used in our study is based on the principle that Fe–EDTA complex reacts with hydrogen peroxide (H_2O_2) by a Fenton–type reaction, generates hydroxyl radicals (OH^-) and superoxides ($O2^-$).[18]

Analytical Procedure: The reaction mixture containing 0.5 ml of phosphate buffer (100mmol/1,pH 7.4), 0.5 ml of sodium benzoate (10 mmol/1) 0.2ml of Fe- EDTA (2mmol/1EDTA+2mmol/1 of $Fe[NH_4]_2SO_4$, 0.2 ml of H_2O_2 (10 mmol/1), and 0.01 ml of saliva was incubated for 60 minutes at 37° C. The reaction was then stopped by addition of 1 ml of 20% acetic acid. 1 ml of thiobarbituric acid (TBA) solution (0.8% in 50 mml/1 NaOH) was added and the solution was heated for 10 min at 100°C .The absorbance of the pink colour thus formed was estimated spectrophotometrically at 532 nm. 0.01ml of uric acid (1mmol/1 in 5mmol/1 NaOH) was used as standard antioxidant for determining the AOA activity of unknown samples. Proper blanks Ko, Ao, and Uao representing control, unknown and uric acid sample respectively were run under similar experimental conditions.

The antioxidant activity of each sample was calculated as follows:

$$AOA\ (mmol/l) = (CU)\ (K-A)/(K-UA)$$

Where,

K= absorbance of control (K- Ko)

A= absorbance of sample (A_1- A_0)

UA= absorbance of uric acid solution (UA_1- UA_0)

CU= concentration of uric acid (mmol/1)

Each sample was tested twice for its AOA and the mean of these two readings was taken as AOA of that sample.

The following chemicals were used in the study: Sodium dihydrogen orthophosphate dihydrate and sodium benzoate (Thomas baker, India), disodium hydrogen phosphate (Sarabhai Chemicals, India), ferrous ammonium sulphate (BDH, Glaxo Laboratories, India), thiobarbituric acid (Sigma chemicals company, USA), acetic acid glacial 100% and ethylene dinitrilotetracetic acid disodium (Merck, India), sodium hydroxide (Glaxo Laboratories, India). All chemicals were of analytic grade and all reagents were prepared in double distilled water.

STATISTICAL ANALYSIS: The all data were analyzed with the help of a Graph pad software. The mean and standard error of mean was calculated for all data. The't'-test and 'p' values among different groups of parameters have been made.

Result: In the control subjects (n=25) the total AOA in serum was found to be significantly higher ($p<0.0001$) than that of the saliva. The results of estimation of AOA of different groups of asthmatic patients are shown in table 1.

The mean AOA of saliva of the patients suffering from intermittent asthma (n=20) was significantly less ($p<0.0001$) as compared to the AOA in saliva of control subjects(table 1) while reduction in AOA is less in serum($p<0.05$). While, in patients with mild persistent group (n=16),the total AOA of both serum and saliva was found to be significantly ($p<0.0001$) decreased as compared to control subjects(table 1)

In patient of moderate persistent group, the total AOA of both serum and saliva was found to be

significantly decreased(p<0.0001) as compared to control subjects. Total AOA of serum and saliva of three asthmatic categories were also significantly different(p<0.0001).

Follow- up groups: The progression / regression of the disease and the levels of AOA at these different times were followed up in several patients. These results are depicted in tables 2.

Table -1 AOA In Bronchial Asthma Patients With Different Groups

Groups	n	Total AOA(mmol/l)	
		Serum	Saliva
Control	25	1.69 ± 0.02^{a}	1.42 ± 0.02
Asthma Patients (45)			
A	20	$1.63 \pm 0.02^{b\#}$	1.25 ± 0.01^{c}
B	16	$1.35 \pm 0.01^{d\#}$	0.92 ± 0.03^{d}
C	9	$1.10 \pm 0.05^{e\#}$	0.60 ± 0.02^{e}

Values are mean ± SEM, ^{a}p<0.0001 as compared to that of saliva of control, ^{b}p<0.05 as compared to that of serum of control, ^{c,d,e}p<0.0001 as compared to their respective values of control,# p<0.0001 as compared to that of saliva of control. A-Intermittent, B-Mild Persistent, C-Moderate Persistent group

Intermitted asthma patient: Sixteen out of twenty patients of this group were followed up for their total AOA level after three months of therapy. Total AOA activities in saliva (p<0.0001) was found to be significantly increased (table 2)but there was insignificant change in AOA of serum as compared to before treatment values(table 2).

Mild persistent asthma patients : Thirteen out ofsixteen patients of this group were followed up after taking treatment for 3 months. The increase in AOA of saliva following drug treatment was significant (p<0.01)(table 2)but no significant change in the serum AOA as compared to the level before treatment was detected(table 2).

Moderate persistent asthma patients: Seven out of nine patients were followed up who were taking treatment for 3 months. After drug treatment, significant recovery in the level of salivary total AOA was observed(p<0.01) (table 2) while the decrease in AOA in serum persisted. (table 2).

Table 2. Total AOA in the Serum and Saliva of the Follow-up Group of Patients with Bronchial Asthma

Groups	n	Serum total AOA(mmol/l)		Saliva total AOA(mmol/l)	
		Before treatment	After treatment	Before treatment	After treatment
Control	25	1.69 ± 0.02	-	1.42 ± 0.02	-
Asthmatic Patients followed up (36)					
A	16	1.61 ± 0.02	1.65 ± 0.01	1.24 ± 0.02	1.39 ± 0.01^{a}
B	13	1.34 ± 0.03	1.35 ± 0.02	0.88 ± 0.02	0.98 ± 0.04^{b}
C	07	1.09 ± 0.05	1.11 ± 0.04	0.60 ± 0.03	0.87 ± 0.03^{c}

Values are mean ± SEM, a, p<0.0001, b,c, p<0.01 as compared to before treatment values of total AOA in saliva, A-Intermittent, B-Mild Persistent, C-Moderate Persistent Group of Bronchial Asthma

Discussion: Bronchial asthma is a chronic, episodic, inflammatory condition of the airways characterized by recurrent episodes of respiratory symptoms, variable airflow obstruction and presence of airway hyper-reactivity. Several mediators including leukotrienes, prostaglandins and ROS have been implicated in the pathophysiology of bronchial asthma. Oxygen free radicals are known to play an important role in cell degeneration in tissue under oxidative stress and have also been implicated in the causation of bronchial asthma. High levels of ROS and oxidatively modified proteins have been measured in the airways of patient with asthma[5]. Another reactive species, nitric oxide (NO), has also been found to be increased in airway of asthmatic patients[14] . Abnormalities of NOS-I and NOS-II genotype and their expression in patients of bronchial asthma have also been reported[15]. An oxidized product of arachidonic acid, 8- isoprostane, which is a $PGF_{2\alpha}$ analogue, is increased 3-4 fold in patients with asthma compared to that observed in healthy subjects[16].

Numerous attempts have been made in the past to estimate the AOA in patients of bronchial asthma. However, most of the studies have used individual markers like superoxide release and cellular glutathione peroxidase (cGPx) activity in granulocytes and monocytes , nitric oxide and lipid peroxidation as a measure of oxidative stress[16.]
Isolated studies have, in the past, tried to establish a relationship between bronchial asthma and the AOA. Mani et al.,[17] and Picardo et al.,[18]reported an increased production of oxidants and decreased AOA of serum in cases of bronchial asthma.

Bentur et al.,[19] stated that children with acute asthma attacks exhibit a decrease in the activity of the most important salivary antioxidant peroxidase enzyme which is accompanied by other salivary composition alterations. Hence, acute asthma is manifested by salivary changes. This implies systemic oxidative stress in asthma, which may be reflected in salivary analysis.

We carried out the present study in asthmatic patients with two objectives- one, to find out if a correlation exists between the severity of disease process and the total AOA in the body as measured in the serum and salivary samples. Secondly, to determine which, if any, the serum or salivary total AOA, was a better index of the disease process.

The total AOA was measured in two body fluids, serum and saliva. In normal (control) person, the total AOA in serum was found to be significantly higher than that in saliva as was also observed by Koracevic et al,.[20].

In patient of bronchial asthma, significant decrease in the serum and salivary total AOA was detected which correlated well with the severity of their disease process (table 1). The decrease in salivary total AOA was much more in magnitude as compared to the decrease in serum AOA. Also, there was a significant difference in total AOA of serum & saliva in the three categories of asthmatic patients.

Following 3 months treatment, the patients were re-examined and total AOA of both serum and saliva was measured. The serum total AOA level in case of mild persistent and moderate persistent cases remained depressed as it was before treatment while that of the intermittent group, approached to control group. No significant recovery could be observed in the AOA levels of serum in the mild persistent and moderate persistent groups. It is interesting to note that a similar decrease in serum AOA has been reported by Picardo et al.,[18] which did not exhibit any recovery towards normal values even after the administration of micronutrients/ antioxidants.

The salivary levels of AOA however, showed remarked recovery following three months treatment in all the three groups as compared to the serum AOA. The salivary AOA level in intermittent group returned to near control level after three months of treatment for bronchial asthma. In contrast, the other two groups exhibited partial recovery of AOA in the saliva after treatment. They showed a decrease of 30% in the mild persistent group and 38.7% in the moderate persistent group as compared to control levels.

Conclusion: We, therefore, conclude that the total AOA level of serum and saliva is decreased in bronchial asthma patients. This decrease seems to be greater with the severity of the disease and a good correlation could be observed between the disease process and the total AOA level. More interestingly, an obvious recovery towards control level could be detected in accordance with the improvement in the disease process due to pharmacotherapy. Therefore, though both serum and salivary total AOA level could be used as markers for mapping the severity of the disease process and its progression/regression, the salivary marker seems to be of greater relevance since it was changed in all three forms of the disease. Finally, salivary analysis which is non-invasive and much easier to perform as compared with serum analysis, is suggested as a new and effective diagnostic tool in bronchial asthma patients.

Acknowledgement : The authors are thankful to the medical superintendent of the Institute for providing necessary facilities and environment required for this research work. We are especially thankful to the patients for their cooperation.

References:

1. Peter JB. Asthma. Braunwald E, Frauci S, Kasper DL, Hauser SL, Lango DL, Jameson JL . Harrison's Principles Of Internal Medicine, 17th ed : Mc Graw-Hill, 2008: 1596-1606.
2. Caramory G, Papi A. Oxidants and Asthma. Thorax 2004; 59; 1703.
3. Humbert M, Ying S, Corrigan CJ, Robinson DS. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma. Immunol Today 1999; 20: 528- 33.
4. Ahmed N, Hanumanthrao G, Chhabra S. Increased oxidative stress in acute exacerbations of asthma. Journal of Asthma 2005; 1: 45-50.
5. Hisatoshi S, Masakazu I . Oxidative & nitrative stress in bronchial asthma. Antioxidants & redox signaling 2008; 10 (4); 785- 798
6. Zhao JJ, Shimizu Y, Dobashi K, Kawata T, Ono A, Yanagitani N. The relationship between oxidative stress and acid stress in adult patients with mild asthma. J Investing Allergol Clin Immunol 2008; 18(1): 41-45.
7. Fujisawa, T. Role of oxygen radicals on bronchial asthma. Current drug targets- Inflammation & allergy 2005; 4 : 505–509
8. Nadeem A, Chhabra SK, Masood A, Raj HG. Increased Oxidative stress and altered levels of antioxidants in asthma. J allergy Clin Immunol 2003; 111(1): 72-78.
9. Carlos K and Bucalen F. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. Journal of cell and molecular biology 2008; 7(1): 1-15.
10. Radhakrishnan S, Narayanasamy A, Jyotirmay B, Sivaramakrishnan R. Total antioxidant capacity in Eale's disease, uveitis & cataract. Indian J Med Res 134, July 2011; 83-90.
11. Ruchi K, Anil Kumar S, Sunil G, Bashir A, Prabhat S. Antioxidant activity in children with ADHD –a comparison in Untreated and treated subjects with normal children. The International Medical Journal Malaysia, june 2011; 10,1.
12. Meucci E, Littarru C, Deli G, Luciani G, Tazza L, Littarru GP. Antioxidant status and dialysis; plasma and saliva antioxidant activity in patients with fluctuating urate levels. Free Radic Res 1998; 29(5): 367-76.
13. Moore S, Calder KA, Miller NJ. Rice–Evans CA. Antioxidant activity of saliva and periodontal disease. Free Radic Res 1994; 21 (6): 417-25.
14. David WR, David PJ, Bryce F, Chris W, Hayden W. Exhaled nitric oxide continues to reflect airway hyperresponsiveness and disease activity in inhaled corticosteroid- treated adult asthmatic patients. Respirology 2003; 8: 479.
15. Fabio L, Ricciardolo M, Peter J, Sterk, Benjamin G, Folkerts G, Nitric oxide in Health and Disease of the Respiratory Systems. Physiol. Rev 2004; 84: 731-765.
16. Wood LG, Gibson PG, Garg ML. Biomarkers of lipid peroxidation, airway inflammation and asthma. Eur Respir J 2003; 21: 177-186.
17. Mani SK, David ML, Serpil CE. Asthma. Department of Pulmonary Allergy and Critical Care Medicine 2005. The Cleveland Clinic.
18. Picardo C, Deulofeu R, Lleonart R, Agusti M, Mullol J, Quinto l, et al. Dietary micronutrients/ antioxidants and their relationship and bronchial asthma severity. Allergy 2001; 56(1): 43-49.
19. Bentur L, Mansour Y, Brik R, Eizenberg Y, Nagler R. Salivary oxidative stress in children during acute asthmatic attack & during remission. Respiratory medicine 2006; 100(7): 1195- 1201.
20. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Methods for the measurements of antioxidant activity in human fluids; J Clin Pathol 2001; 54: 356-61.