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Full Length Research Paper

Markers of Oxidative Stress and C-reactive Protein Levels in Asthmatics

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Abstract

Oxidative stress and inflammation are possible consequences of cellular activation in many disease conditions. There is a dearth of information on the status of these products of cellular activation in Nigerian asthmatics. This study assessed the levels of markers of oxidative stress (products of cellular activation) and C-reactive protein (a marker of inflammatory response) in Nigerian asthmatics. Twenty-two known asthmatics (12 males; 10 females; age=16.3±2.8 years) with resent allergic reaction volunteered to participate in this study. Twenty apparently normal individual (12 males; 8 females; age=15.1±3.2 years) without history of asthma or resent allergic reactions were selected as controls. Plasma levels of total plasma peroxide (TPP), total antioxidant potential (TAP), malondialdehyde (MDA) and C-reactive protein (CRP) were determined in the two groups using spectrophotometric methods and immunodiffusion (Macinni) methods respectively. Oxidative stress index (OSI) was determined as the percent ratio of TPP and TAP. Significantly higher levels of TPP ($p<0.02$), OSI ($p<0.001$), MDA ($p=0.01$) and CRP ($p<0.01$) were observed in the asthmatics when compared with controls. There was no significant ($p>0.2$) change in the plasma level of TAP in the asthmatics when compared with the controls. Cellular activation-induced oxidative stress and inflammation are possible features of asthma.

Keywords: Oxidative stress, inflammation, asthma.

INTRODUCTION

Asthma is a multi-factorial airway disease that arises from a relatively common genetic background, inter-phased with exposures to allergens and airborne irritants (Gilmour et al., 2006). The collagen surrounding airways, blood vessels and vascular smooth muscle exhibit increased immunoreactivity after exposure to antigen challenge (Talati et al., 2006). Asthma is characterized by production of lymphokines, transformation of B-lymphocytes to IgE-producing plasma cells, mast cell sensitization, release of degranulation products of mast cell (histamine, heparin, prostaglandin D2, tryptase, β-hexosaminidase, β -glucuronidase, arylsulphatase, myeloperoxidase and superoxide dismutase) and recruitment of various inflammatory cells in the airway mucosa (Klein, 1989). IL-6 released during cellular activation induces the hepatocytes to produce C-reactive protein (Sahoo et al., 2009).

It has been reported that upon activation of the aggregated phagocytes in the lungs, their oxygen uptakes increase markedly above the baseline levels, leading to the production of free radicals (Klein, 1989). Exogenous oxidants such as cigarette smoke and ozone enhance oxygen radical production and worsen the asthma exacerbation (Sahoo et al., 2009; Fujisawa, 2005). Excessive production of free radicals beyond the detoxification capacity of the antioxidant system causes oxidative stress with detrimental consequences such as airway smooth muscle contraction (Rhoden and Barnes, 1989), induction of airway hyper-responsiveness (Katsumata et al., 1990; Weiss and Bellino, 1986), mucus

hypersecretion (Adler et al., 1990), epithelial shedding (Doelman et al., 1990), vascular exudation (del Maestro et al., 1981) and induction of cytokine and chemokine production through oxidative stress sensitive transcription of nuclear factor-κB in bronchial epithelial cells (Biagioli et al., 1999). Immunologic factors causing asthma has always being the concern of the patients and some health workers. Meanwhile, adequate knowledge of the patho-physiology of the disease may suggest better treatment strategies. This study determined the status of markers of oxidative stress and C-reactive protein in the asthmatics during acute exacerbations.

METHODS

Twenty-two asthmatic patients volunteered to participate in this study. Another twenty age-matched, apparently healthy individuals without history of asthma or resent allergic reactions served as controls. Five (5) milliliters of blood was withdrawn from each patient into a lithium heparin bottle. The blood samples were centrifuged and the plasma separated and stored at -20^{0}C until analyzed.

Determination of CRP

CRP 0was quantified by single radial immunodiffusion method. A volume of an optimally diluted anti-CRP antiserum was mixed with noble agar and poured on glass plate. Wells of equal diameters were cut in the antibody-agar mixture. The wells were filled with test or standard sera. After incubation, the diameters of precipitin rings were measured using a Hyland viewer with a micrometer eyepiece.

Determination of Malondialdehyde (MDA)

Level of lipid peroxidation was determined by measuring the formation MDA using the method of Varshney and Kale, 1990. The principle is based on the fact that malondiahydehyde (MDA) produced from the peroxidiation of membrane fatty acid reacts with the chromogenic reagent; 2-thiobarbituric acid (TBA) under acidic conditions to yield a pink–coloured complex measured spectrophotometrically at 532nm. 1, 1, 3, 3-tetramethoxylpropane was used as standard.

Determination of Total Antioxidant Potential (TAP)

TAP was determined using the ferric reducing / antioxidant power (FRAP) assay (Benzie and Strain, 1996; Harma et al., 2003). 1.5 ml of working pre-wormed (37^{0}C) FRAP reagent (300mM acetate buffer - $_p$H 3.6, 10mM 2,4,6- tripyridyl-s-triazine in 40mM HCl and 20mM $FeCl_3$ at ratio 10:1:1) was vortex mixed with 50µl of test sample and standards. Absorbance was read at 593 nm against a reagent blank. The result was reported as µmol Trolox equiv. / L.

Determination of total plasma peroxide (TPP)

Principle

Ferrous-butylated hydroxytoluene-xylenol orange complex reacts with plasma hydrogen peroxide to form a color complex measured spectrophotometrically at 560mm. H_2O_2 was used as standard. 1.8ml of reagent 6 (F0X2) was mixed with 200µ1 of plasma. This was incubated at room temperature for 30 minutes. 100µMol H_2O_2 was used as standard. The mixture was centrifuged and the supernatant separated for reading at 560nm (Benzie and Strain, 1996).

Determination of oxidative stress index (OSI)

OSI, an indicator of the degree of oxidative stress is the percent ratio of the TPP to the TAP (Benzie and Strain, 1996).

Statistical analysis

The data were presented in the form of Mean and Standard deviation. Student (t) test was used for comparison between groups. The p-values of less than 0.05 were considered significant.

RESULTS

In table 2, significantly higher levels of TPP ($p<0.02$), OSI ($p<0.001$), MDA ($p=0.01$) and CRP ($p<0.01$) were observed in asthmatics when compared with the controls. There was no significant ($p>0.2$) change in the plasma level of TAP in the asthmatics when compared with the controls.

DISCUSSION

The present study show significantly higher levels of TPP and MDA in the asthmatics. This could be associated with excessive cellular activation commonly encountered in asthma. Klein, 1989 stressed that hypersensitivity reactions in the asthmatics enhance aggregation and activation of phagocytes around the site of inflammation in the lungs. The oxygen uptakes of the activated phagocytes increase markedly above the baseline levels, leading to the production of free radicals. Excessive production of free radicals beyond the detoxification capacity of the antioxidant system causes oxidative stress in our asthmatics. These free radicals in the

Table 1. Physical Characteristics of Asthmatics and Controls

	N	Age (years)	Height (Meter)	Weight (Kg)	BMI (Kg/Meter^2)
Controls	20	15.1±3.2	1.44±0.15	40.2±7.58	20.2±2.10
Asthmatics	22	16.3±2.8	1.51±0.15	41.5±7.45	19.1±2.08
p-values		>0.2	>0.2	>0.2	>0.2

N- number of subjects in the group
BMI- body mass index.

Table 2. Markers of Oxidative Stress and CRP in Asthmatics and Controls

	N	TPP (µMol/l of H_2O_2)	TAP (µMolTrolox equiv./L)	OSI (%)	MDA (nMol/ml)	CRP (Mg/L)
Controls 20		10.1±4.5	1100±250	0.9±0.6	2.0±0.8	3.2±1.3
Asthmatics	22	41.5±10.0	950±300	4.1±0.9	6.0±2.6	12±3.8
p-values		<0.02*	> 0.2	<0.001*	0.01*	<0.01* .

* = significantly different from the control.
N- number of subjects in the group

tissues have the potentials to abstract hydrogen atoms from the methylene groups (CH_2 group) of long-chain polyunsaturated fatty acids (LC-PUFA) which results in lipid peroxidation (Halliwell and Chirio, 1990; Miranda et al., 2004). The data previously published by Wood et al., 2000 and Montuschi et al.,1999 indicate that asthma severity is related to the extent of lipid peroxidation with a positive association between 8-isoPGF$_{2\alpha}$ concentrations and disease severity. Our result is consistent with some previous findings where higher levels of lipid peroxidation were reported in asthmatics (Gilmour et al., 2006; Ochs-Balcom et al., 2006). Misso and Thompson, 2005 also reported significantly higher levels of reactive nitrogen intermediates in asthma patients. This finding also corroborates the reports of Kirkham and Rahman, 2006 who associated the higher levels of reactive oxygen species and lipid peroxidation in asthma to the inflammatory response at many levels through its impact on signal transduction mechanisms, activation of redox-sensitive transcriptions factors, and chromatin regulation resulting in pro-inflammatory gene expression. Elevated products of lipid peroxidation (i.e. 8-isoPGF$_{2\alpha}$) have also been reported in the breath (Montuschi et al., 1999), urinary and bronchoalveolar lavage (BAL) fluid (Dworski et al., 1999) of allergen-induced asthma. Talati et al., 2006 reported that these products of lipid peroxidation can be suppressed by dietary vitamin E supplement.

Despite higher levels of TPP and MDA in the asthmatics, the antioxidant status was not significantly reduced. This could be associated with the antioxidant potentials of certain enzymes (i. e. myeloperoxidase and superoxide dismutase) released during degranulation of mast cells of the airway. Previous reports show higher activity of SOD and CAT in asthmatic patients (Mak and Chan-Yeung, 2006; Rahman et al., 2006). Our finding seems to agree with Ercan et al., 2006 who reported significantly higher level of GSH that correlated positively with increase in severity of asthma. These activities of antioxidant enzymes have been associated with periodical release of degranulation products of mast cell (histamine, heparin, prostaglandin D2, tryptase, β-hexosaminidase, β -glucuronidase, arylsulphatase, myeloperoxidase and superoxide dismutase) into the plasma during asthmatic attack. The present finding contradicts the reports of Nadeem et al., 2005 that plasma total antioxidant capacity and total protein sulfhydryls decreased significantly in asthma. Though, they also reported that there were no significant changes in the plasma glutathione peroxidase, protein carbonyls, total nitrates, red cell anti-oxidative enzyme activities, superoxide anion released from leukocytes, and total blood glutathione.

The CRP has been described as the surrogate marker for the activity of pro-inflammatory cytokines including tumor necrotic factor alpha (TNF-alpha) and interleukin 6 (IL-6) (28). Synergistic effect of TNF-alpha and IL-6 induces the hepatocytes to produce C-reactive protein (28, 29). Higher level of CRP observed in our asthmatics could be linked with increased cellular activation that might enhance the release of these pro-inflammatory cytokines in the asthmatics. Our study corroborates that of Sahoo et al., Sahoo et al., 2009 who reported higher levels of hs-CRP in asthmatics. In their study, they concluded that certain degrees of systemic inflammation and local bronchial inflammation occur in asthmatics (Sahoo et al., 2009). Fujita et al., 2007 stressed that the

mean serum hs-CRP levels increase significantly in asthma patients with or without attacks. They reported significant negative correlations between serum hs-CRP levels and forced expiratory volume in 1 second/forced vital capacity in all asthmatic patients.

In conclusion, oxidative stress and inflammation are possible features of asthma.

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