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Research Article

REDUCED PLASMA ANTIOXIDANT LEVELS IN SMOKING ASTHMATICS

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ABSTRACT
The imbalance in antioxidants is thought to play an important role in the pathogenesis of bronchial asthma. The present study was undertaken to evaluate the status of lipid peroxidation and antioxidants as biomarkers in human plasma. The extent of lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substances (TBARS) and the status of the antioxidants by reduced glutathione (GSH) in the smokers with and without asthma. The mean TBARS levels were higher among smokers with asthma (2.97±1.09) when compared with control smokers (0.75 ±0.81). The mean GSH level were higher in control non-smokers (0.172±0.21) compared to asthma smokers (0.032±0.03) and non smokers (0.061±0.06). Measured parameters confirm that cigarette smoke mediated oxidative stress could represent the primary causative factor in the pathogenesis of asthma.

KEY WORDS: Oxidative stress; Bronchial asthma; Thiobarbituric reactive substances; Total reduced glutathione.

INTRODUCTION

Smoking is considered to be one of the primary etiological factors of many respiratory diseases. Tobacco smoke contains complex mixture of over 4700 chemical compounds, including high concentrations of oxidants, capable of interacting with cell constituents. Experimental and clinical records suggest that oxidants play a role in the pathogenesis of respiratory disorders, including bronchial asthma. Impaired oxidant defense systems have been reported in adult smokers as compared with non-smokers, [1] the lower activities of the enzymes in smokers leads to oxidative stress. Oxidants also induce increased apoptosis of bronchial epithelial cells in asthmatic subjects [2, 3]. Inflammation driven by increased oxidative stress occurs in the airways of patients with asthma [4]. Increasing evidence reported that the chronic airway inflammation typical of asthma results in increased oxidative stress in the airways, as indicated by elevated levels of oxidative products in asthma patients [5]. Oxidative stress in the context of this study was assayed by lipid peroxidation (TBARS), reduced glutathione in peripheral blood as biomarkers of oxidative stress.

The interaction between genetic predisposition of the host and other environmental factors such as cigarette smoke and its effect on the imbalance between oxidants and antioxidants may probably be the explanation for these differences. We, therefore, prospectively studied the plasma levels of TBARS, an end-product of lipid peroxidation and GSH as indices of oxidative stress in chronic smokers and non smokers with or without stable asthma.

MATERIALS AND METHODS

Study Population

Eighty male subjects were enrolled in the study which includes cigarette smokers with asthma (n=20); non smokers with asthma (n=20); control smokers (n=20); and control non-smokers (n=20). The smoking history was measured by the unit of pack-year. A pack year history was calculated as: [(the number of cigarettes smoked per day/20) × the number of years smoked)]. Patients with COPD, atopy, tuberculosis, other respiratory disorders and other systemic diseases were excluded from the study. All control subjects were healthy individuals who were not receiving current medication or nutritional supplements and who had no history of lung and other major disorders.

Informed consent was procured from all participants. For the estimation of TBARS and GSH, 5 ml of peripheral venous blood samples were collected in heparin screw cap tubes and centrifuged. Plasma was separated and stored at -70^0C for processing.

TBARS assay

The TBARS levels were estimated as per the spectrophotometric method described by Ohkawa *et al* [6]. Briefly, to each test tube, 0.5 ml of plasma, 0.5 ml of normal saline, 1 ml of 20% trichloroacetic acid (TCA) and 0.25 ml of TBA reagent (200 mg of thiobacbituric acid in 30 ml distilled water and 30 ml of acetic acid) were added. The test tubes were kept for boiling at 95ºC for one hour. To each of the test tubes, 3 ml of n-butanol was added and mixed well. The tubes were centrifuged at 3000 rpm for 10 minutes. The separated butanol layer was collected and read in a spectrophotometer against reagent blank at 535 nm Thiobarbituric reactive substances concentration was expressed in terms of nmol of malondialdehyde per millilitre of plasma.

Reduced glutathione assay

The GSH levels were estimated by the method described by Moron *et al* [7]. Briefly, 0.1 ml of plasma was precipitated with 5% TCA and the precipitate was removed by centrifugation. To an aliquot of the supernatant, 2 ml of 5-5'- Dithiobis, 2-nitrobenzoic acid (DTNB) reagent was added to make the final volume three milliliter. Absorbance was read at 412 nm against a blank containing TCA instead of the sample. The amount of reduced glutathione was expressed as µmol/ mg of protein.

Statistical Analysis

Numerical values were reported in terms of mean and standard deviation. Statistical analysis was performed in ANOVA using the statistical package (SPSS).

RESULTS

A total of 40 adults with asthma and 40 age and sex matched healthy controls were recruited for the study. The demographic and clinical characteristics of the subjects are summarized in (Table-1). In all of the subjects, there were no significant differences in the mean age and smoking status.

The mean TBARS and GSH concentrations among asthma patients and control subjects are shown in (Table- 2). The mean values of TBARS levels were higher among smokers with asthma in comparison with smokers without asthma and control smokers and non smokers.The mean GSH levels were significantly higher in control non-smokers compared to asthmatics (p<0.001). The level of reduced glutathione was compared between smokers and nonsmokers, a significant decrease (p<0.001) in the levels of reduced glutathione was observed among smokers in both groups.

DISCUSSION

Smoking is an escalating health problem especially in developing countries such as India. Cigarette smoking leads to the uptake of many hazardous compounds and their metabolites extracted from burning tobacco. The prevalence of smoking in India varies from 15% to 50% among men [8]. However, smoking is less common among women [9]. Cigarette smoking leads to the uptake of many hazardous compounds and their metabolites extracted from burning tobacco. These substances may be electrophilic and react with biological molecules, and give rise to oxidative stress through the formation of reactive species in the membranes[10]. Smoking is a major public health issue due to its direct and indirect effects on health out come [11-13], which leads to oxidative stress. Smoking appears to result in elevation of several biomarkers of oxidant stress. Cigarette smoking impairs endothelial function, which may partly attributable to oxidant stress. Oxidative stress and its significance occur as a consequence of an imbalance between the productions of reactive oxygen species (ROS) and the available antioxidant defense against them. The generation of ROS has several undesirable consequences, including the impairment of cellular energetic [14] and defense [15] systems and the promotion of malignant transformation [16].

Tobacco smoke is a rich source of oxidants and increased production of reactive oxygen species associated with smoking may exceed the capacity of oxidant defense system, resulting in oxidative damage [17, 18]. Oxidative stress is the result of an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant system in favour of the former[19]. The potential damage that can be caused by free radicals is normally minimized by a combination of biological antioxidant systems including enzymatic and non-enzymatic reactions. In smokers, several arms of the oxidant defense system have been reported to be impaired as compared with non-smokers which leads to the lower activities of these enzymes in smokers due to oxidative stress. In the present study, the mean level of GSH was lower in both the group of smokers compared with non-smokers. However, the mean GSH levels were comparable in smokers with and without asthma. But TBARS levels were higher among smokers with and without asthma when compared with control smokers and non-smokers. These observations reflect increased lipid peroxidation because of oxidative stress due to smoking.

CONCLUSION

In conclusion, we found that smoking induced oxidative stress was associated with asthma, as reflected by the TBARS and GSH levels. Investigation of oxidative stress is an important area of research with latent applications for the monitoring of asthma and for development of new therapies. Our findings suggest that oxidative stress in the respiratory system may represent different mechanisms contributing to the pathogenesis of asthma. Hence prevention and quitting smoking are major public health goals.

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Table: 1 Demographic characteristics and smoking status of the study subjects

Parameters	Asthma subjects (40)		Controls (40)	
	Smokers (20)	Non smokers(20)	Smokers (20)	Non smokers(20)
Sex (M)	20	20	20	20
Age (years)	47.15 ± 4.31	44.8 ± 6.36	39.7 ± 9.64	49.1 ± 8.88
Weight (kg)	67.65 ± 7.83	65.24 ± 11.13	60.15±8.08	72.3 ± 9.98
Duration of asthma (years)	5.65 ± 2.34	6.25 ± 1.83	-	-
Smoking status (years)	15.4 ± 3.04	-	18.25 ± 4.97	-

All data are shown as mean ± SD

Table: 2 Plasma TBARS and GSH levels in the study population

Study parameters	Asthma subjects (40)		Controls (40)	
	Smokers (n=20)	Non-smokers (n=20)	Smokers (n=20)	Non-smokers(n=20)
TBARS (nmol of MDA/ml of plasma)	2.97±1.09*	1.17±0.94*	0.75±0.81	0.58±0.10
GSH (µmol/mg of protein)	0.032±0.03	0.061±0.06	0.013±0.01	0.172±0.21*

All data are shown as mean ± SD;
TBARS= thiobarbituric reactive substances; GSH=total reduced glutathione.

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