Indian J Physiol Pharmacol 2005; 49 (1): 95–98

# OXIDATIVE STRESS AND PROTEIN GLYCATION IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

MADHURI PARIJA[1], ZACHARIAH BOBBY[1]*,
VINOD KUMAR S.[2] AND SARADHA B[1].

*Departments of [1]Biochemistry and [2]Tuberculosis and Chest Disease, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry – 605 006*

**( Received on July 15, 2004 )**

**Abstract :** Several studies have indicated the presence of increased oxidative stress as a critical feature in the pathogenesis of chronic obstructive pulmonary disease (COPD). Another biochemical complication leading to pathogenesis is protein glycation. The nexus between oxidative stress and protein glycation in various pathological conditions is being unraveled. Increased oxidative stress can lead to enhanced protein glycation by a process of auto – oxidative glycation. No information is available in the literature regarding protein glycation among COPD patients. Eleven non – diabetic COPD patients were included in the study and equal number of age and sex – matched healthy individuals were enrolled as controls. The whole - blood reduced glutathione was found to be less among the patients while lipid peroxides and fructosamine were elevated in comparison to control. The present study confirmed oxidative stress and enhanced protein glycation among the COPD patients. Antioxidant therapy may be considered as part of the treatment regimen for COPD patients.

**Key words :** COPD lipid peroxidation protein glycation

## INTRODUCTION

While considerable progress has been made in development of drugs for asthma, there have been few advances in the treatment of chronic obstructive pulmonary disease (COPD) (1). The inflammatory response in COPD is essentially steroid resistant and differs markedly from that of asthma so that alternative anti – inflammatory therapy is needed. New therapeutic approaches will arise out of better understanding of the disease process at cell and molecular level. Oxidative stress is implicated in the pathogenesis of several disease processes. An increasing amount of research has focused on the proposal that an oxidant/antioxidant imbalance occurs in smokers and in patients with COPD as part of the pathogenesis of the condition (2–4). The presence of increased oxidative stress is a critical feature in the pathogenesis of COPD, since it results in inactivation of antiproteinases, airway epithelial injury, mucus hypersecretion, increased sequestration of neutrophils in pulmonary microvasculature

*Corresponding Author :

and gene expression of pro-inflammatory mediators. Oxidative stress also has a role in enhancing the airway inflammation. Oxidative stress, measured as thiobarbituric acid reactive substances (TBARS) in plasma, has also been shown to correlate inversely with the percent predicted $FEV_1$ in a population study, indicating that lipid peroxidation is associated with airflow limitation in the general population (5). An association between dietary intake of antioxidant vitamins and lung function has been demonstrated in the general population (6). Another biochemical complication leading to pathogenesis is protein glycation (7). It is also implicated in the acceleration of normal aging process (8). The nexus between oxidative stress and protein glycation in various pathological conditions is being unraveled. Increased oxidative stress can lead to enhanced protein glycation by a process of auto-oxidative glycation (9). Although *in-vitro* experimental evidences are available in support of the hypothesis (10), not much information is available from *in-vivo* pathological situations. We have observed that in cases of non-diabetic renal failure, nephrotic syndrome, hyperthyroidism and rheumatoid arthritis, oxidative stress could enhance protein glycation (11–13). The present study was carried out to find out if COPD is associated with enhanced protein glycation and to elucidate the nexus between oxidative stress and protein glycation if any.

## METHODS

**Selection of cases and control:** Eleven patients with COPD attending the Out Patients Department of Tuberculosis and Chest Disease, JIPMER hospital was included in the study. The diagnosis of COPD was based on clinical history, pulmonary function tests and X-ray. Eleven age and sex-matched control samples were collected from healthy individuals. Diabetic patients were excluded from the study. Ethics clearance for the study was obtained from Research Council, JIPMER, Pondicherry. The study was carried out with the blood samples collected for the routine biochemical investigations required by the clinicians for the patients. Five milliliters of fasting blood samples were collected in EDTA bottles for the study.

**Assessment of biochemical parameters:** Estimation of lipid peroxidase was carried out with thiobarbituric acid (14). Whole blood reduced glutathione (GSH) (Reference range: $2.02 \pm 0.32$ mg/g Hb) was estimated with Ellman's reagent and the results were expressed as mg per gram of hemoglobin (15). Plasma fructosamine (Reference range: 1.6–2.8 mmol/L) was estimated by p-indo-nitrotetrazolium violet kinetic method using the Raichem kits (Hemagen diagnostics, San Diego, CA, USA) adapted to 550 express plus autoanalyzer (Ciba Corning Diagnostics, Oberlin, OH).

**Statistical analysis:** The data between control and test groups was compared using unpaired student's t test.

## RESULTS

The whole blood reduced glutathione was found to be significantly reduced among the COPD patients in comparison to the age and sex-matched controls. The plasma lipid peroxides and plasma fructosamine were

TABLE I: Mean and standard deviation of age, oxidative stress parameters and fructosamine concentrations in controls (n=11) and non-diabetic COPD patients (n=11).

| *Parameters* | *Control group* | *COPD group* | *'p' value* |
|---|---|---|---|
| Age (years) | 54.72 (7.52) | 56.54 (8.0) | 0.58 |
| Male/Female | 9/2 | 10/1 | – |
| Whole blood GSH (mg/g Hb) | 1.79 (0.24) | 1.37 (0.24) | 0.00094 |
| Plasma lipid peroxides (μM/l) | 5.04 (0.64) | 7.13 (1.99) | 0.00363 |
| Plasma fructosamine (mmol/l) | 2.80 (0.552) | 3.55 (0.716) | 0.0109 |

A 'p'<0.05 was considered significant.

raised in the patients in comparison to the control group. The above results are given in Table I.

## DISCUSSION

The results obtained indicate enhanced oxidative stress and protein glycation in the COPD patients. Since all the COPD subjects included in the present study were non-diabetic, enhanced protein glycation cannot be attributed to impaired glucose tolerance. Another cause for protein glycation is the prevailing oxidative stress. In vitro experimental evidences indicate that pro-oxidative forces can enhance protein glycation by autoxidative glycation and glycoxidation (16). *In vitro* study by Jain et al has indicated that the process of protein glycation can be promoted by malondialdehye (MDA), an end product of lipid peroxidation (17). However, the mechanism by which MDA enhances glycation of proteins has not been clearly defined. It is presumed that the aldehyde groups of MDA act as an anchor between sugar and protein moieties, thereby enhancing the formation of glycated proteins (17). In-vitro experiments have indicated that antioxidants such as vitamin C and glutathione play a possible role in preventing the process of protein glycation (18). An inhibiting effect of vitamin C on glycosylation of proteins has been reported (19). In our earlier studies in non-diabetic patients with undialyzed chronic renal failure, pediatric nephrotic syndrome, hyperthyroidism and rheumatoid arthritis, we found a nexus between oxidative stress and protein glycation (11–13). In the present study with non-diabetic COPD patients, we found a similar significantly enhanced protein glycation that could be attributed to the oxidative stress. A significant correlation between oxidative stress and protein glycation in the COPD patients could not be established and this might be due to the fact that they were under treatment. It will be interesting to know the effect of an early inclusion of antioxidant therapy in the treatment regimen on the level of protein glycation among the COPD patients.

## REFERENCES

1. Barnes PJ. Potential Novel Therapies for chronic obstructive pulmonary disease. *Novartis Found Symp* 2001; 234: 255–272.

2. Rahman I, MacNee W. Role of oxidants/antioxidants in smoking-induced lung diseases. *Free Radic Biol Med* 1996; 21: 669–681.

3. Tsukasoshi H, Shimizu Y, Iwamae S, et al. Evidence of oxidative stress in asthma and COPD; potential inhibitory effect of Theophylline. *Respir Med* 2000; 94(6): 584–588.

4. Rahman I, Swarska E. Is there any relationship between plasma antioxidant capacity and lung function in smokers and in patients with chronic obstructive pulmonary disease? *Thorax* 2000; 55(3): 189–199.

5. Schunemann HJ, Muti P, Freudenheim JL, Armstrong D, Browne R, Klocke RA, Trevisan M. Oxidative stress and lung function. *Am J Epidemiol* 1997; 146: 939–948.

6. Britton JR, Pavord ID, Richards KA, et al. Dietary antioxidant vitamin intake and lung function in the general population. *Am J Respir Crit Care Med* 1995; 151: 1383–1387.

7. Brownlee M, Cerami A, Vlassara H. Advanced glycosylation end products in tissue and the biochemical basis of diabetic complications. *N Eng J Med* 1998; 318: 1315–1321.

8. Monnier VM. Nonenzymatic glycosylation, the Maillard reaction and the aging process. *J Gerontol* 1990; 45: 105–111.

9. Wolff SP. Diabetes Mellitus and free radicals: free radicals, transition metals and oxidative stress in the aetiology of diabetes mellitus and complications. *Brit Med Bull* 1993; 49: 642–452.

10. Wolff SP, Dean RT. Glucose autoxidation and protein modification: potential role of autooxidative Glycosylation in diabetes. *Biochem J* 1987; 245: 243–250.

11. Selvaraj N, Zachariah Bobby, Ashok Kumar Das, Ramesh R, Bidhan Chandra Koner. An evaluation of level of oxidative stress and protein glycation in nondiabetic undialyzed chronic renal failure patients. *Clin Chim Acta* 2002; 324: 45–50.

12. Balamurugan R, Zachariah Bobby, Selvaraj N, Nalini P, Bidhan Chandra Koner, Sen SK. Increased protein glycation in non-diabetic pediatric nephrotic syndrome: Possible role of lipid peroxidation. *Clin Chim Acta* 2003; 337: 127–132.

13. Mohan Kumar KM, Zachariah Bobby, Selvaraj N, et al. Possible link between glycated hemoglobin and lipid peroxidation in hyperthyroidism. *Clin Chim Acta* 2004; 342: 187–192.

14. Yagi K. Methods of enzymology. London, Academic Press. 1984; 105: 328–331.

15. Beutle E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med* 1963; 61: 882–888.

16. Lindsay RM, Smith W, Lee WK, Dominiczak MH, Baird JD. The effect of delta Gluconolactone, an oxidized analogue of glucose, on the non-enzymatic glycation of human and rat haemoglobin. *Clin Chim Acta* 1997; 263: 239–247.

17. Jain SK, Palmer M. The effect of oxygen radicals metabolites and vitamin E on glyosylation of proteins. *Free Radical Biol Med* 1997; 22: 593–596.

18. Stolba P, Hatle K, Krnakova A, Streda M, Starka L. Effects of ascorbic acid on nonenzymatic glycation of serum proteins *in vitro* and *in vivo. Diabetologia* 1987; 30: 529.

19. Huby R, Harding JJ. Non-enzymatic glycosylation (glycation) of lens proteins by galactose and protection by aspirin and reduced glutathione. *Exp Eye Res* 1988; 47(1): 53–59.