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Research article

STUDY OF SERUM MALONDIALDEHYDE AND WHOLE BLOOD REDUCED GLUTATHIONE IN EMPHYSEMA PATIENTS

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ABSTRACT

Background: Emphysema is associated with high incidence of morbidity and mortality. The imbalance between oxidants and antioxidants is thought to play an important role in the pathogenesis of Emphysema. **Methods**: A total number of 80 subjects comprising of 40 healthy controls and 40 Emphysema cases were studied. In all the subjects, serum levels of malondialdehyde (MDA) as a biomarker of lipid peroxidation and antioxidant whole blood reduced glutathione (GSH) were estimated. **Results:** The level of whole blood reduced glutathione was significantly decreased in emphysema patients when compared to controls. Serum MDA was significantly increased in Emphysema patients when compared to controls. **Conclusion**: The presence of increased systemic oxidative stress in emphysema patients seems to be associated with current active smoking and systemic inflammation. The decrease in antioxidants levels appears to be mainly a consequence of increased oxidative stress. This suggests that oxidative stress is likely to be involved in pathogenesis of emphysema.

Keywords: Oxidative stress, Antioxidants, Emphysema, Whole blood reduced glutathione, MDA.

INTRODUCTION

Emphysema is a long term progressive disease of the lungs that primarily causes shortness of breath. It is generally considered one of the two forms of Chronic Obstructive Pulmonary Disease (COPD) and Emphysema is associated with cigarette smoke–induced COPD.[1]

American Thoracic Society defines Chronic Obstructive Pulmonary Disease as a disease state characterized by the presence of air flow obstruction due to chronic bronchitis or emphysema; the airflow obstruction is generally progressive, may be accompanied by airway hyper-reactivity, and may be partially reversible.[2]

Emphysema is a pathologic diagnosis that denotes abnormal permanent enlargement of air spaces distal to the terminal bronchiole, with destruction of their walls without obvious fibrosis.[2]

Two hypothesis accounts for the destruction of alveolar walls are the protease– antiprotease mechanism and imbalance of oxidants and antioxidants.[3]

The protease–antiprotease imbalance hypothesis is based on genetic deficiency of the antiprotease, α-1 – antitrypsin which is a major inhibitor of protease (particularly elastase) secreted by neutrophils during inflammation.α-1–antitrypsin deficiency develop symptomatic panacinar emphysema which occurs at an earlier age and increases with severity if individual smokes. About 1% of all patients with emphysema have this defect.[3]

Smoking plays an important role in disturbing the oxidant antioxidant balance which leads to the pathogenesis of emphysema. Normally, the lung contains a healthy complement of antioxidants (superoxide dismutase, glutathione) that keeps oxidative damage to a minimum. Tobacco smoke contains abundant reactive oxygen species (free radicals) and activated neutrophils released due to smoking also add to the pool of reactive oxygen species in the alveoli, which deplete these antioxidant mechanisms, leading to tissue damage. A secondary consequence of oxidative injury is inactivation of native antiproteases, resulting in "functional"α-1 – antitrypsin deficiency even in patients without enzyme deficiency[3]

The reduced form of Glutathione present in red blood cells functions as an efficient scavenger of hydrogen peroxide and plays an important role in the prevention of peroxidative lung damage.[4] The reduced form of glutathione is an efficient antioxidant of both intracellular and extracellular medium. Cells are thought to be protected by extracellular GSH from oxidants produced and released by inflammatory cells, and by intracellular GSH from oxidants produced in normal biochemical processes and from xenobiotics.[5, 6]

Malondialdehyde is the organic compound with the formula $CH_2(CHO)_2$. This reactive species occurs naturally and is a marker for oxidative stress. Reactive oxygen species degrade polyunsaturated lipids, forming Malondialdehyde. This compound is a reactive aldehyde and is one of the many reactive electrophilic species that cause toxic stress in cells and form covalent protein adducts which are referred to as advanced lipoxidation end products (ALE). The product of this aldehyde is used as a biomarker to measure the level of oxidative stress in an organism.[7]

Present study is undertaken to evaluate antioxidant whole blood reduced glutathione and serum Malondialdehyde as a marker of oxidative stress in controls and in emphysema patients.

MATERIALS AND METHODS

A cross sectional study of whole blood reduced glutathione and serum malondialdehyde in controls and emphysema patients were carried out from April 2009 to April 2010. Controls and emphysema cases were selected from Bapuji Hospital and Chigateri General Hospital, Davangere. Each gave an informed consent and this study was approved by the ethical and research committee of J.J.M Medical College, Davangere to use human subjects in the research study. The patients and controls voluntarily participated in the study.

A total number of 80 subjects were participated in the study, of which 40 were healthy controls and 40 were emphysema cases. Among 40 controls, 30 were male and 10 were female and their mean age was 57.5 ± 7.4 years and among 40 emphysema cases, 32 were male and 8 were female and their mean age was 60.7 ± 5.8 years. There were no significant differences in age among cases and controls.

Inclusion criteria:

i) Cases: 40 clinically and radiologically diagnosed cases of emphysema were included.

ii) Controls: 40normal healthy individuals without any history of smoking and chronic lung disease were included.

Exclusion criteria: Patients with pneumonia, asthma or other chronic respiratory disease, history of diabetes mellitus, hepatic disease, cardiac failure, recent surgical intervention and renal disease

Collection of blood samples: About 5ml of blood was collected from large peripheral vein

under aseptic precaution after overnight fasting. Out of which 2ml was taken in an anticoagulant (EDTA) bulb for estimation of whole blood reduced glutathione (GSH) and 3ml in a plain bulb for estimation of serum malondialdehyde (MDA).

Estimation of Whole Blood Reduced Glutathione: Whole blood reduced glutathione was estimated by Ernest Beutler et al., Method. [8] It is based on the principle that all of the non-protein sulphydryl groups of red blood cells are in the form of reduced glutathione (GSH). 5,5[1] - dithiobis-2-nitrobenzoic acid (DTNB) is a disulphide compound, which is readily reduced by sulphydryl compounds, forming a highly colored yellow compound. Optical density measured at 412nm and is directly proportional to the GSH concentration.

Estimation of Serum Malondialdehyde : Serum malondialdehyde estimated by Kei Satoh Method.[9] It is based on the principle of auto-oxidation of unsaturated fatty acids involves the formation of semistable peroxides, which then undergo a series of reactions to form malondialdehyde (MDA). MDA reacts with thiobarbituric acid (TBA) to form pink colored chromogen. The resulting chromogen is extracted with 4.0ml of n-butyl alcohol and the absorbance of which is measured at 530 nm.

Statistical analysis: Results are expressed as mean ±SD and range values. Unpaired't' test is used for comparing different biochemical parameters between cases and controls. p value of < 0.05 was considered as statistical significance.

RESULTS

Table 1: Comparison of Serum MDA and Whole Blood Reduced Glutathione in Controls and Emphysema cases

Groups	MDA (nmol/ml)	GSH (mg/dl)
Control	2.52 ± 0.42	32.65 ± 2.23
Emphysema	5.79 ± 0.42	25.39 ± 1.70
Mean difference	3.27	7.26
t*	28.11	17.17
p	< 0.001	< 0.001

* Unpaired t- test

Table 1 shows biochemical characteristics of the study subjects. Serum mean level of malondialdehyde a biomarker of lipid peroxidation was significantly (p< 0.001) increased in emphysema patients (5.79±0.42 nmol/ml) when compared to controls (2.52 ±0.42 nmol/ml) t Mean level of whole blood reduced glutathione was significantly (p< 0.001) decreased in emphysema patients (25.39 ±1.70 mg/dl) than in controls (32.65 ±2.23 mg/dl). These results indicate that increase in oxidative stress and decrease in antioxidant level in emphysema cases when compared to controls

DISCUSSION

Oxidative stress plays an important role in the pathogenesis of emphysema. Antioxidants transform free radicals into less reactive species, thereby limiting their toxic effects. Even when present at low concentrations compared with those of an oxidizable substrate, antioxidants

significantly delay or prevent oxidation of that substrate.[10]

These results indicate that there is increase in oxidative stress and decrease in antioxidant levels in emphysema patients when compared to controls. When compared to controls, emphysema patients have significantly decreased (p value < 0.001) level of GSH. This is in accordance with the study of Madhuri Parija et al[11],Mukadder calikoglu et al [12]andMacNee W et al.[13]

Glutathione exists in reduced (GSH) and oxidized (GSSH) states. In the reduced state, the thiol group of cysteine is able to donate a reducing equivalent ($H^+ + e^-$) to other unstable molecules, such as reactive oxygen species. In donating one electron, glutathione itself becomes reactive, but readily reacts with another reactive glutathione to form glutathione disulfide (GSSG). In healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form and less than 10% exists in the disulfide form (GSSG). An increased GSSG to GSH ratio is considered indicative of oxidative stress[14]

The airways of smokers are exposed to highly reactive components and the lung is always at the risk of oxidative injury. Most of the intracellular glutathione is stored in the reduced form (GSH). During increased oxidative stress, the free sulfhydryl (- SH) groups become oxidized resulting in loss of GSH. The gaseous phase of cigarette smoke may also irreversibly react with GSH to form GSH derivatives that cannot be reduced back, thereby depleting the total available reduced glutathionepool. [15]

After exposure to cigarette smoke condensate(CSC) The activities of glutathione synthesis and redox system enzymes such as glutathione peroxidase, gamma-glutamyl cysteine synthetase and glucose-6-phosphate dehydrogenase were transiently decreased in alveolar epithelial cells, possibly as a result of the action of highly electrophilic free radicals on the active site of enzymes. Thus there is a time dependent depletion of intracellular soluble GSH, concomitant with the formation of GSH conjugate [16]

The enzymatic redox cycle, which is normally activated after oxidative stress and the formation of GSSG, could not be activated because of the depletion of GSH into non reducible glutathione components, with the loss of the GSH pool. This exhaustion of the pool of reduced GSH may induce a chronic lack of antioxidant protection. Persistent smokers may inhale more reactive oxygen species than can be scavenged by the residual antioxidants, resulting in increased vulnerability to oxidative stress. This makes the synthesis of GSH essential for cellular survival and protection of the lung.[15]

When compared to controls, emphysema patients have significantly (p value < 0.001) increased level of MDA. This is in accordance with the study of M.K. Daga et al [17], and Gamze kirkil et al[18]

MDA is a lipid peroxidation product which is formed during oxidative process of PUFA by reactive oxygen species. All of the major classes of biomolecules may be attacked by free radicals but lipids are the most susceptible. Cell membranes are rich sources of polyunsaturated fatty acids which are readily attacked by oxidizing radicals. The oxidative destruction of PUFA by deleterious free radical reactions is known as lipid peroxidation. Lipid peroxidation has been implicated in a wide range of cell and tissue damages, diseases, biological variables and life habits.[19]

Oxidative stress has been implicated in the pathogenesis of tobacco smoke induced chronic obstructive pulmonary disease and Cigarette smoke exposes the lung to extreme levels of oxidative stress. Reactive oxygen species present in the tobacco smoke may cause damage to human alveolar epithelial cells by lipid peroxidation of cell membranes. Increased MDA concentration in patients with emphysema may be due to increased production of reactive oxygen species and hence more lipoxidation products.[20]

CONCLUSION

In conclusion there is an oxidant and antioxidant imbalance in emphysema patients when compared to healthy controls and this imbalance play an important role in the pathogenesis of emphysema. Hence further studies are required on beneficial effect of consumption of diet rich in antioxidants and their effect on preventation of oxidative damage in emphysema.

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