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Study of Serum Malondialdehyde and Vitamin C in Chronic Bronchitis Patients

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Abstract: The oxidative stress caused by free radicals and reactive oxygen species released during smoking can play a significant role in pathogenesis and development of Chronic Bronchitis. This study is conducted to evaluate serum levels of Malondialdehyde (MDA) a lipid peroxidation product as a marker of oxidative stress and vitamin C major antioxidant in body in chronic bronchitis patients and healthy controls. Comprised of 80 subjects, 40 healthy controls and 40 chronic bronchitis cases were included. Kei Satoh method used for measuring MDA and Serum vitamin C was estimated by method 2,4-Dintrophenyl hydrazine method Serum MDA a lipid peroxidation product from cell membrane considered as marker of oxidative stress was elevated in chronic bronchitis patients when compared to healthy controls where as serum level of vitamin C major antioxidant significantly decreased in chronic bronchitis patients when compared to controls.. MDA maker is of oxidative injury significantly increased in chronic bronchitis cases which is likely associated with active smoking habit and also be due to systemic inflammation. The decrease in level of antioxidant capacity in chronic bronchitis patients appears likely consequence of higher oxidative stress and increased consumption of antioxidants. By stopping smoking and advising antioxidant rich food may halt the further progression of chronic bronchitis disease.

Keywords: Chronic bronchitis, MDA, Vitamin C, Free radicals.

INTRODUCTION

Chronic Bronchitis is described by chronic inflammation of the lungs mainly in bronchi. It includes one of the two variants of Chronic Obstructive Pulmonary Disease (COPD). COPD patients are higher in areas where smoking habit is common. In India consumption of cigarette and tobacco is high so chronic bronchitis is thought to be one of the major health concerns [1]. Thoracic Society of America defines Chronic Obstructive Pulmonary Disease as "A disease state that characterized by the obstruction to airflow caused due to chronic bronchitis or emphysema; here airflow obstruction is progressive and accompanied hyper-reactivity of airways in lungs and may be reversible partially[2]".

Chronic bronchitis is clinically diagnosed by increased bronchial mucus secretion and is featured by productive cough daily for continuous 3 months or more in at least two consecutive years [2]. A present hypothesis in pathogenesis of COPD is due to excess oxidative stress consequence of current active smoking practice and also due to spilling of reactive oxidative compounds from airspace WBCs which are not precisely counter balanced by respiratory antioxidant defense mechanism, leading to in elevated oxidative stress in lungs. Increased formation of oxidative compounds lead to increased pro inflammatory gene expression and also tissue injury by oxidants proceeds to COPD formation [3].

Malondialdehyde with the chemical formula CH_2 (CHO) [2]. Free radicals and other oxidants hydrolyse PUFA molecules present in cell membrane leadind to formation of Malondialdehyde. MDA is very reactive and is one of the electrophilic compounds that can cause toxic damage in tissues. MDA induces damage to tissues by formation reactive compounds which are considered as ALE (advanced lipoxidation end products)[4].

On other side deficiency of antioxidants also leads increase in oxidative stress. Antioxidants protect body from highly reactive oxygen and nitrogen compounds and also bring changes in the inflammatory reactions that can cause a significant role in development of chronic bronchitis [5]. Lipid soluble antioxidant vitamin E is body's major defense against oxidative stress in preventing cell membrane injury. Vitamin C is a water soluble

antioxidant, can directly scavenge free radicals like reactive oxygen and nitrogen species and helps to reduce the oxidant burden created by active smoking habit [6]. The trial is conducted study serum levels Malondialdehyde and serum vitamin C in controls and in chronic bronchitis patients.

MATERIALS AND METHODS

Present study of serum Malondialdehyde and serum vitamin C was carried out in controls and Chronic Bronchitis cases from medicine outpatient department, JJM medical college and Bapuji hospital Davangere. Institutional ethics committee approved study and informed consent taken from patients.

Inclusion criteria

- Cases: 40 chronic bronchitis cases which were diagnosed included
- Controls: 40 normal healthy individuals without any past history of smoking habit

Exclusion criteria

- Patients with chronic lung disease such as asthma
- Patients with history of recent surgical operation
- Patients with history of DM
- Patients with recent history of major illness

Collection of blood samples

About 5ml of venous blood collected under aseptic conditions in plain vial for biochemical tests at admission. Immediately after collecting sample serum is separated properly stored.

The following tests carried out in cases and controls

- Serum Malondialdehyde(MDA)
- Serum Vitamin C

Estimation of serum Malondialdehyde

Serum Malondialdehyde estimation carried out by method Kei Satoh [7]. It is characterized by principle of auto-oxidation of PUFA molecules present in cell membrane leading to formation of semistable peroxides, these reactive compounds undergo a series chain reactions to form malondialdehyde (MDA). MDA undergo reaction with compound TBA (thiobarbituric) to produce pink colored substance. This resulting colored substance is extracted with n-butyl alcohol and the absorbance is measured at 530 nm

Estimation of serum Vitamin C

Serum vitamin C was measured by 2, 4 – dinitrophenyl hydrazine method [8]. Here ascorbic acid is oxidized by copper ions to form compounds dehydroascorbic acid and diketogulonic acid. These compounds are treated with 2, 4-dinitrophenyl hydrazine (DNPH) to form the derivative bis-2,4-dinitrophenyl hydrazone. This product in strong sulfuric acid, undergoes modification to form a colored product which is measured at 520nm.

STATISTICAL ANALYSIS

Results are expressed as mean ±SD. The unpaired‘t’ test is used for comparing different biochemical parameters between cases and controls. The ‘P’ value of < 0.05 was considered as statistical significance.

RESULTS

A total number of 80 subjects were included in the study of which 40 consist of cases and 40 were controls. Among 40 controls, 25 were male and 15 were females and among 40 chronic bronchitis cases, 28 were male and 12 were females.

Table-1: Comparison of Serum Malondialdehyde and vitamin C in Controls and chronic bronchitis cases

Groups		MDA (nmol/ml)	Vitamin C (mg/dl)
Controls	Mean ± SD	2.42 ± 0.47	1.18 ±0.21
Chronic bronchitis	Mean ± SD	4.66 ± 0.46	0.72 ±0.14
Controls vs. chronic bronchitis	Mean difference	2.24	0.46
	t*	19.15	17.65
	P	< 0.001	< 0.001

* Unpaired t- test, P value < 0.001 considered as highly significant

Table 1 shows biochemical characteristics of the study subjects. Serum mean level of MDA a marker of lipid peroxidation was significantly (p< 0.001) elevated in chronic bronchitis patients when compared to controls. Mean level of vitamin C major antioxidant was significantly (p< 0.001) decreased in chronic bronchitis patients than in controls

DISCUSSION

The findings from present trial observed that there is an elevated oxidative stress and decrease in antioxidant levels in chronic bronchitis cases when related to healthy controls. Oxidative stress caused by free radicals released from active smoking habit involved in tissue injury and development of COPD [9].

Smoking is one of the major factors in pathogenesis and formation of chronic bronchitis. Smoking directly associated with chronic bronchitis cases worldwide. In developing countries such as India 40% of chronic bronchitis cases are related with non

smoking causes such as fuel burning examples include usage of wood and cow dung and burning crop wastages from farm which releases of reactive oxygen compounds into the atmospheric air [10].

Airway obstruction is vital clinical finding in identification of chronic bronchitis. In development of disease initially elastin tissue in lungs undergo hydrolysis leading to decrease in elastic recoiling capacity in the lungs, Flow of air in lungs mainly dependent on elastic recoil capacity , the injury to elastin tissue in chronic bronchitis causes airway obstruction air-trapping in lungs and fibrosis of the airways also causes airway narrowing. Further Consequences of these changes leading to development of chronic bronchitis disease [11, 12].

Chronic bronchitis is also associated with systemic inflammation. The systemic inflammation which happens in chronic bronchitis disease is due to release of inflammatory compounds from the lungs into the systemic circulation through the thin layered respiratory vasculature that can expose the other tissues of the body to inflammatory consquences [13].

MDA is produced during oxidation of PUFA molecules in cell membrane by free radicals. Free radicals can damage all organic and inorganic compounds in body but PUFA present in cell membranes are the most susceptible. Cell membranes are rich in PUFA molecules which are attacked by free radicals and reactive oxygen species [14]. Chronic bronchitis patients are associated with increased oxidative stress and higher level of MDA. When correlated with healthy controls, chronic bronchitis cases have significantly (P value < 0.001) higher level of MDA. Results here are in conferring with the research done by M.K. Daga *et al.* [15], and Gamze kirkil *et al.* [16]. When associated to healthy controls chronic bronchitis cases have significantly reduced (P value <0.001) level of vitamin C. This is in conferring with the research done by Mukuddar colikoglu *et al.*[17].

Vitamin C executes its function by by preventing cellular molecules from getting oxidized by donating its electrons. But in this action of vitamin C by donating electrons gets oxidized itself. This newly formed chemical product formed after loss of electron is ascorbyl radical which is more stable with half life of few seconds and is relatively nonreactive which explains the antioxidant function carried out by ascorbic acid. The mechanism involved in the decreased level of vitamin C level in cases may be due rapid utilization of ascorbic acid by reactive oxygen and nitrogen species. The inverse relationship between vitamin C and MDA may be due to the depletion of vitamin C when the oxidant burden is elevated because of its excess utilization [18].

Tobacco smoking causes release of reactive free radicals which is directly associated in the pathogenesis and progression of Chronic Bronchitis disease [15]. Increased MDA concentration and reduced level of antioxidant vitamin C in patients with chronic bronchitis is due to excess formation of reactive oxygen species because of current active smoking and inflammatory reaction and decrease level of antioxidants due its excess utilization for neutralizing free radicals.

CONCLUSION

In present study we have observed that there is increased oxidation of biomolecules in chronic bronchitis patients mainly consequence of current active smoking habit and decrease in antioxidant capacity when related to healthy controls. This study demonstrates the possible effects of imbalance in oxidative stress and antioxidant capacity in pathogenesis of chronic bronchitis.

Clinical significance

By stopping tobacco smoking and consumption of food rich in antioxidants may prevent the further oxidative stress induced alveolar damage in chronic bronchitis patients.

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