Tobacco, oxidative stress and protein carbonyl content

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Abstract- The highest risks of exposure of tobacco for both adults and adolescents, the major concern is that tobacco consumption is associated with certain other behaviors, long range consequences and show characteristic facial changes. Definite role for reactive oxygen species (ROS) in the tissue destruction that characterizes in tobacco addicts and protein carbonyl is the most widely used biomarker for oxidative damage to proteins, and reflects cellular damage induced by multiple forms of ROS. The purpose of this study is to determine malondialdehyde and protein carbonyl content in tobacco addicts with Otorhinological manifestations and to find an association between them.

Material and Methods : - Total 300 subjects were selected. 150 were healthy control group and 150 patients were presented with one or more otorhinolaryngological manifestations. In addition to demographic data, information regarding tobacco use was documented in the subjects.

Results : - Both benign and malignant conditions of ENT were common in male patients. MDA, marker of oxidative stress and protein carbonyl content were increased in both benign and malignant conditions of otorhinolaryngological. Both values were higher in malignant lesions than benign lesions. There was significant positive correlation between MDA and protein carbonyl levels in cancer patients.

Conclusion : - Increase in the oxidative stress (MDA) to such an extent that damages the DNA and increase in the protein carbonyl content would leads to cancerous condition. Protein Carbonyl estimation can be done in the unknown primary lesions and dysplastic lesions.

I. Introduction

The most widely distributed and commonly used drug in the world is 'Tobacco'. Tobacco is the most easily accessible legally available addictive substance which contributes significantly to premature death and long term suffering, being a major risk factor for cardiovascular diseases, chronic obstructive pulmonary diseases, cancers, reproductive outcomes and oral diseases (Sauvaget et al., 2008; Zarocotas, 2011; Sarkar and Reddy, 2012).In addition, one third of the global burden of oral cancer is predominantly attributed to high prevalence of tobacco consumption within India (Byakodi et al., 2012; Gupta et al., 2013).

Tobacco addiction is leading preventable cause of deaths both in the India and the World. Tobacco addiction includes addiction to tobacco and tobacco related products like cigarettes, bidis, gutkas, pan masalas. Reactive oxygen species were generated by tobacco smoke (1) leads to increase in the oxidative stress. Tobacco causes increase in oxidative stress which is duration dependent (2). Diagnosis of the Benign and malignant head and neck conditions has been based upon clinical, pathological and radiographic examination of the lesion. These parameters are of limited use in prognosis and prevention of lesions. Increase in oxidative stress leads to free radical damages genome, membranes and macromolecules of macrophage cells to increased carbonyl contents of proteins which affect their biological functions as enzymes, hormones, transport proteins and immune proteins etc.

This clinico-biochemical study was undertaken to estimate the protein content levels in benign and malignant head and neck conditions and to assess the role of MDA as a marker of oxidative stress in cancer patients

II. Aims and Objectives

The present project was designed so as to study carbonyl content and oxidative stress. Our aim was to study whether there is correlation development between ENT diseases like oral sub mucus fibrosis, stomatitis, and gingivitis, leukoplakia, erythroplakia, carcinoma and the biochemical parameters.

1) To study the oxidative stress in the different tobacco consumption pattern.

2) To study incidence of benign and malignant conditions in male and female population.

3) To study whether there is significant correlation between biochemical parameters and ENT diseased states.

III. Materials and Methods

In this study tobacco consuming patients were selected with tobacco consumption in the form of tobacco, tobacco with lime, tobacco consumption in the pan(quit), tobacco mishari application on teeth, tobacco smoking in the form of bidis and cigarettes, ghutka, mawa, jerda, tobacco used as snuff, etc. all were included. Patients included in this study population consumed tobacco products daily for more than 2 yrs duration. The selection of patients was done at random. Clinical examination of patients was done which included ear, nose, and throat examinations. Informed consent was taken before the blood investigations. Healthy control group who had balanced diet and not having addiction to tobacco, alcohol, etc. Equal male and female population was selected in the control group. MDA by Buege and Aust, and Protein carbonyl by Levine assay using spectrophotometer JASCO 670.

Inclusion criteria-

1. Tobacco consumers with benign and malignant ear, nose, throat lesions between the age group 15 yrs to 60 yrs.
2. Non tobacco consumers without ear, nose, throat diseases between the age group 15 yrs to 60 yrs.
3. Age group selected was 15 to 60 yrs and both sexes.
4. Patients using tobacco in the chewing form such as ghutka, pan, tobacco, tobacco and lime, mawa, etc on daily basis for more than 2 yrs with benign or malignant lesion
5. Patients using tobacco in the smoking form such as shisha smoking, cigarette smoking, bidis smoking, sinus, etc on daily basis for more than 2 yrs with benign or malignant lesion.
6. Patients using tobacco in the smoking and or chewing form with occasional alcohol drinker.

Exclusion criteria:-

1. Patients with diabetes mellitus, hypertension, pancreatic diseases, liver diseases, kidney diseases, heart diseases and H.I.V positive patients.
2. Patients with upper and lower respiratory tract infection and known genetic disorders..

Blood collection

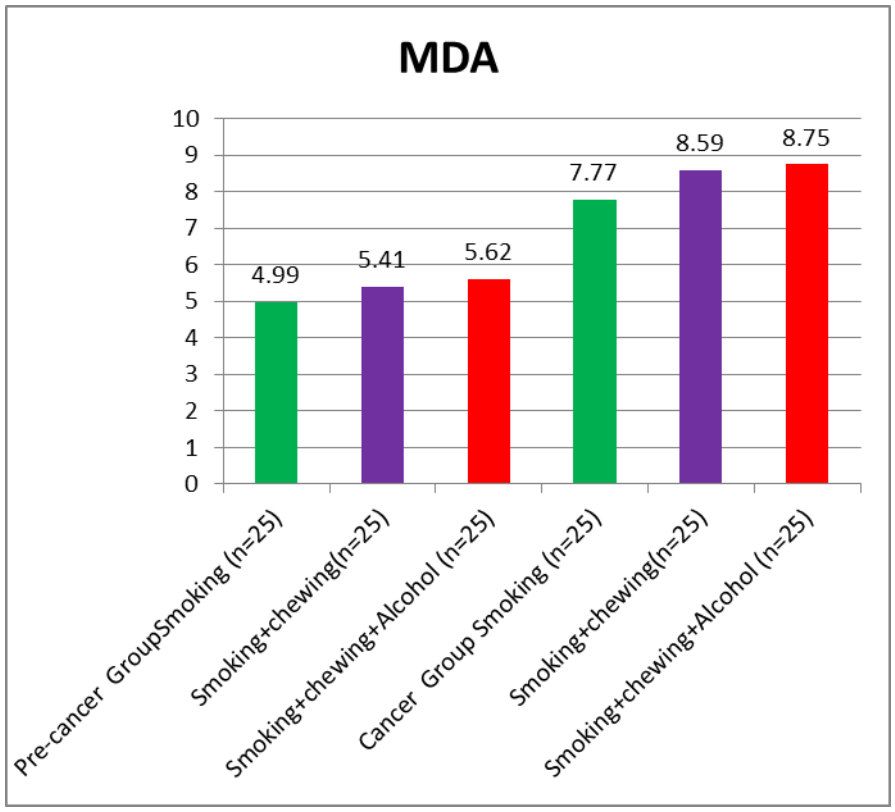
Venous Blood samples were collected after overnight fasting from the antecubital fossa by venipuncture using a 20-gauge needle and immediately transferred to the laboratory. The blood sample was allowed to clot at room temperature and after 1 hour serum was separated from blood by centrifuging at 3000rpm for 5 min. All samples were stored in deep freezer under -70^0 C temperature and the estimations were carried out.

Results

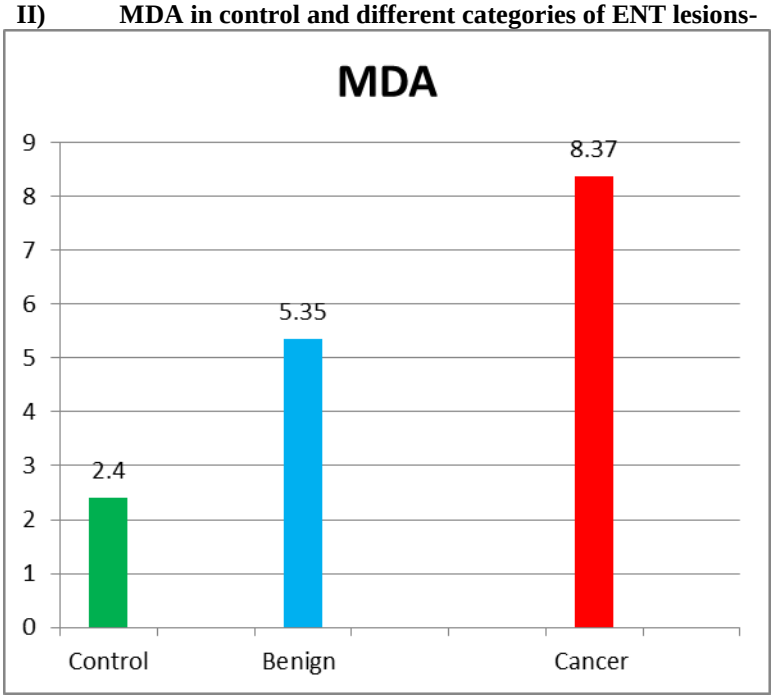
Table 1 Male and Female Distribution in Study Population-

Sex	Benign	Malignant	Control
Male	64	66	38
Female	11	09	37

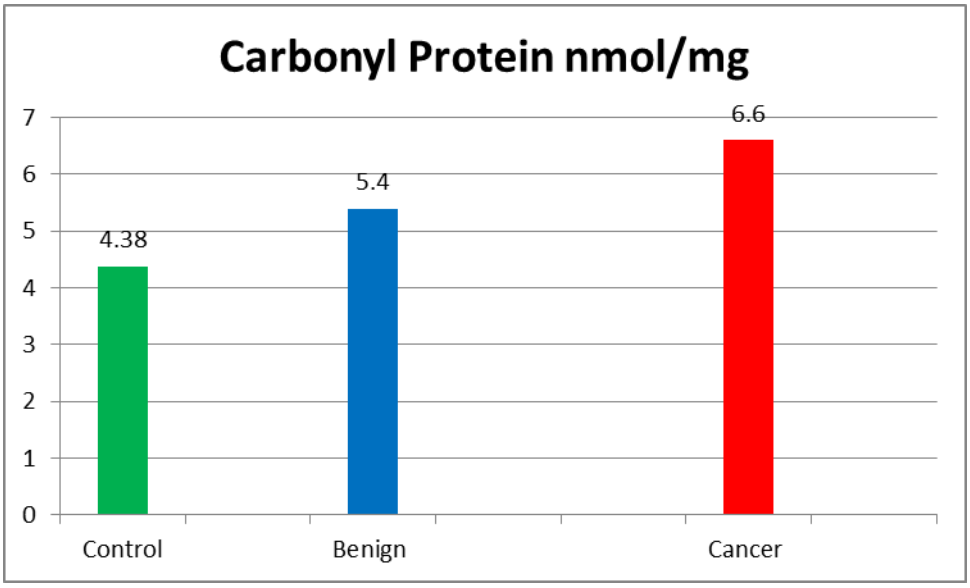
I) Malondialdehyde (MDA) in the different Tobacco consumption pattern-



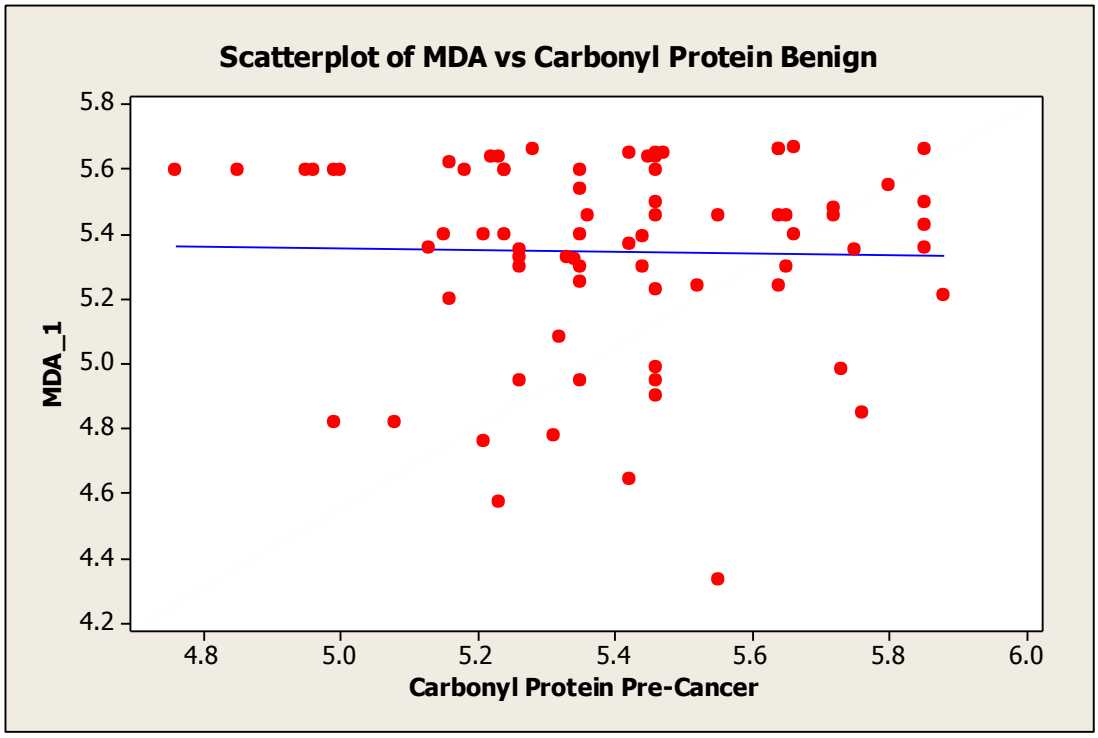
II) MDA in control and different categories of ENT lesions-



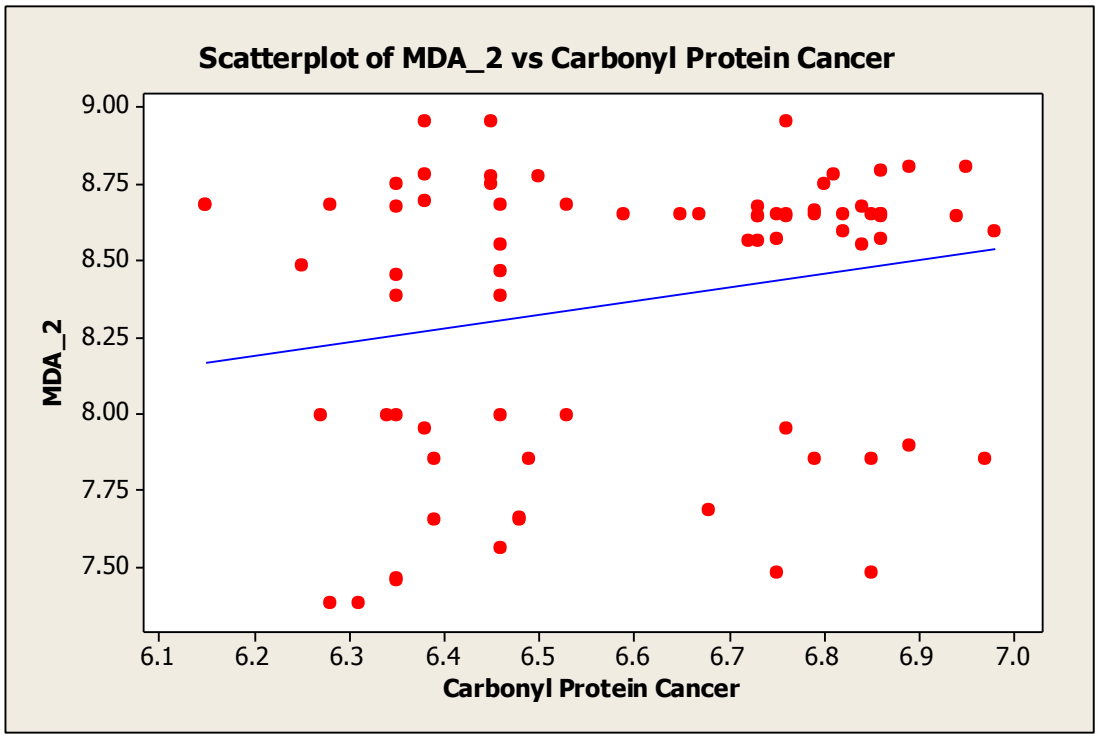
III) Protein carbonyl content in control and different categories of ENT lesions-



Graph I



GraphII



IV. DISSCUSSION

Oxidative stress is indicative of physiological disequilibrium within a cell or tissue or organ. Oxidative stress is increased when there is disequilibrium between the levels of free radicals in a cell and its antioxidant defenses (3). Near about 1-3 billion reactive oxygen species (ROS) are generated per cell per day. ROS includes the superoxide radical, hydroxyl radical, and nitric oxide radical species, and non-radical derivatives of oxygen, such as hydrogen peroxide and hypochlorous acid. ROS can react directly with the protein or they can react with molecules such as sugars and lipids leading to the production of reactive carbonyl species which then react with the protein. Direct oxidation of proteins by ROS produces highly reactive carbonyl derivatives (4). Protein carbonyl is the most widely used biomarker for oxidative damage to proteins (5). Excess productions of ROS are responsible for increase in oxidative stress. In present study, MDA and protein carbonyl was estimated in the different tobacco consumption pattern in both benign and malignant conditions with common ENT problem in male patients. Males had a higher prevalence and comprised 86.6% of the sample. That may be because of increased quantity of tobacco consumption in multiple forms and more exposure of tobacco to males.simillar study by Sujatha D, Hebbar PB, Pai A in 2012 (6).

Malondialdehyde (MDA) is the marker of oxidative stress. MDA estimation was done in control group (Group I), patients with benign conditions such as stomatitis, submucus oral fibrosis, leukoplakia, erythroplakia, gingivitis (Group II) and patients with malignancy (Group III). MDA level was significantly high in both benign and malignant group with lesions of ENT as compared to control group. Maximum increase in the MDA levels in the patients with addiction to tobacco chewing and smoking and habit of alcohol intake followed by patients with combined habit of smoking and chewing. Smokers have higher levels of MDA seen in both benign and malignant conditions as compared to control group i.e. Dhouha Haj Mouhamed et al, Kashinakunti SV in2011, Lykkesfeldt J, in 2004, Mahdavi R,in 2009 (6,7,8,9).

MDA and protein carbonyl shows significant positive correlation in the cancer patients. It indicates that increase in the oxidative stress increases to such an extent that damages the DNA and increase in the protein carbonyl content would leads to cancerous condition. In the patient with benign lesions there was not significant correlation between MDA levels and protein carbonyl content. Study done by Megson IL, et al in 2013 had shown the effect of passive smoking on the elevated levels of MDA and protein carbonyl content (10).

Conclusion-

Both benign and malignant conditions of ENT were common in male patients. MDA marker of oxidative stress and protein carbonyl was increased in both benign and malignant lesions of otorhinolaryngology. MDA levels were higher in malignant lesions than benign lesions. In the patient with benign lesions there was not significant correlation between MDA and protein carbonyl content. But in patients with the malignant ENT lesions there was significant positive correlation between MDA levels and protein carbonyl content. Increase in the oxidative stress to such an extent that damages the DNA and increase in the protein carbonyl content would leads to cancerous condition. Protein Carbonyl is helpful in the unknown primary lesions and dysplastic lesions.

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