Introduction
Oxidative stress is tied to mitochondrial oxidation of foodstuff and the generation of the energy necessary to sustain life occupies a place of central importance. Oxidative stress is a state of altered physiological equilibrium within a cell, tissue, or organ. It is a condition arising when there is a serious imbalance between the levels of free radicals in a cell and its antioxidant defences. It is estimated that 1-3 billion reactive oxygen species (ROS) are generated/cell/day. Therefore the body’s antioxidant defence system for the maintenance of health is important. Tobacco also causes increase in oxidative stress. Tobacco products have no safe level of consumption. It is not only tobacco related products alone, but also local Indian products like bidis, gutkas and pan masalas, which are the culprits.

The role of tobacco in alteration of enzymatic activity (SOD, GTR, GTP, and MAD) and their association with development of benign and malignant condition was studied.

Aims and Objectives
1) To study the activity of enzymes malondialdehyde (MDA) superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, Superoxide-Dismutase (SOD), Catalase in ENT lesions.

Material and Method
A Group of people was selected and both tobacco consumers and non consumers were compared for biochemical parameters. Exclusion criteria were patients with diabetes mellitus, hypertension, pancreatic diseases, liver diseases, kidney diseases and heart diseases, H.I.V. positive patients, and genetic disorders. Patients were selected between 18-60 yrs age group.
Venous Blood samples were collected after overnight fasting. Hemolysate prepared from heparinised blood specimens were used for estimation of activities of catalase (CAT), Superoxide dismutase (SoD), glutathione peroxidise (GHS-PX), glutathione reductase (GR) and Melondialdehyde (MDA).

Citrated blood collected was utilized for estimation of blood glutathione (GSH).

All samples were stored in refrigerator and the estimations were done within 24 hours of specimen collection.

A) Serum malondialdehyde:-

Method: Buege and Aust

Malondialdehyde (MDA) is a highly reactive three carbon dialdehyde, produce from lipid hydroperoxide. It can, however, also be derived by the hydrolysis of pentose’s, deoxyribose, hexoses, from some amino acids and from DNA. MDA has most frequently been measured by thiobarbituric acid reaction. MDA is measured as an index of lipid Peroxidation.

Principle:- Serum sample is first treated with TCA for protein precipitation and then treated with thiobarbituric acid. The mixture is heated for 10 minutes in boiling water bath. One molecule of MDA reacts with two molecules of thiobarbituric acid. The resulting chromogen is centrifuged and intensity of colour developed in supernatant is measured spectrophotometrically at 530nm. MDA levels are expressed in nmol/mL.

Reagents:
- a) 40% Trichloroacetic acid (TCA).-40 gms of TCA in 100 mL of distilled water.
- b) 0.67 % Thiobarbituric acid (TBA) 0.67 gm of TBA in 100 ml of distilled water in boiling water bath.
- c) Standard Malondialdehyde (MDA).

Stock MDA is Prepared from the 1,1,3,3 tetraethoxy propane by acid hydrolysis. A solution containing 0.1105 ml 1,1,3,3 tetraethoxy propane in 50 mL distilled water and 0.5mL 0.1 M HCl is warmed at 50ºC for 1 hour and volume adjusted to 100 mL with distilled water. The concentration of free MDA was determined spectrophotometrically at 267nm, using a molar absorption coefficient of 31,800.

Sample processing:-

The above reaction mixture was heated in boiling water bath for 10 minute. It was then cooled at R.T. and centrifuge. The absorbance of supernatant ar 530 nm was noted. The result was calculated from standard graph.

B) Superoxide-Dismutase Activity (SOD)

Method:- Arthur JR, Boyne R

Principle:- The role of superoxide dismutase (SOD) is to accelerate the dismutation of the toxic superoxide radical (O2), produced during oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with,

2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (I.N.T.) to form a red formazan dye. The superoxide dismutase activity is then measured by the degree of inhibition of this reaction. One unit of SOD is that which causes a 50% inhibition of the rate of reduction of INT under the conditions of the assay.

Xanthine + O2 + H2O2 + NADPH + H+ → Formazan + H2O2 + SODOR

C) Glutathione Peroxidase (GSH-PX)

Method:- Paglia Donald E & Valentine William N.

Principle:- This enzyme has been shown to catalyse with high specificity the in vitro detoxification of hydrogen peroxide by the oxidation of reduced glutathione according to following reaction:

Reaction Principle

GPX

2GSH + ROOH → ROH + GSSG + H2O

D) Glutathione Reductase

Method:- Goldberg DM. & Spooner RJ [6]

Principle:- Glutathione reductase (E.C.1.6.4.2) catalyses the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to NADP+. The decrease in absorbance at 340 nm is measured.

GR

NADPH + H+ + GSSG → NADP+ + 2GSH

E) Catalase (CAT)

Method:- Aebi [7]

Principle:- In the UV range H2O2 shows a continual increase in absorption with decreasing wavelength. The decomposition of
H2O2 can be followed directly by the decrease in extinction at 240nm.

Reagents:-
1. Phosphate buffer (50 mM, pH 7.0)
   a) Dissolved 6.81 gms of potassium dihydrogen phosphate (KH2PO4) in glass distilled water and volume made to 1 liter.
   b) Dissolved 8.90 gms of disodium hydrogen phosphate (Na2HPO4) in glass distilled water and volume made to 1 liter.
2. Hydrogen Peroxide (30 mM)
   Diluted 0.34 ml of 30% H2O2 solution with phosphate buffer to 100ml which was prepared just before use.

Assay system Calculation:

\[
\text{1 Unit} = \dfrac{2.3 \times \text{Log A1} - 1000 \times \text{Log A2}}{6.93 \times \text{Co}}
\]

\[\text{Co} = \text{Concentration of the original enzyme sample in assay system.} \]

A1A240 at t=0 and A2--A240 at t=15 sec.

Result was expressed by converting in Units/gm of Hb.

Observations-
Glutathione Reductase, Glutathione Peroxidase, Superoxide-Dismutase (SOD), Catalase in control and different categories of ENT lesions were studied. Glutathione Reductase U/g Hb was significantly lower in diseased states. In control group Glutathione Reductase U/g Hb was 11.49977±1.972828. In benign conditions the Glutathione Reductase U/g Hb values were 5.8068 ±0.876812 while in cancer patients Glutathione Reductase U/g Hb was 3.8948±0.735391.

Correlations between Malondialdehyde (MDA) and Glutathione Reductase, Glutathione Peroxidase, Superoxide-Dismutase (SOD), Catalase in ENT lesions were studied.

In benign Otorhinolaryngological conditions R values for MDA/ Glutathione Reductase, MDA / Glutathione Peroxidase, MDA / SODU and MDA / Catalase for were between -0.925 and -0.981. In cancer group R--value was between -0.784 and -0.965. P values for correlation between MDA/Glutathione Reductase, MDA/ Glutathione Peroxidase, MDA / SODU and MDA / Catalase in benign and cancer group was 0.00.

There is significant negative correlation between Malondialdehyde (MDA) and Glutathione Reductase, Glutathione Peroxidase, Superoxide-Dismutase (SOD), Catalase in ENT lesions.

Discussion
Tobacco contain carcinogens like polycyclic aromatic hydrocarbons, aldehydes, benzo[a]pyrene, ethylene oxide, 4-aminobiphenyl and nitrosamines which are metabolically activated by hydrolysis, reduction, or oxidation by xenobiotic metabolism through phases I and II enzymes. Therefore in tobacco consumer there are elevated levels of enzymes indicative of increased oxidative stress. Oxidative stress (OS) can also result from conditions like excessive physical stress, exposure to environmental pollution and xeno-biotics. Oxidative stress, as a pathophysiological mechanism, has been linked to numerous pathologies, poisonings, and the ageing process. Accordingly, from the point of view of routine clinical-diagnostic practice, it would be valuable to routinely analyze OS status parameters to earlier recognize potential disease states and provide the basis for preventative advance treatment with appropriate medicines.

The role of tobacco in alteration of enzymatic activity (SOD, GTR, GTP, and MAD) is associated with development of carcinoma in the oral sub mucus fibrosis. Tobacco also causes increase in oxidative stress which is duration dependent.

Reactive oxygen species and reactive nitrogen species, endogenously or exogenously produced, can readily attack all classes of macromolecules (protein, DNA, unsaturated fatty acid). The disrupted oxidative-reductive milieu proceeds via lipid per oxidation, altered antioxidative enzyme activities and depletion of non-enzymatic endogenous antioxidants, several of which can be detected in the pre-symptomatic phase of many diseases. These biochemical parameters can be used as biomarkers for certain disease states.

Different ear, nose, throat diseases were studied in detail according to staging and biochemical parameters. During the study it is observed that the ENT diseases seen in tobacco consumers were dose and duration dependant. Also certain factors like addition of lime, betal nuts along with use of tobacco were more prone for the disease process. Also alcohol intake along with tobacco increases disease severity and staging.

There is significant negative correlation between Malondialdehyde (MDA) and Glutathione Reductase, Glutathione Peroxidase, Superoxide-Dismutase (SOD), Catalase in ENT lesions. Chronic exposure to tobacco smoke aggravated eosinophilic inflammation and promoted airway remodeling and nasal polyp formation in a murine model of ERSwNPs. But there was no significant allergic complaints and hearing loss seen in tobacco consumers.

The malignant transformation rate of OSF has been reported to be around 7.6% over a 17-year period.

Conclusion
Oxidative stress parameters were increased in tobacco chewers and both benign and malignant conditions of ear, nose and throat. There is significant negative correlation between Malondialdehyde (MDA) and Glutathione Reductase, Glutathione Peroxidase, Superoxide-Dismutase (SOD), Catalase in ENT lesions. There were no significant allergic complaints and hearing loss seen in tobacco consumers.

Further study is required to see the malignant transformation of these benign lesion with continued and discontinuation of tobacco consumption.

References
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