Role of Vitamins in Prevention of Carcinoma: Biochemical Analysis

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Abstract

Objective: **1) To study the blood vitamin level in non tobacco chewers and tobacco chewers. 2) To study the correlation Correlations between Malondialdehyde (MDA) and Vitamins in ENT lesions. 3) Vitamins (Anti-oxidants) in control and different categories of ENT lesions.**

Material and methods: **Total 75 patients with tobacco chewers with benign and malignant ENT lesions were selected. Patients who gave history of tobacco smoking or used non-smoked tobacco (chewing, snuff, etc.) were considered as tobacco users. Blood sample was taken for estimation of Vitamin A, E and C levels. Serum Melanodealdehyde estimation was done for assessment of Oxidative stress. The biochemical parameters such as oxidative stress, antioxidants(vitamins) were studied in tobacco chewers and nontobacco chewers.**

Introduction

Tobacco chewing and smoking are leading preventable causes of death. It is not only tobacco related products alone, but also local Indian products like bidis, gutkas, pan masalas, which are the culprits. Bidis are Indian cigarettes, wrapped in tendu or temburini leaf and secured with a string at one end. The smoke of bidis contains more than three times the amount of nicotine and five times of the amount of tar than regular cigarette smoke.[1]

Elevated free radical production and/or insufficient anti oxidative defence results in cellular oxidant stress responses. Sustained and/or intense oxidative insults can overcome cell defences resulting in accumulated damage to macromolecules, leading to loss of cell function, membrane damage, and ultimately to cell death. Oxidative stress (OS) can result from conditions including excessive physical stress, exposure to environmental pollution and xeno-biotics, and smoking. Oxidative stress, as a pathophysiological mechanism, has been linked to numerous pathologies, poisonings, and the ageing process. 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

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many diseases. Therefore, they could represent markers of altered metabolic and physiological homoeostasis. Accordingly, from the point of view of routine clinical-diagnostic practice, it would be valuable to routinely analyse OS status parameters to earlier recognise potential disease states and provide the basis for preventative advance treatment with appropriate medicines.[2] Tobacco also causes increase in oxidative stress which is duration dependent.[3]

Material and methods

The study is carried out in Government medical college hospital.

Inclusion criteria

1. Tobacco consumers with benign and malignant ear, nose, throat diseases between the age group 15 yrs and 60 yrs.
2. Tobacco and alcohol consumers with benign and malignant ear, nose, throat diseases between the age group 15 yrs and 60 yrs.
3. Non tobacco consumers without ear, nose, throat diseases between the age group 15 yrs and 60 yrs.

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.

A group of people are selected and both tobacco consumers and non consumers were compared for biochemical parameters.

All patients considered in study were examined in detail according to proforma.

Observations

A) Serum Malondialdehyde (MDA):-

Method: Buege and Aust

Malondialdehyde (MDA) is a highly reactive three carbon dialdehyde, produced 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 530 nm. 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 50°C 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

Reagents	Quantities
Serum	1.0 mL
40% TCA	1.0 mL
0.67 % TBA	2.0 mL

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 530 nm was noted. The result was calculated from standard graph.

B.) Vitamin-E and Vitamin A

Method:- Baker.H and Frank.

Principle:-The proteins in the serum were precipitated by an equal volume of absolute Ethanol; the whole mixture was subjected to extraction by an equal volume of n-heptane. The 2,2-dipyridyl was then added to an aliquot of the upper layer to estimate the principle interfering substance such as beta-carotene and measured at 460 nm. Further by Emmer Engel reaction the extracted tocopherol reduces ferric to ferrous ions. The reduced ferrous ions then forms a red coloured complex with 2, 2-dipyridyl. This was read at 520 nm. The results were calculated from standard graph.

C.) Vitamin-C

Method:- Ayekyaw.

Principle:- The Phosphotungstate (PTA) was found to be specific and sensitive for ascorbic acid determination in the serum with good reproducibility. Phosphotungstate serves not only as protein precipitant and ascorbic acid extractant but also as the colour developing agent.

Observations

Table 1 : Vitamins (Anti-oxidants) in control and different categories of ENT lesions.

Mean (SD)	Vit A µmol/L	Vit E µmol/L	Vit C µmol/L
Control	1.285867 **±0.070711**	22.7112 **±2.545584**	14.80093333 **±4.504270196**
Benign	1.080133 **±0.120208**	11.2152 **±0.636396103**	11.12106667 **±1.103086579**
Cancer	0.706933 **±0.042426**	6.6072 **±1.400071**	7.6752 **±1.103087**

In present study vitamin levels were significantly lower in cancer patients.

Table 2: Correlations between Malondialdehyde (MDA) and Vitamins in ENT lesions.

Correlations	MDA/Vit A	MDA/ Vit E	MDA/ Vit C
Pre Cancer			
R- value	-0.846	-0.933	-0.925
P- value	0.000	0.000	0.000
Cancer group			
R- value	-0.823	-0.847	-0.913
P- value	0.000	0.000	0.000

There is significant negative correlation between Malondialdehyde (MDA) and Vitamins in ENT lesions. It indicates that in low blood vitamin levels oxidative stress increases. So the enzymes like melanodealdehyde (MDA) which is indicative of oxidative stress increases.

Discussion

Tobacco contain carcinogens like polycyclic aromatic hydrocarbons, aldehydes, benzo [alpha] 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.[4] Tobacco is the major cause of oral cancer. Other causes also

considered are alcohol consumption, genetic predisposition and diet lacking in micronutrients. Alcohol intake is a risk factor that acts synergistically with tobacco. A recent study showed that the cause of chronic inflammation in cancer patients was chronic infection in 20%, tobacco smoking and inhaled pollutants in 30% and 35% to dietary factors.[5]

Imbalance between the Prooxidants and Antioxidants in favour of prooxidants is called oxidative stress. This causes the malignant transformation of cells leading to cancer development. The burst of ROS (Reactive Oxygen species) has been implicated in the development of oral cancer in tobacco chewers and smokers. Smoking may enhance oxidative stress not only through the production of reactive oxygen radicals in cigarette tar and smoke but also through weakening of the antioxidant defence systems. Caused by smoking prooxidants / antioxidant imbalance elevates oxidative stress which is accompanied by increase of lipid peroxidation, oxidative Deoxyribonucleic acid (DNA) damage and disturbances of enzymatic antioxidant defence.

The case-control study, conducted in Italy and French-speaking Switzerland, included 749 patients with incident cancer of the oral cavity and pharynx, and 1772 hospital controls with acute, non-neoplastic conditions. The interviews used a validated food frequency questionnaire. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using multiple logistic regression. Compared with low folate intake, a consistent reduction in risk was already observed from intermediate levels of intake, suggesting that cancer risk may be related to relative folate deficiency.[6]

Epidemiological studies worldwide have implicated dietary and nutritional factors in the development of oral and pharyngeal cancer. Dietary information in these case-control studies generally was collected through food-frequency questionnaires. Consistently, studies observed a protective effect of a diet high in fruit intake, reflected in a 20-80% reduction in oral cancer risk. A high intake of foods considered to be dietary staples in particular cultural groups, possibly indicating a generally impoverished diet, has been linked to excess risk. Indigenous dietary practices in single studies that were found to increase risk include a high intake of chilli powder and wood stove cooking. Supplementation with various vitamins has been protective in a few studies. Chemoprevention trials generally have found that chemo preventive agents reduce the size of oral leukoplakia lesions or the frequency of second primary oral cancers. The most consistent dietary findings across multiple cultural settings are a protective effect of high fruit consumption and the carcinogenic effect of high alcohol intake.[7] The issue of dose-response is important, and some evidence suggests that a very low intake of fruits and vegetables, e.g. less than 2 servings or 200 g/d, is related to increases in risk compared with higher intakes, but that there may be little additional benefit for intakes higher than about 400 g/d. Also, fruits and vegetables are extremely heterogeneous; studies have suggested

that intake specific food or vegetables lower particular cancer risk. Intake of fruits and vegetables is not only factor but the bioavailability of micronutrients in fruits and vegetables and antagonistic factors present in body are also important. So the dose-response of vitamins is important issue.[8]

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Temporomandibular disorders

After odontogenic pain, temporomandibular disorders (TMDs) are one of the most common causes of pain in the mouth and face and also have the potential to produce persisting (chronic) pain. Chronic or persistent (myogenous) TMDs can be associated with other chronic pain conditions, including migraine, fibromyalgia, and widespread pain.

The probability of first onset TMDs is also strongly associated with concurrent headaches and body pain.

TMDs can present with any or all of the following signs and symptoms: pain in the temporomandibular joint or muscles of mastication, which may radiate or refer to local or distant structures; clicking, popping, or crepitus of the temporomandibular joint on any of its movement with or without locking of the joint; headache limited to the temporal region; and otalgia or tinnitus, or both in the absence of aural disease.

A simple method to screen for painful TMDs (as a physical entity) is to use a six item self complete questionnaire developed in tandem with the Diagnostic Criteria for TMD.

Convincing evidence shows that psychosocial factors are influential in the development of general chronic pain states and of TMD specifically.

An international consensus reached in 1995 and revised slightly in 2010 was that reversible, conservative treatments should generally form the first line intervention for TMDs.

After an initial diagnosis of acute TMD the first approach to management should be a careful explanation of the diagnosis alongside education about the condition and its largely benign course. This can be supplemented by advice on appropriate, time limited, simple analgesic use and altered dietary consistency for acute exacerbations plus information on self management techniques.

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