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Original Article

Lipid peroxidation and antioxidant enzyme status in oral carcinoma patients

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Abstract
Objective: To measure the lipid peroxidation and endogenous antioxidant enzyme status in oral carcinoma and the protective role of exogenous antioxidants.
Meterial and methods: 20 new cases of histologically proven oral squamous cell carcinoma, 20 of leukoplakia and 20 age and sex matched healthy conrols were included. Intra oral pH of patients and controlled were measured by quantitative litmus paper test and serum was analysed for malonialdehyde (MDA), super oxide bismutase (SOD), catalase and glutathione peroxidase (GP). Patients with leukoplakia were treated with exogenous antioxidants for 3 months and the same were reassessed.
Results: Oral pH of oral cancer patients was neutral (PH-7) but that of leukoplakia and controls were mildly acidic (6.64 and 6.58 respectively). Serum malonialdehyde levels were highest in oral cancer group. With antioxidant enzymes super oxide bismutase, catalase and glutathione peroxidase different pattern was noticed. Antioxidant enzymes remained almost the same ($P > 0.005$ each) in patients with leukoplakia after 3 months of vitamin A,C and E. but there was marginal increase in catalase level ($P<0.05$).
Conclusion: This study shows the positive benefit of vitamin (A,C,E) and nutrition supplementation on the antioxidant enzyme defense system hence prevention of oral carcinogenesis in patients with leukoplakia.

Keywords: Lipid peroxidation, malonialdehyde, catalase, glutathione peroxidase, superoxide dismutase, oral cancer

A free radical is a molecular fragment that contains an odd number of unpaired electrons in the valence shell (ie radical) and is capable of existing freely (ie free). Most free radicals are highly reactive and short lived. They may interact with cellular micromolecules such as DNA, protein, lipid and carbohydrate to initiate or promote inflammatory, toxic or carcinogenic processes. Free radical mediated lipid peroxidation has recently been proposed as a basic mechanism of injury responsible for a wide variety of diseases and conditions like atherosclerosis, SIRS, MODS, malnutrition and cancer .

The role of oxygen free radicals in the initiation, promotion and progression of carcinogenesis and the protective role of anti-oxidant enzymes has been the subject of much speculation with conflicting reports in literature. The aim of this study was to measure the free radical stress and endogenous anti-oxidant enzyme levels in serum of patients of oral cancer, leukoplakia and healthy age and sex matched controls. Malonialdehyde (MDA) which is a stable end product of free radical induced lipid peroxidation was used as a surrogate marker for oxidative damage to tissues. The endogenous anti-oxidant enzymes measured were superoxide dismutase (SOD), catalase and glutathione peroxidase (GP).

Materials and methods

The study was conducted in the Department of Surgery. Banaras Hindu University over a period of 3 years from March 2000 to February 2003. Twenty new cases of histologically proven oral squamous cell carcinoma, 20 of leukoplakia and 20 age and sex matched healthy controls were included. None of the patients had received radiotherapy or chemotherapy prior to inclusion in the study. The intra oral pH of patients and controls was measured by quantitative litmus paper test. The serum of the subjects were obtained taking full precaution to avoid hemolysis. Biochemical analysis of malonialdehyde (Bergmeyer2), superoxide dismutase (Marklund3), catalase (Aebi4) and glutathione peroxidase (Beutlers) was done in the serum samples in the biophysics laboratory of Banaras Hindu University.

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The values obtained were correlated with the intra oral pH, clinical profile of oral cancer patients and within the 3 groups. Patients with leukoplakia which is a potentially premalignant condition received oral supplementation of Vitamin A, 5000 IU., Vitamin C, 500 mg., and Vitamin E, 400 IU., for 3 months following which repeat estimation of MDA, SOD, catalase and GP levels were done.

Statistical analysis was done using the one way ANOVA test, paired `t' test and chi square test (x2). A `p' value of <0.05 was considered significant.

Results

The mean age of oral cancer patients, leukoplakia patients and controls was 54.6 years, 45.2 years and 47.4 years respectively. All patients of oral cancer (100%), 161eukoplakia patients (80%) and 11 controls (55%) gave a history of either tobacco chewing or smoking. Stage wise, among oral cancer patients, 3 had stage I, 6 had stage II, 9 had stage III and 2 had stage IV disease.

Serum malonialdehyde levels were highest in oral cancer patients (0.67f0.57 nmol/L versus 0.32f0.06 nmol/L in controls, p<0.001). The levels of MDA in leukoplakia patients was similar to controls (p>0.05). With anti oxidant enzymes superoxide dismutase, catalase and glutathione peroxidase a different pattern was noticed. The serum levels of a113 enzymes were significantly depressed in both oral cancer and leukoplakia patients as compared to control subjects (cancer versus control, p<0.00 1, leuoplakia versus control, p<0.01). (Table 1 and 2).

To evaluate the influence of tobacco usage on the production of these enzymes, their levels were compared among tobacco users (n=11) and non tobacco users (n=9) in the control group. The levels of MDA was higher in tobacco users than non users (p<0.05). Of the anti oxidant enzymes, the levels of SOD was similar in both groups (p>0.05) while catalase and GP were significantly depressed in the tobacco users (p<0.01). (Table 3).

The intra oral pH was recorded with quantitative litmus paper test. While the pH of oral cancer patients who were all tobacco users was neutral (7.00), that of leukoplakia patients and controls was slightly acidic (6.64 and 6.58 respectively), (Table 4). No statistical relationship could be established between the intra oral pH and serum enzyme levels in either patients or controls.

The patients with leukoplakia received a 3 months supplementary treatment with Vitamin A (5000 IU.), Vitamin C (500 mg.) and Vitamin E (400 IU). Following this treatment a marginal increase in catalase levels was noted (p<0.05) while the values of MDA, SOD and GP remained almost the same (p>0.05 each) (Table 5).

Table 1: Levels of various enzymes (mean ± SD) in the serum of various study groups.

	MDA	SOD	Catalase	GP
	(nmol/L)	(unit/mg	(pmol H202	(pmol of GTNB
		protein)	decomposed/mg	conjugate/ mg
			protein)	protein)
Oral Cancer	0.67±0.57	0.06±0.12	41.7±22.2	0.056±0.022
(n=20)				
Leukoplakia	0.37±0.07	0.10±0.09	58.7±16.6	0.053±0.013
(n=20)				
Controls	0.3210.06	0.43±0.95	191.4±149.9	0.134±0.157
(n=20)				

Table 2: Statistical analysis of serum enzymes levels in various groups by SNK test and their significance.

Group	MDA	SOD	Catalase	GP
Comparisons				
Control vs	P<0.001	P<0.001	P<0.001	P<0.001
Oral Cancer				
Control vs	p>0.05	P<0.01	P<0.01	P<0.001
Leukoplakia	(NS)			
Leukoplakia vs	P<0.001	p>0.05	p>0.05	p>0.05
Oral Cancer		(NS)	(NS)	(NS)

NS = Not significant

Table 3: Levels of various enzymes (mean ± SD) in the serum of controls according to habits of tobacco usage.

Group	MDA	SOD	Catalase	GP
Comparisons	(nmol/L)	(unit/mg	(pmol H202	(p,mol of GTNB
		protein)	decomposed/mg	conjugate/ mg
			protein)	protein)
Tobacco	0.37±0.09	0.42±0.31	154.7±82.5	0.082±0.098
Users (n=11)				
Non tobacco	0.26±0.12	0.44±0.47	223.2±108.7	0.164f0.118
Users (n=9)				
P – value	p>0.05	p>0.05	P<0.01	P<0.01
	(NS)	(NS)		

NS = Not significant

Table 4: Mean f SD levels of pH in different study groups

Groups	pH
Oral Cancer	7.00±0.23
Leukoplakia	6.64±0.27
I Control	6.58f0.32

Table 5: Changes in mean ± SD levels of serum enzymes in leukoplakia patients following 3 months treatment with Vitamins A, C and E

Variables	Pre treatment	Post treatment	P- value
MDA	0.37±0.07	0.36±0.1	p>0.05 (NS)
SOD	0.10±0.09	0.13±0.13	p>0.05 (NS)
Catalase	58.7±16.6	69.8±18.6	P<0.05
~ GP	~ 0.053±0.013	0.06±0.01	p>0.05 (NS)

NS = Not significant

Discussion

In aerobic life cycle, oxygen free radicals (OFR) are formed in normal cell metabolism from molecular oxygen. Despite anti oxidant defenses, these OFR cause constant damage to oxidisable molecules which are repaired or replaced in a dynamic equilibrium. Oxidative stress arises either from the overproduction of OFR or from the deficiency of anti oxidant defense or repair mechanisms and results in reversible or

irreversible tissue injury[6]. Examples of short term oxidative stress are the ischemia- reperfusion injury and acute inflammation.

The role of oxidative damage in carcinogenesis is increasingly being speculated. Given the long term evolutionary development of cancer, these conditions are not normally expected to cause cancer unless they are the source of a primary mutagenic event. Some important exogenous causes of oxidative stress involved in carcinogenesis are tobacco smoke in broncogenic carcinoma, ultraviolet light in skin cancer, fatty acids in food for colorectal cancer and ethanol for hepatocellular cancer[7].

We chose to study the role of oxidative stress and the protective influence of endogenous anti oxidant enzymes in oral cancer. Leukoplakia is a well recognized premalignant condition with a 5-7% malignant transformation rate[8]. The role of antioxidants in the prevention of oral cancer is well known9. Therefore we decided to investigate the causative role of oxidative stress in leukoplakia and the potential of altering the lipid peroxidation- anti oxidant enzyme milieu in these patients with essential nutrient and vitamin supplementation for 3 months.

We found that leukoplakia patients had similar levels of MDA as controls ($p>0.05$), but the levels of all 3 antioxidant enzymes were markedly depressed in these patients ($p<0.01$). Oral cancer patients had markedly elevated MDA levels ($p<0.001$) in addition to the suppressed antioxidant enzymes. (Table 1 & 2). Among control subjects we found that the enzymes catalase and GP were significantly lower ($p<0.01$) among tobacco users compared to non tobacco users (Table 3). The levels of MDA and superoxide dismutase were similar in both groups of controls ($p>0.05$). Clearly the use of tobacco had caused suppressed production of the antioxidant enzymes which was evident among leukoplakia patients as well, 80% of whom were tobacco users. Therefore the oxidative stressantioxidant enzyme equation has adversely been affected in tobacco using controls and leukoplakia patients. Oral cancer patients had in addition elevated levels of MDA which could be the cause or effect of the carcinogenesis process. A more reasonable explanation could be that there are genetic factors in play which decide which of the tobacco users would respond by excessive lipid peroxidation as evidenced by increased MDA production and which of these subjects will not. Thus there could be a close internetworking between genetic susceptibility-tobacco usage and oxidative stress induced carcinogenesis in oral cancer patients.

We found that the intra oral pH of patients with oral cancer was more alkaline compared to controls (7.0 versus 6.58). Most of our patients who chewed tobacco used slaked lime as well in combination. The intra oral pH of patients immediately after they had spit out the tobacco quid was found to be in the range of 8-10. Nair et al[10] have demonstrated that nitrosation of both tobacco specific nitroso compounds (TSNA) and areca specific alkaloids at pH 7.4 can occur in the mouth of betel quid chewers. The role of alkaline pH in the generation of oxygen free radical is quite probable and requires further investigation.

The chemo preventive role of retinoids, minerals and vitamins in carcinogenesis is increasingly being appreciated". Remission of oral leukoplakia in betel nut chewers by nutrient intervention has been documented[12]. The 20 patients suffering from leukoplakia in our study received a 3 months supplementation with Vitamins A, C and E. At the end of this period there was a significant elevation of serum catalase levels ($p<0.05$) while levels of MDA, SOD and GP were almost same.

Hristozov et a1[13] found significantly higher levels of lipid peroxidation products (MDA) in early and advanced cancers in comparison to control volunteers. They report similar SOD activity among cancer patients and controls and an adaptive increase in catalase activity in cancer patients. Oberley et al la using immunohistochemical techniques demonstrated early cancers to have low antioxidant enzyme expression. Both catalase and GP levels were low suggesting that most cancer cell types cannot detoxify hydrogen peroxide. Casado et al[l] found the SOD activity in patients with cancer not to be significantly different form the SOD activity observed in the normal population. Catalase activity was lower in patients with cancer compared to normal population. Yamaguchi et a1[16] used Northern Blot analysis on livers of mice with a rat tumor to demonstrate that catalase gene expression was lower in a tumor size dependent fashion. Removal of the implanted tumor resulted in restoration of the reduced gene message to the normal level. Chen et al[17] examined the relationship of sex specific mortality rates for selected cancers with the biochemical indicators of antioxidant status including SOD, catalase and GP enzyme status. They found that the plasma levels of antioxidant enzymes were consistently negatively correlated with cancer mortality rates.

Sun[18] proposes that free radicals are involved in both initiation and promotion of multistage

carcinogenesis. They cause DNA damage, activate procarcinogens and alter the cellular antioxidant defense system. Antioxidant enzymes are inhibitors of initiation, promotion and transformational stage of carcinogenesis and protect cells against oxidative damage. They found tumor cells to be always low in SOD and catalase activity compared to normal tissue. GP activity was found to be highly variable. Correspondingly Balcerska et a1[19] observed a decrease of total antioxidant barrier during the initiation of a neoplastic disease. An increase of SOD, GP and catalase activity was noticed in children with clinical remission. Faber et a1[20] found the concentration of free radical metabolite thiobarbituric acid higher in plasma of patients with cancer than controls and was further increased after chemotherapy.

They implicated free radical induced lipid peroxidation for cardio toxicity caused by Adriamycin.

Das[21] suggests that tumor cells have relatively low amounts of SOD which quenches superoxide anion and as a result of a higher level of aerobic metabolism, higher concentration of hydroxyl ion compared to normal cells. He also suggests that ionizing ration radiation and chemotherapeutic agents like Anthracyclines and Bleomycin exert their anticancer effect by the production of free radicals. Kong et a1[22] have also expressed similar views regarding the dual effects of oxygen free radicals and antioxidants. They say that oxygen free radicals play a critical role in anti cancer therapies. The use of antioxidants such as SOD was found to decrease the efficacy of anti tumor therapies which depend on free radical generation for their action. In addition increased antioxidant activity can often be utilized by tumor cells to favors increased growth. Additionally they hypothesize that the appropriate administration of anti oxidant inhibitors and / or free radical generating compounds may be a useful strategy in the treatment of solid tumors[23].

Our work demonstrates that tobacco induced oral carcinogenesis could be mediated by its influence on the free radical generation- antioxidant enzyme system. Even normal controls who used tobacco had suppressed antioxidant enzymes. Leukoplakia patients had decreased antioxidant enzymes but their MDA levels were comparable to controls. Patients with oral cancer had elevated MDA levels in addition to the low antioxidant enzymes. Thus free radical mediated tissue and DNA injury was maximal in these patients while their antioxidant defense mechanisms were compromised. Not all patients who use tobacco have elevated MDA levels or develop cancer. It seems reasonable to presume that genetic factors come into play which determine the outcome of tissue exposure to tobacco vis a vis free radical generation and free radical induced cell damage. Additionally we were able to demonstrate the positive benefit of vitamin and nutrient supplementation on the antioxidant enzyme defense system in leukoplakia patients. Thus the role of vitamins in primary prevention of oral cancer especially among tobacco users needs to be further emphasized.

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