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Original Research Article

Estimations of various antioxidants in oral cancer patients in comparison with smokers and non-smokers - a biochemical study

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ABSTRACT

Background: Oral cancer is one of the most fatal health problems faced by the mankind today. Oral cancer ranks as the eighth most common cancer in developing countries. A high incidence of oral cancer is observed in the Indian sub-continent which accounts for one third of the world burden. In India, because of cultural, ethnic, geographic factors and the popularity of addictive habits, the frequency of oral cancer is high. It is estimated that about 56 thousand new cases are reported each year in India resulting in about one lakh individuals suffering from the disease at any given time. Cancer or precancer states can be found in individuals who are non-smokers and quid chewers. The possibility of alteration of various antioxidant levels due to presence of carcinogens in the blood plays a key role in the development of malignant states.
Methods: The material for the present study comprise of 50 subjects selected at randomly. After recording history regarding habits, thorough clinical examination the patients clinically diagnosed as oral cancer underwent biopsy to confirm histopathologically. A total of 2.5ml ml of blood was drawn from the mid-cubital vein with necessary aseptic precautions and transferred to sterile vacutainers. Serum was separated immediately by centrifugation at 2000rpm for 15mins for the estimation of antioxidants namely ceruloplasmin, malondialdehyde, glutathione using photometric method.
Results: The mean values of Ceruloplasmin (the mean values for controls 32.87, oral cancer 40.49), Glutathione (controls 4.46 oral cancer 5.55), malondialdehyde (controls 2.97, oral cancer 4.34) were compared using students t-test. The mean values of antioxidants were significantly increased in cancer compared to controls (pvalue < 0.05), It can be observed from the results that values of enzyme increased from controls to Oral cancer.
Conclusions: It can be stated with our study that the antioxidant defence mechanism constitute a very important defence system against the free radical injury of the cells. The antioxidants come into play not only during minor free radical injurious process but also when the malignancy develops. Hence the antioxidants play a vital role as valuable markers in diagnosing oral cancer.

Keywords: Antioxidant, Ceruloplasmin, Glutathione and Malondialdehyde oral cancer spectrophotometric method

INTRODUCTION

In most people's minds there is no scarier diagnosis than that of cancer. Cancer is often thought of as an untreatable, unbearably painful disease with no cure. However, Cancer is undoubtedly a serious and potentially life-threatening illness.[1] Oral cancer is one of the most fatal health problems faced by the mankind today.[2] In India, because of cultural, ethnic, geographic factors and

the popularity of addictive habits, the frequency of oral cancer is high.[3]

This may be due to high prevalence of the premalignant lesions and conditions of oral cavity.4Cancer or precancer states can be found in individuals who are non-smokers and quid chewer.[5] Cancer is a biological variation in which the cells have acquire in a high power of uncontrollable development and multiplication, but have lost the power of contact.[6] Tobacco smoke is a complex mixture of carcinogenic compounds and it contains numerous substrates such as benzopyrene, ethylene oxide and 4-amino biphenyl.[7]

Chemical carcinogenesis is a progressive process involving two distinct sequential stages viz initiation and promotion. During the phase of initiation, a normal cell undergoes irreversible DNA damage by covalent binding of electrophiles and reactive oxygen species generated by carcinogens is termed as INITIATED CELL. During the phase of promotion there is a clonal proliferation of these altered cells forming neoplastic cell.[8,9]

An antioxidant is a substance that when present in low concentration relative to the oxidable substrate significantly delays or reduces oxidation of the substrate.[10] The body has developed several endogenous antioxidant systems to deal with the production of ROS (reactive oxygen species). These systems can be divided into enzymatic and non-enzymatic groups. The enzymatic antioxidants include superoxide dismutase (SOD) which catalyses the conversion of O_2 to H_2O_2 and H_2O, catalase, which then converts H_2O_2 to H_2O and O_2 and glutathione peroxidase, which reduces H_2O_2 to H_2O.[11]

In addition, the trace elements Selenium, Manganese, Copper and Zinc also play important roles as nutritional antioxidant cofactors. Selenium is a cofactor for enzyme glutathione peroxidase and Manganese, Copper and Zinc are cofactors for superoxide dimutase. Zn also acts to stabilize the cellular metallothionein in pool, which has direct free radical quenching ability. The complex interaction of these different antioxidants system may imply that therapeutic strategies will depend on combination therapy of various antioxidants rather than a single agent.[11]

Aims of the study

To measure the levels of Glutathione, Ceruloplasmin, Malondialdehyde) in oral cancer and compare with smokers and nonsmokers.

Objectives of the study

- Estimation of Glutathione in oral cancer and its comparison with levels among controls
- Estimation of Ceruloplasmin in oral cancer and its comparison with levels among controls.
- Estimation of Malondialdehyde in oral cancer and its comparison with levels among controls.

By using spectrophotometric method.

Glutathione

Glutathione is a ubiquitous tripeptide found virtually in all cells. It also protects cells against the destructive effects of reactive oxygen intermediates and free radicals. The diverse functions of glutathione thus are relevant to enzymology, radiation biology, cancer, toxicology, etc. Glutathione is known to have a role in regulation of enzymes and as a cell protective agent against oxidative stresses.[12] Its functions are:

- Antioxidant defense
- Scavenging free radicals and other reactive species
- Removing hydrogen and lipid peroxides
- Preventing oxidation of biomolecules
- Metabolism and Regulation.

Ceruloplasmin

Ceruloplasmin is a glycoprotein with its polypeptide chain including 1046 Amino acid residues. It is a principal copper containing protein of plasma. The following have all been plausibly proposed as physiological functions of ceruloplasmin.[13] Plasma Ferroxidase Activity: Iron Homeostasis, Ascorbate Oxidase Activity, Copper Transport and Storage, Degradation of Organic Substrates, Antioxidant Activity: Defence Against Oxidant Stress, Pro-oxidant Activity, Oxidation of Nitric Oxide: The Metabolism of Nitroso thiols. Chromatographic studies of human serum led to the proposal that ceruloplasmin is the primary antioxidant and barrier against free radicals in the bloodstream.

Malondialdehyde

Lipid peroxidation of polyunsaturated lipids is a facile process. Malondialdehyde (MDA) is a carbonyl compound generated by lipid peroxidation and during arachidonic acid metabolism for the synthesis of Prostaglandins.[14] The erythrocyte membrane is particularly susceptible to oxidative stress due to its high content of polyunsaturated fatty acids, which are more vulnerable to oxidative damage. The normal erythrocyte is however highly resistant to free radical damage because of its multilevel defense mechanisms against lipid peroxidation. A high level of lipid oxidation products can be detected in cell degradation after cell injury or disease.

METHODS

The material for the present study comprise of 50 subjects selected at random from among the patients attending the department of Oral Medicine and

Radiology, Government Dental College and Hospital, Hyderabad and cancer patients attending MNJ Cancer institute, lakadikapool, Hyderabad.

All the subjects included were examined using mouth mirror and probe under adequate artificial light. History including habits and clinical findings were located in a specially prepared standard proforma. After taking informed consent from the patient, the patient clinically diagnosed as oral cancer underwent biopsy and initial clinical diagnosis was confirmed histopathologically. Materials: Tourniquet, 5ml syringes, Sterile siliconized tubes

EDTA

A total of 2.5ml ml of blood was drawn from the mid-cubital vein with necessary aseptic precautions in a 5-ml disposable syringe. It is transferred to sterile vacutainers containing Clot activators. Serum was separated immediately by centrifugation at 2000rpm for 15mins for the estimation of antioxidants namely ceruloplasmin, malondialdehyde, glutathione. These subjects were categorized into 2 groups:

Group I 25 Healthy subjects- controls (smokers and nonsmokers). Group II 25 patients who were clinically and histopathologically diagnosed as cases of oral cancer.

- Estimation on ceruloplasmin by Ravin method by using para phenylene diamine.[16]
- Estimation of glutathione by spectroflourimeter Testmethod: Mauriz et al. (2007).[17]
- Estimation of malondialdehyde by Test Method: Gavino e t al (1981).[15]

Statistical analysis

Data collected are tabulated and subjected to statistics using student's t test.

RESULTS

Study is aimed at the estimation of levels of antioxidants namely Glutathione, Ceruloplasmin, Malondialdehyde) in oral cancer and controls. The materials for present study comprised of 50 subjects who were categorized into 2 groups 25 healthy subjects free of lesions (controls), 25 patients of Oral cancer.

Table 1: Student's t test and p values of ceruloplasmin in different groups.

Group ceruloplasmin	Number of patients	Mean	Sd	Difference from control Mean±sd	T value	P value
Control	25	32.87	3.69			
Cancer	25	40.49	2.17	7.65±1.52	-8.901	0.000 s

The mean values of Ceruloplasmin in different groups were compared using students t-test. The mean values of enzyme were significantly increased in cancer compared to controls (p-value < 0.05), the mean values for controls 32.87, oral cancer 40.49. It can be observed from the results that values of enzyme increased from controls to Oral cancer.

Table 2: Student's t test and p values of glutathione in different groups.

Group glutathione	Number of patients	Mean	SD	Difference from control Mean±SD	t value	P value
Control	25	4.46	0.39	--	--	--
Cancer	25	5.55	0.91	1.09±0.52	-5.494	0.000 S

Table 3: Student's t test and p values of malondialdehyde in different groups.

Group MDA	Number of patients	Mean	Sd	Difference from control Mean±sd	T value	P value
Control	25	2.97	1.09	--	--	--
Cancer	25	4.34	1.69	1.37±0.60	-3.399	0.001s

The mean values of glutathione in different groups were compared using students t-test. The mean values for controls 4.46 oral cancer 5.55 enzyme was significantly increased in oral cancer compared to controls (p-value < 0.05). It can be observed from the results that values of enzyme increased from controls to Oral cancer.

Figure 1: Carcinoma.

Figure 2: Centrifugation machine.

Figure 3: Spectrophotmeter.

The values of malondialdehyde in different groups were compared using students t-test. The mean values of enzyme controls 2.97, oral cancer 4.34. was significantly increased in oral cancer compared to controls (p-value < 0.05). It can be observed from the results that values of enzyme increased from to Oral cancer.

DISCUSSION

Tobacco use is a well-known risk factor for cancer and preneoplastic states. At least six alkaloids are present in the betel nut itself, of which arecoline and arecadine have been suggested as possible carcinogens.[18]

Lipid peroxidation induced by free radicals has been implicated in the pathogenesis of various diseases, and the extent of cellular damage induced by free radicals can be modulated by antioxidant defense mechanisms.[19] The antioxidant property of the serum is attributed to ceruloplasmin. In normal erythrocyte cell several enzymes are elevated to detoxify the reactive radicals, namely the superoxide dismutase, glutathione, glutathione peroxidase and glucose-6-phosphate dehydrogenase.[20]

Ceruloplasmin

The increase in oxidative stress, there is increase in the ceruloplasmin levels. This has been supported by many authors in precancer states viz Anjali Rao et al and Manjula et al, Ozagur et al, Krishnamurthy et al, Sharma et al in different cancers.[20-23]

In our study on ceruloplasmin, there was significant difference between cancer patients to controls group (p value < 0.0005). Cancer, had higher values of enzyme compared to controls and the values were statistically significant (p value< 0.0005). These observations are in agreement with observations of Jha et.al in oral carcinoma, Sharma et.al in malignant tumours, Ermis et al, in bronchial asthma, Mehmet et.al in oesophageal cancer and Patel et al neuro degrative diseases. The present observations disagree with observations of Krishnamurthy et al, Jha et al in oral cancer and Aroor et al in-brain tumors, Ozagur et al.

However, Rao et.al in their study on leukoplakia and oral cancer have shown that there was decrease in the ceruloplasmin levels in patients with oral cancer compared to control group and precancerous states.[21] However the levels of the enzyme increased following the treatment in patients with oral cancer. They explained that ceruloplasmin probably tries to minimize the pro-oxidant status, favouring a good prognosis in such cases. In contrast to these studies our study shows significant increase in the values of ceruloplasmin in patients with oral cancer compared to controls and precancer states.[21]

It is observed from other studies that not only in oral cancer, but also in other malignancies, increased ceruloplasmin (or serum copper) would function as second tier, of antioxidant protection along with other antioxidants.[24]

Glutathione

It is a ubiquitous tripeptide found in virtually all cells.[12] It

protects cells against the destructive effects of reactive oxygen species. Serum levels of the enzyme are often raised in patients with both cancer and precancerous states. Zhang et al and Krishnamurthy et al in oral cancer.[20,23] The reason for raised levels in precancer and cancer patients has been explained on the basis of the theory that, carcinogen altered cells have acquired increased resistance to cytotoxic effects of xenobiotics and hence can selectively grow in a toxic environment relative to the normal cells.[23]

Over expression of GSH and glutathione peroxidase has been reported in malignant tumors and cell lines. However, this theory has not been completely proven. Despite the decrease in the levels of GGT in overt neoplasias, there is increase in the levels of GSH in the cells.[19] In contrast to this theory, other school of thought states that the levels of this enzyme are reduced in cancer patients due to increased turnover to counteract the oxidative damage. This theory has been supported by works of Anjali Rao et al in oral cancer and Elzbieta et alon colorectal cancer patients.[21,25]

In our study on serum glutathione, there was significant difference between patients with lesions and controls (p value < 0.0005). Significant differences were observed between controls and cancer, (p value < 0.0005). These observations are in agreement with observations of Zhang et al and Krishnamurthy et al.[20]

The raised red cell glutathione values in the subjects with lesions indicate the strong antioxidant defense mechanism in these cells. This is set against the toxic substances released by the tumors.

Malondialdehyde

Malondialdehyde (MDA) is a carbonyl compound generated by lipid peroxidation, during arachidonic acid metabolism for the synthesis of Prostaglandins.[14] The erythrocyte membrane is particularly susceptible to oxidative stress due to its high content of polyunsaturated fatty acids, which are more vulnerable to oxidative damage. Malondialdehyde (MDA) has been recognized as reactive products of membrane lipid peroxidation, and has been demonstrated to play a vital role in the pathogenesis of several diseases and inflammatory processes. The normal erythrocyte is however highly resistant to free radical damage because of its multilevel defense mechanisms against lipid peroxidation. High levels of oxidative stress result in peroxidation of membrane lipidswith the generation of peroxides that can decompose to multiple mutagenic carbonyl products.[26]

In our study on malondialdehyde, statistically significant differences were observed between controls and cancer patients (p value < 0.0005) and controls. The observations are in agreement with Shanmugam et.al on cervical carcinoma, Balasenthil et al on oral cancer and hamster buccal pouch cancer, Elzbieta et al colorectal cancer.[19] With these observations it can be concluded that there is higher oxidative damage in cancer as compared to and controls.

In the present study the raised values of the enzyme in serum ceruloplasmin of the cancer patients compared to controls indicate an increased antioxidant defense in response to the toxic substances released by the tumor and preneoplastic cells hence can be used as reliable marker of oxidative damage in these cells. The increased levels of GSH in erythrocytes may be in response to the toxins released by cancer cells. It is said that these erythrocytes are also resistant to oxidative hemolysis, indicating that these cells were adequately protected against any free radical damage, if serum levels of other antioxidants are suppressed. The elevated MDA levels is due to the increased oxidative stress in erythrocytes of precancer and cancer patient.

CONCLUSION

Ceruloplasmin being an effective antioxidant protein is one of the acute phase reactants, whose concentration in plasma rises after tissue injury. It can be said that Ceruloplasmin levels can be used as reliable marker of oxidative damage.

Glutathione levels in oral cancer is increased may be in the response to the toxins released by cancer cells. It is said that these erythrocytes are also resistant to oxidative hemolysis indicating that these levels were adequately protected against any free radical damage although serum cells of other antioxidants were suppressed.

A high levels of lipid peroxidation production can be detected in cell degradation after cell injury or disease. Therefore, the levels of malondialdehyde can is increased in precancer and cancer.

It can be stated with our study that the antioxidant defense mechanism constitutes a very important defense system against the free radical injury of the cells. The antioxidants come into play not only during minor free radical injurious process but also when the malignancy develops. Hence the antioxidants play a vital role as valuable markers in diagnosing oral cancer.

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