

SALIVARY OXIDANT – ANTIOXIDANT STATUS IN ORAL SQUAMOUS CELL CARCINOMA

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CERTIFICATE

Certified that the dissertation on “**SALIVARY OXIDANT – ANTIOXIDANT STATUS IN ORAL SQUAMOUS CELL CARCINOMA**” done by **Dr. B. DEVI PRIYA**, Part II Post Graduate student (MDS), Branch IV – Oral and Maxillofacial Pathology, Saveetha Dental College and Hospitals, Chennai submitted to The Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment for the M.D.S. degree examination in February 2005, is a bonafide research work done under my guidance and supervision.

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INTRODUCTION

“Apples turn brown. Butter turns rancid. Iron rusts.”-

All are everyday signs of oxidative stress which is, simply stated, the destruction caused by free radical molecules. But none of these nuisances compare to what these unstable molecules can do inside the body!

Free radicals???

A free radical may be defined as any species capable of independent existence that contains 1 or more unpaired electron. (Halliwell 1991)⁸¹. They are highly reactive molecules. They have been implicated in over 100 human diseases ranging from rheumatoid arthritis and hemorrhagic shock to AIDS¹¹⁶. Examples of free radicals are:

1. O₂- ground state oxygen
2. Singlet oxygen
3. Super oxide anion radical
4. Hydroxyl radical
5. Carbon centered radical

Reactive oxygen species???

These are oxygen containing molecules that have a higher reactivity than the ground state oxygen. They are capable of free radical formation in the extra oral and the intraoral environments. (Halliwell and Gutteridge 1990)⁸⁶. They are also termed as pro-oxidants which cause tissue damage by a variety of different mechanisms¹¹⁶ which include

- 1) DNA damage
- 2) Lipid per oxidation
- 3) Protein damage
- 4) Oxidation of important enzymes

5) Stimulation of cytokine release

There are many different sources by which the reactive oxygen species (ROS) are generated.

1. **Exogenous:** Exogenous sources include exposure to cigarette smoke, emission from automobiles and industries, excess alcohol, asbestos, exposure to ionizing radiation and bacterial, fungal or viral infections.
2. **Endogenous:** ROS can form as by-products of normal and essential metabolic reactions, such as energy generation from mitochondria or the detoxification reactions involving the liver cytochrome P-450 enzyme system.

Antioxidants???

These are substances which when present at low concentrations compared to those of an oxidisable substrate and will significantly delay or inhibit oxidation of that substrate⁸⁶. They act as defense mechanisms against the free radicals.

Antioxidant mechanisms

Antioxidants (AO's) are classified according to their mode of action.

1. **Scavenging AO's** prevent oxidative damage by literally scavenging radicals as they form.
2. **Preventive AO's** function largely by sequestering transition metal ions and are largely proteins by nature.
3. **Enzyme AO's** are systems that function by catalyzing the oxidation of other molecules.

They may be:

1. **Cellular antioxidants** such as Glutathione GSH, α - tocopherol and Ascorbate.

2. **Extra cellular antioxidants** such as albumin, uric acid, ferritin, ceruloplasmin
3. **Antioxidant enzymes** which may be further divided into primary and secondary enzymes.

The **primary** enzymes include Super oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase.

The **secondary** enzymes include Glutathione – S-Transferase, Glutathione reductase (GR), Glucose– 6 – Phosphate dehydrogenase (G6PD)

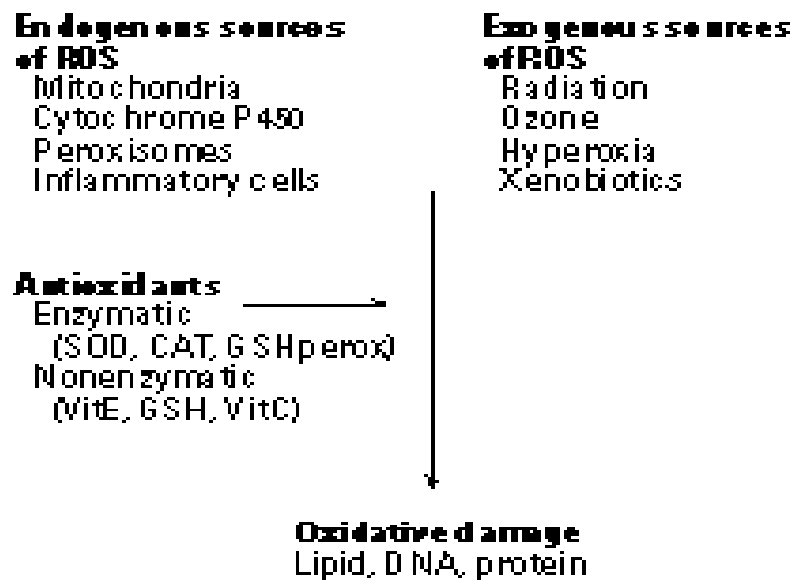


Figure 1. Reactive oxygen species production and disruption of cellular homeostasis. ROS can be produced by both endogenous and exogenous sources. The normal antioxidant defense system acts against these substances. CAT, catalase; GSH, reduced glutathione; GSHperox, reduced glutathione peroxidase; SOD, super oxide dismutase; VitC, vitamin C; VitE, vitamin E.

Oxidative stress:

Oxidative stress is the term applied to the disturbance in the balance between the production of reactive oxygen species and antioxidant defenses which may lead to tissue injury. Oxidative stress results when the balance between the production of reactive oxygen species (ROS) overrides the antioxidant capability of the target cell. The formation of oxidative stress may result in damage to critical cellular macromolecules including DNA, lipids, and proteins.

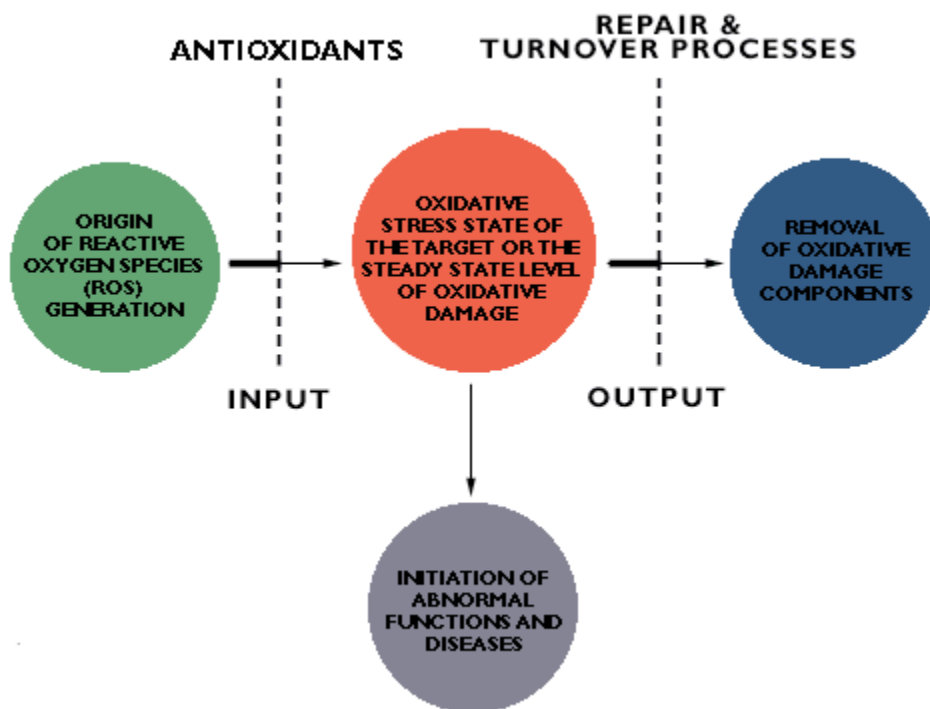


Figure 2.

Virtually all diseases thus far examined involve free radicals. In most cases, free radicals are secondary to the disease process, but in some instances free radicals are causal¹¹⁶. Thus, there is a delicate balance between oxidants and antioxidants in health and disease. A few examples of pathological conditions include heart disease, cancer, atherosclerosis, rheumatoid arthritis, periodontal diseases, cataract, diabetes mellitus,

inflammatory bowel disease, retinal ischemia, AIDS, ARDS and neurodegenerative diseases such as stroke, Parkinson's disease and Alzheimer's disease¹¹⁶.

Cancer:

The most merciless and dreaded disease of the human body is cancer. A neoplasm is defined as "An abnormal mass of tissue whose growth exceeds and is uncoordinated with that of normal tissues and which persists in the same excessive manner after cessation of the stimuli which evoked it¹⁵⁷." Cancer is progressively becoming a more and more important health problem as more people are becoming aware of its vital role in affecting the health of the human race.

Oral squamous cell carcinoma or oral cancer is the sixth most common malignancy and is a major cause of cancer morbidity and mortality world wide¹⁰¹. Globally about 500000 new oral and pharyngeal cancers are diagnosed annually and three quarters of these are from the developing world. It is already established that oral carcinomas in India constitute 47% of total carcinomas affecting the body.

Over the past few years there has been no change in incidence but there has been a slight decrease in mortality.

Oxidative Stress in the Cancer Process: Oxidative stress has also been implicated in the cancer process^{79, 179} either by an increase in the formation of free radicals or a decrease in the antioxidant defense mechanism.

Carcinogenesis is generally divided into 3 stages as

- Initiation
- Promotion
- Progression

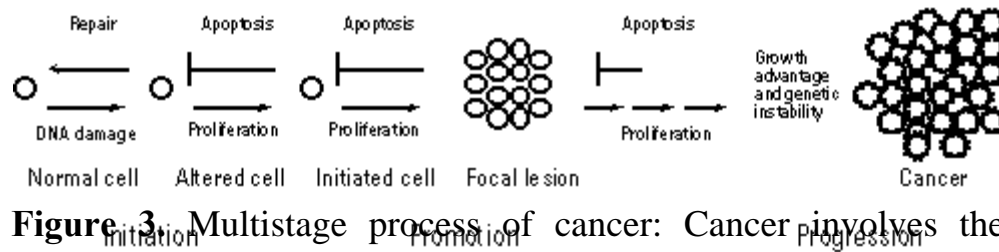


Figure 3. Multistage process of cancer: Cancer involves the formation of an altered cell that becomes a mutated initiated cell after a round of DNA synthesis. This initiated cell may clonally grow through either the induction of cell proliferation or the inhibition of apoptosis to a focal lesion. Subsequent additional DNA damage and genetic instability may allow selective focal lesions to progress to the neoplastic stage.

Oxidative stress interacts with all three stages of the cancer process⁹⁸:

1. **Initiation stage:** Oxidative DNA damage may produce gene mutations and structural alterations of the DNA, resulting in a heritable mutation.
2. **Promotion stage:** ROS and oxidative stress can contribute to abnormal gene expression, blockage of cell-to-cell communication, and modification of second messenger systems, resulting in an increase in cell proliferation or a decrease in apoptosis in the initiated cell population. This results in the clonal expansion of the initiated cells to preneoplastic focal lesions.
3. **Progression stage:** ROS impart further DNA alterations to the initiated cell population. These changes may result in changes in enzyme activity and make the lesions resistant to normal growth control.

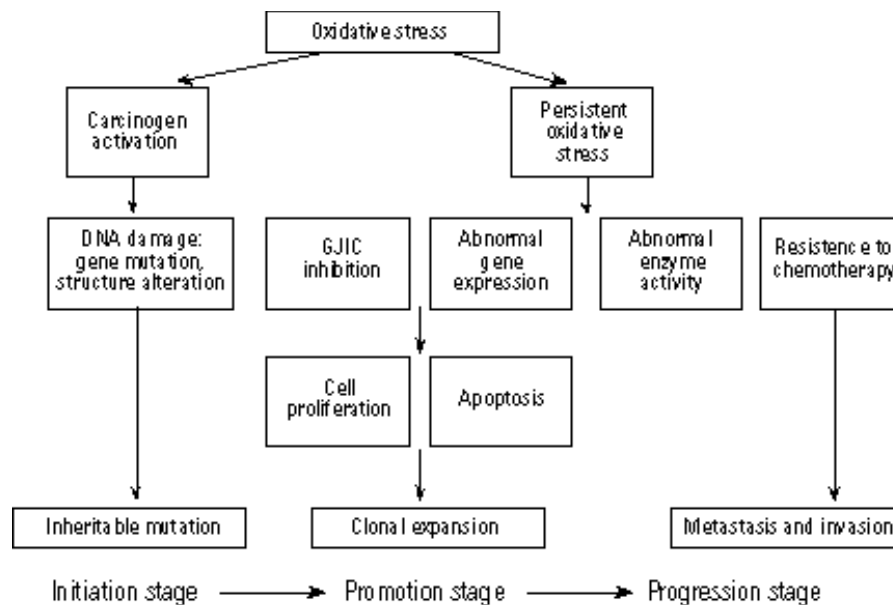


Figure 4: GJIC, gap junctional intercellular communication.

Research is now being directed towards assays for antioxidant capacities of biological fluids. Numerous studies have been performed in serum and tissues with regard to antioxidant levels.

Saliva, a heterogeneous biological fluid comprising of proteins, electrolytes, small organic molecules, compounds transported from the blood constantly bathes the oral cavity. Saliva could contribute to a first line of defense against free radical mediated oxidative stress²⁴. Saliva has pro-oxidant¹⁶² and antioxidant properties²⁴.

In the last 10 years the use of saliva as a diagnostic fluid³⁹ has become somewhat of a translational research success story.

Hence we were interested in studying lipid peroxidation which is a measure of free radical production and the levels of uric acid, albumin and glutathione detectable in saliva of oral squamous cell carcinoma patients and comparing them with normal controls, thus assessing the oxidant-antioxidant status in oral squamous cell carcinoma {OSCC} and hence to explore the potential usability of saliva as a diagnostic tool.

REVIEW OF LITERATURE

Irwin Fridovich (1978)⁹³ has reviewed the biology of oxygen toxicity and suggested that super oxide radicals cause the cellular damage and the cell acts against the free radicals with defense mechanism of the antioxidative enzymes like Super Oxide Dismutase, Catalase and Peroxidase.

Rebecca Gerschman, Dan Gilbert¹⁵⁶ and their colleagues suggested that oxygen is toxic because it makes free radicals.

Corroll E Cross, Albert Van der Vliet *et al* (1994) studied reactive oxygen species affecting lung epithelium and antioxidative actions against the oxygen species by lung epithelial tissue-antioxidative enzymes and vitamins.

Wulf Droge (2001)²¹⁴ explained in his publication about release of free radicals in physiological cell metabolism and their control by the cell with antioxidants.

S. Bhaskaran, S. Lakshmi (1999)²¹ studied effects of cigarette smoking on lipid peroxidation and antioxidative enzymes like Super Oxide Dismutase, Glutathione Peroxidase and Catalase in albino rats. Their results suggest that cigarette smoking induces lipid peroxidation and the antioxidant enzyme levels were enhanced in order to protect the tissues against the deleterious effects of the oxygen-derived free radicals. The depletion of reduced glutathione in these organs could be due to its utilization by the tissues to mop off the free radicals.

Cross C.⁵⁰ stated that Free radicals (FR) cause oxidative damage to nuclear DNA and consequently somatic mutations such as base changes, deletions and chromosomal strand breaks are developed.

Feig DI, Reid TM, Loeb LA.⁶⁶ Stated that FR also activate oncogenes and inactivate tumor suppressor genes such as p53 by mutational changes.

Studies show that stage I-III of HIV disease are characterized by significant impairment of antioxidative defenses provided by selenium, GSH-Px and GSH.

Chapple (1997), Battino et al (1999)⁴⁴ claimed that the imbalances in levels of free radicals and reactive oxygen species with antioxidants may play an important role in the onset and development of several inflammatory oral pathologies.

Battino et al (1999)²³ reviewed the current evidence for oxidative damage in the chronic inflammatory periodontal diseases and the possible therapeutic effects of antioxidants.

Several studies confirmed the reactivity and associated toxicity of ROS may be major contributors to the pathogenesis of several chronic degenerative diseases.

R.J. Waddington, R. Moseby, G. Emberg (2000)²²¹ provided further evidence for the role of ROS in tissue destruction associated with inflammatory periodontal diseases.

Elena M.V. de Cavanaugh, Alba E. Honegge, Erica Hofer et al⁶¹ underscored the importance of antioxidant therapy to protect cells from further damage.

Mascie R.D., Murphy M.E. et al (1991)¹²⁵ in their article gave an excellent overview of oxidative stress in which anaerobic metabolism entails the generation of oxygen species capable of damaging DNA, proteins, carbohydrates and lipids. These potentially deleterious reactions were

controlled by a system of enzymatic and nonenzymatic antioxidants which eliminated pro-oxidants and scavenged free radicals.

Pryor W.A. (1991)¹⁵⁴ stressed the role of free radicals in aging and that improvement in immunocompetence was caused by dietary antioxidant nutrient and any one of these nutrients could play a role as a reducing agent without necessarily behaving either as an antioxidant or a free radical scavenger.

ILC Chapple (1997)⁴⁴ reviewed the role of ROS and antioxidant defense mechanisms in inflammatory diseases and explained the role in periodontal disease.

Davies K.J. (1988)⁵³ proposed a comprehensive classification of antioxidant defense system.

Irwin Fridovich and Joe McCord (1969)⁹³ a specific free radical in the spotlight, super oxide.

Johnson N reported that tobacco smoke stimulates H₂O₂ and hydroxyl radicals in addition to direct carcinogenic effects on the epithelial cells of the oral mucous membrane.

Terala LS, Repine JE¹⁹⁵ free radicals are defined as a molecule or atom with an unpaired electron, thus reduction of oxygen (O₂) by 1,2, or 3 electrons causes formation of super oxide anion, hydrogen peroxide and hydroxyl radical (OH) respectively.

Lunce F, Blake D¹¹⁷: Polyunsaturated lipids, proteins, DNA and carbohydrate are susceptible to free radical attack.

N.M. Boyd and P.C. Read (1988)³² have reviewed the factors associated with carcinogenesis considering familial or genetic factor, dietary factors, age and gender.

N.M. Boyd and P.C. Read (1988)³³ have reviewed three types of carcinogenic stimulus namely Chemical, Physical and Viral and their possible mechanism in general and their possible relevance to the induction of oral mucosal carcinoma in particular.

A.I. Zavas, C.W. Douglass *et al* (2001)²²⁰ studied the role of smoking and alcohol in the etiology of oral squamous cell carcinomas in Greece and found increased risk of oral cancer in combination of smoking and alcoholism than individual habit. They further noticed that the risk is more in female comparing with males with these habits.

Rustgi AK, Podolsky DK.¹⁶⁶ Stated that procarcinogens ingested by foods are activated by host enzymes most often in the liver to yield electrophilic carcinogens that may then bind covalently to DNA and lead to mutations.

Babu KG¹⁶ Suggests that the location of tumors within the oral cavity correlates with tobacco smoking as well as the site where the betel quid is kept for prolonged hours.

M.E. Rahbani-Nobar, Rahimi Nobar *et al* (1999)¹⁵⁸ studied plasma total antioxidant capacity and changes in the activity of two antioxidant enzymes Super Oxide dismutase and glutathione peroxidase in 125 diabetic patients and compared with 120 normal control group. Authors found reduction in total antioxidant capacity and decreased activity of Super Oxide Dismutase and glutathione peroxidase and suggested that in case of low activity of SOD and GPx patients supplemented with trace elements such as Selenium, Copper, Zinc and Manganese, which are the essential components of the enzymes.

Dr. Warwick, D. Reymont *et al* (1997)²⁰⁹ described cell damage by free radicals causing cancer and action of various types of natural

antioxidative enzymes like Super Oxide Dismutase, Glutathione Peroxidase and Catalase and antioxidative vitamins Vitamin A, C and E against the free radicals.

J.H. Jeng, M.C. Chang and L.J. Hahn (2001)¹⁰² reviewed the role of areca nut in betel quid and its association with chemical carcinogenesis. Many of literature based studies revealed that areca nut extract may induce pre neoplastic and neoplastic lesions and contents of areca nut were highly suspected carcinogens. Reactive oxygen species produced during auto oxidation of areca nut poly-enols in the betel quid of chewer's saliva, which are crucial in initiation and promotion of oral cancers. Arecadine and areca nut extract are further suggested to be tumor promoters. Anti oxidants such as Glutathione and N-acetyl L-cysteine can potentially prevent such cell damage.

Sabitha. K.E., Shyamaladevi. C.S. (1998)¹⁶⁸ studied the effect of radiation on Oral Cancer patients using the activity of SOD, GPx and Catalase. These enzymes in blood showed significant decrease representing lack of antioxidative defense and suggested that the degree of radiation affects the decrease of antioxidative enzyme activities and increase in lipid peroxidation.

H. Mukundan & A.K Bahadur *et al* (1999) determined plasma Glutathione and erythrocyte Glutathione Peroxidase in 30 patients with invasive cervical carcinoma before initiation and completion of radiotherapy and found that the levels of Plasma Glutathione, erythrocyte Glutathione peroxidase levels are decreased when compared with normal controls. The study indicates a change in the antioxidants in relation with the Glutathione system among patients with invasive carcinoma of uterine cervix.

Dr. A.J. Vanisree and Dr. C.S. Shyamaladevi (1999)²⁰⁷ studied eighty patients with lung cancer and non malignant lung diseases for antioxidative enzyme activity of Super Oxide Dismutase, Glutathione Peroxidase and Catalase and found that the antioxidative enzymes were decreased in lung malignant pleural exudates when compared to those of non-malignant effusions.

R. Subapriya, R. Kumaraguruparan, C.R. Ramachandran et al²²² compared the extent of lipid peroxidation and the status of antioxidants in tumor and venous blood of patients with OSCC at different intraoral sites. They found no significant difference in different sites but observed differences between the tumor and blood with respect to their susceptibility to lipid peroxidation and antioxidant status.

J. Yang, E.W.N.Lam, H.M. Hammad et al⁹⁴ concluded that detection of antioxidant enzymes may be a useful future marker in the molecular diagnosis of the oral cancer and that it may be possible to monitor oral cancer and that it may be possible to monitor the effectiveness of chemo preventive and therapeutic strategies in oral cancer and tumor recurrence.

Elena M.V. de Cavanaugh, Alba E. Hon egger, Erica Hofer et al.⁶¹ evaluated oxidative stress in peripheral blood plasma (PBP) and bone marrow plasma (BMP) from lung carcinoma and breast carcinoma patients and concluded that untreated cancer patients presented an imbalance between oxidant generation and lipid soluble antioxidant levels in favor of the former.

Stawein H.B. Fred et al (1991) stated that amongst the various mechanisms thought to influence carcinogenesis, free radical formation was particularly important as free radicals could damage DNA structural proteins, enzymes and membranes and lead to toxic products and that body's

defense system against free radicals depended heavily on antioxidants, vitamins and carotenoids.

Enuonwu C.D. and Mechs V.I. (1995) stated that active oxygen species and other reactive free radicals long considered to be mutagenic were then believed to mediate other phenotypic and genotypic alteration that led from mutation to neoplasia. Burst of reactive oxygen species have been implicated in the development of oral cancer in tobacco and beetle quid chewers. Many maternal mutagens and carcinogens found in human diet might act through generation of oxygen radicals.

Meydani M and Meisler J.G. (1997)¹³¹ stated that oxidation appeared as a constant and natural process with resultant atoms or nucleus with unpaired electrons, known as free radicals. They were by products of normal metabolism that lead to shifts and exchanges of electron during chemical reaction in the body. Those charged compounds produced by exposure to radiation and environmental agents as well as by normal body function were highly unstable. They further stated that according to Ames and Colleagues about 1% to 2% of oxygen consumed by the cells in the body escaped during normal metabolism forming reactive oxygen free radicals. On any given day, the DNA in every human cell may be bombarded 10,000 or more times by oxidative reactions. If this constant attack on DNA was not neutralized, cells might undergo mutation and uncontrolled division leading to cancer.

Singal PK, Petkau A, and Hrushovetz S, Foerster J et al¹⁷⁹ commented that oxidative stress has a role in the process of cancer and inflammation.

Guyton KZ, Kenster TW⁷⁹: Evidence supporting the role of free radicals in the etiology of cancer.

Feig DI, Reid Tm, Loel LA⁶⁶: The reactive oxygen derivatives are involved in the pathogenesis of 3 common tumors: lung cancer, hepatocellular carcinoma and carcinoma of prostate.

Kang DH¹⁰⁴ Oxidative stress is a disturbance in the balance between the production of reactive oxygen species (ROS) and antioxidant defenses. It occurs when excessive production of ROS overwhelms the antioxidant defense system or when there is a significant decrease or lack of antioxidant defenses. Oxidative stress, in turn, is known to cause DNA damage and mutations of tumor suppressor genes that are critical initial events in carcinogenesis.

Pelicanoh, Carney D, Huang P¹⁵¹. Reactive oxygen species (ROS) are constantly generated and eliminated in the biological system, and play important roles in a variety of normal biochemical functions and abnormal pathological processes. Growing evidence suggests that cancer cells exhibit increased intrinsic ROS stress, due in part to oncogenic stimulation, increased metabolic activity, and mitochondrial malfunction. The escalated ROS generation in cancer cells serves as an endogenous source of DNA-damaging agents that promote genetic instability and development of drug resistance.

Behrend L, Henderson G. Zwacka Rm²⁵. An elevated oxidative status has been found in many types of cancer cells, and the introduction of chemical and enzymological antioxidants can inhibit tumour cell proliferation, pointing to a critical role of ROS in mediating loss of growth control.

Barbara Kochanska, Ryszard T. Smolenski and Narcyz Knap¹⁸ analyzed the profile and normal concentration of nucleotide metabolites in human saliva and concluded that the determination of nucleotides and their

catabolites in saliva due to its simplicity and reproducibility may be a clinical value in diagnosis of local or systemic disorders.

M. Battino, M.S. Ferzcuo I, Gallardo (2002)²⁴ reviewed the antioxidant capacity of saliva and concluded that systematic studies of saliva are still lacking.

Terao and Nagao (1991)¹⁹⁶ proposed that saliva may constitute a first line of defense against free-radical mediated oxidative stress.

Studies confirm that saliva is rich in antioxidants, mainly uric acid, with lesser contributions from albumin and glutathione.

Terao and Nagao (1991)¹⁹⁶ demonstrated that saliva has a role in suppressing the lipid peroxidation of ingested foods.

Lenora R. Biglev, Charles et al¹¹⁴ indicated that C-erb B-2 protein expression in saliva may be a very useful diagnostic tool for measuring patient response to chemotherapy and /or surgical treatment and their disease.

Reznick AZ, Hershkovich O, Nagler RM¹⁶² suggested that the saliva always intervenes the cigarette smoke and prevents direct contact with the mucosa. They demonstrated a synergistic effect of CS and saliva on oral cancer cells. This synergism is based on the reaction between redox active metals in saliva and low reactive free radicals in cigarette smoke, which results in the production of highly active hydroxyl free radical. Thus when exposed to cigarette smoke, salivary behavior is reversed and the saliva loses its antioxidant capacity and becomes a potent prooxidant milieu.

Boyle et al (1994)³⁵ examined the possible value of p53 in saliva as a marker for squamous cell carcinoma. They detected and identified tumor specific mutations in p53 in preoperative salivary samples of individuals

suffering from head and neck squamous cell carcinoma. Positive findings were observed in 71% of the patients studied.

Tavassoli et al (1998)¹⁹³ found salivary antibodies to P53 elevated among patients with oral carcinomas.

Kerlikowske et al (1995)¹⁰⁷ reported that a saliva test used in conjunction with imaging may increase the overall diagnostic value of the latter test and reduce the number of false positive and negatives currently associated with imaging.

Jenzano et al (1988)¹⁰⁰ reported the use of saliva to detect kallikrein in patients with solid tumours that were remote from the oral cavity.

Di-Xia, Schwartz and Fan-Qun (1990)⁵⁷ found that saliva contained LA125, a glycoprotein complex that is often used marker for ovarian cancer. Their studies suggested that the salivary CA125 assay had a better and diagnostic value than comparable serum assay.

Navarro et al (1997)¹⁴⁰ showed that epidermal growth factor concentrations were higher in saliva of women with primary breast cancer or recurrence.

Streckfies et al¹⁸⁶ found that the protein product of the oncogene c-erb B-2 is elevated in saliva of women with breast cancer.

CF Streskfus, LR Bigler³⁹ et al have reviewed the use of saliva as a diagnostic fluid.

Dean V. Sculley and Simon C. Langley. Evans (2002)⁵⁴ reviewed the salivary antioxidants and periodontal disease status.

Y.M.C. Henskens, U Vander Velden, E.C.I. Veerman et al²¹⁵ investigated that salivary protein, albumin and cystatin concentration in saliva of individuals with gingivitis or periodontitis and found that the protein and albumin concentrations in saliva of individuals with gingivitis or

periodontitis were significantly increased compared with the healthy subjects.

Diab-Ladki R, Pellat B, Chahine R⁵⁵. studied the role of free radical induced tissue damage and the antioxidant defense mechanism of saliva in periodontal disease.

The total antioxidant capacity of saliva is significantly decreased in these patients.

They concluded that periodontal diseases are associated with an imbalance between oxidants and antioxidants in favor of the former due to both an increase in free radical production and a defect in the total antioxidant activity of saliva.

Moore S, Calder KA, Miller NJ, Rice-Evans CA¹³³. antioxidant potential of saliva does not appear to be compromised in patients with periodontal disease but this may relate to the antioxidant flow from the GCF.

Nagler RM, Klein I, Zarzhevsky N, Drigues N, Reznick AZ¹³⁸. demonstrated the existence of much higher concentration of various salivary molecular and enzymatic antioxidant parameters in the parotid saliva compared with the submandibular/sublingual saliva.

Akkuş I⁴. stated that Lipid peroxidation is a biological pathway concerning peroxidation of cell membrane phospholipids and polyunsaturated fatty acids (PUFA) by reactive free oxygen radicals. Hence, lipid peroxy and hydroperoxy radicals are formed. Aldehyde products such as malondialdehyde and 4 hydroxynonenal are produced consequently.

Some researchers detected that LP products (malondialdehyde and 4 hydroxynonenal) in tumour tissues and body fluid samples (serum, urine, bile etc.) of cancer patients were increased.

Nair J et al¹³⁹. reported that L P products derived from diet rich in PUFA form promutagenic exocyclic DNA adducts in human cells and contribute to cancer development.

Firatti E, Unal T, Onan U et al (1994)⁶⁸ have discussed that lipid peroxidation of biological membranes was related to inflammation. The oxidation of gingival tissues during inflammation was enhanced by bacterial or host derived hydroxyl radicals, free oxygen radicals, singlet molecular oxygen and super oxide anions.

Szuster – Ciesielska A. et al¹⁹⁰ studied the production of ROS –super oxide anion and hydrogen peroxide by neutrophils isolated from blood of laryngeal carcinoma patients. The serum activity of super oxide dismutase, catalase as well as total peroxidase activity in serum also was estimated. The production of ROS is increased in laryngeal carcinoma and increased parallel with tumor stage.

The serum activity of catalase and peroxidase was also increased in patients with advanced laryngeal carcinoma. Their studies indicate the existence of oxidative stress in blood of patients with laryngeal carcinoma and its significant decrease after partial or total laryngectomy.

Cejas P, Cascado E, Belda – Iniesta C, Castro J D⁴⁰ stated that ROS mediated lipid peroxides are of critical importance because they participate in chain reactions that amplify damage to bio molecules including DNA and that the status redox is of great importance for oncogenetic process activation and it is also implicated in tumor susceptibility to specific chemotherapeutic drugs.

Park JE, Yang JH, Yoon SJ, Leeth et al.¹⁴⁶ suggest that lipid peroxidation resulted in a pro-oxidant condition of U937 cells by the depletion of GSH and inactivation of antioxidant enzymes, which

consequently leads to a decrease in survival and oxidative damage to DNA. The results indicate that the peroxidation of lipid is probably one of the important intermediary events in oxidative stress – induced cellular damage.

Boyd NF, McGuine V.³⁴ Compared the urinary excretion of the mutagen malonaldehyde in premenopausal women at different risks for breast cancer as determined by the breast parenchyma on mammography and suggested that mammographic dysplasia may be associated with lipid peroxidation and raise the possibility that mutagenic products generated by this process may influence breast cancer risk.

B. Ozturk, A Ozet, M. Guven.¹⁵ An increase in LPO may play a role in testes carcinomas.

Uzun k, Vural H, Ozturk J et al.²⁰⁴ The oxidation stress and LP are markedly increased in malignant lung diseases. They concluded that determination of MDA level may be a useful addition to the test currently available in the diagnosis of malignancies.

Serum MDA levels were higher in patients with lung cancer.

Mean serum MDA levels in the malignancies were significantly higher than those of control group and benign lung diseases.

Petruzzells S, Hictanen E, Bartsch H, Camus AM, Mussi et al¹⁵²: Free radicals and lipid peroxidation are associated with lung cancer. LP products and ROS have been found to be very active in binding to DNA to cause mutations and initiate cancers. MDA levels increased in lung cancer.

Richard C, Lemonyer F, Thubult M.¹⁶⁴ LP is prevented in biological membranes by antioxidants such as catalase, super oxide dismutase and glutathione peroxidase.

Floyd RA^{69, 70} Oxidative damage to membrane lipids initiated lipid peroxidation.

Skrzydłewska E, Stankiewicz A, Sulkowska M.¹⁸¹ Significant changes in antioxidant capacity of colorectal cancer tissues, which lead to enhanced action of oxygen radicals, resulting in lipid peroxidation.

The activity of super oxide dismutase, glutathione peroxidase and glutathione reductase was significantly increased while the activity of catalase was significantly decreased in cancer tissue.

The level of non enzymatic antioxidant parameters (glutathione, Vitamin C and Vitamin E) was significantly decreased manifested by a significant rise in malondialdehyde and 4 hydroxynonenal levels.

University of Southern California²⁰². Science Blog May 2002: Suggest a possible cause for kidney cancer – lipid peroxidation might be key mechanism for mysterious malignancy.

Annie apple seed project⁹. Kidney cancer – Lipid peroxidation is the main culprit.

Ender Inci, Sabiha Civelek, and Arzu Seven et al⁶². Investigated the oxidative stress parameters in laryngeal cancer and cancer free adjacent tissues. Laryngeal cancer tissue exhibited higher lipid peroxidation than cancer free adjacent tissue.

They concluded that the detection of antioxidant status may be useful to determine the tissue resistance to therapy, to choose the correct radiotherapy, increase chemotherapy and to monitor the effectiveness of the therapeutic strategy.

Turkdogan MK, Sekeroglu R, Hekun H²⁰⁰. Serum MDA levels are increased in gastric cancer and esophageal cancer.

Concluded that lipid peroxidation plays a role in the etiopathogenesis of upper GI cancers.

Rahman I, Macnee W¹⁵⁹ Free radical attack essentially affects polyunsaturated fatty acid in cell membrane, producing lipid peroxidation which generate hydro peroxide and long lived aldehydes. The end products of these reactions are MDA, ethane and pentane.

Schwartz J.L. Shklar G. (1996) stated that previous studies have shown that reduced glutathione (GSH) inhibited experimental oral carcinogenesis in hamster buccal pouch model. The significant inhibition of oral carcinogenesis associated with GSH administration was correlated with the increased levels of the valid type tumor suppressor gene, suggesting its possible use as a biomarker for GSH chemoprevention. The inhibition of oral carcinogenesis by reduced GSH was also related to a very significant inhibition of tumour angiogenesis defined by factor VIII staining. Thus angiogenesis inhibition may be an additional mechanism for antioxidant chemoprevention and this suggested another possible biomarker for antioxidant chemoprevention.

Gibson et al (1985) suggested the existence of GSH dependent factors that inhibited lipid peroxidation in membranes.

YU B.P. (1994) stated that as with most antioxidant defenses the level of GSH fluctuated under various physiological conditions including aging and some neoplastic diseases.

Several authors have suggested that increased levels of glutathione might be meaningful predictors of poor responsiveness to chemotherapy in several human cancers.

Zappacosta B, Persichilli S, De Sole P, Mordente A, Giardina B²¹⁹ stated that smoking a single cigarette induced a significant reduction in glutathione concentration { $p < 0.05$ }

Moore et al (1994)¹³³ reported that uric acid is the major antioxidant in saliva accounting for more than 85% of the total antioxidant activity of resting and stimulated saliva from both healthy and periodontally compromised patients.

1. Salivary urate concentration varies from 40 and 240 μ M.
2. Salivary albumin concentration is about 10 μ M.
3. Salivary glutathione concentration is about 2 μ M.

Howell and Wyngarden (1960) reported the scavenging of OH by innate mechanism in vitro. Uric acid has traditionally been considered merely an end product of purine metabolism in human and higher apes.

Ames et al (1981)⁶ proposed the role of uric acid as a potential physiological protective defense mechanism both intracellularly and extracellularly although not proved; it has been proposed that uric acid may act by preserving plasma Ascorbate.

Ames BN, Cathcast R, Schwiens E, Hochstein P.⁶ hypothesised that Uric acid is a powerful antioxidant and is a scavenger of singlet oxygen and radicals. The plasma urate levels in humans (about 300 μ M) are considerably higher than the Ascorbate level, making it one of the major antioxidants in humans.

Hasegawa T, Kuroda M.⁸⁷ Suggested that Allantoin is generated from uric acid attacked by free radical, especially by the hydroxyl radical and uric acid plays a role as an antioxidant in plasma of patients with chronic renal failure.

Yardim – Akaydin S, Sepici A, Ozkany etal²¹⁶. Concluded that Uric acid is a free radical scavenger and thus is converted to allantoin. An increase in allantoin levels suggests possible involvement of free radicals in rheumatoid arthritis.

Seppo Lehto MD; Leo Niskanen MD, Tapani Ronnema.¹⁷³
Concluded that Hyperuricemia is a strong predictor of stroke events in middle aged patients with NIDDM independently of other cardiovascular risk factors.

Kondakova I, Lissi EA, Pizarro M¹¹¹. Suggested no statistical difference between uric acid in saliva of smokers and nonsmokers. Also smoking one cigarette does not modify the levels of antioxidants present in saliva.

Pedercen LM, Milman N.¹⁴⁸ **Presence** of microalbuminuria was associated with advanced disease stage and host survival.

Dreilich M, Bergstrom S, Wagenius G et al⁵⁸. Performance status, smoking habits, swallowing function, localization of tumour, leukocytes and albumin levels and stage of disease were prognostic factors.

Pedersen LM, Sorensen PG.¹⁵⁰ Elevated Urinary Albumin Excretion UAE – highly sensitive –indicates clinical behavior of low grade lymphoma. Both response to treatment and time to progression were predicted by levels of UAE.

Pedersen LM, Sorensen PG.¹⁴⁹ Demonstrates a high frequency of albuminuria in patients with breast cancer. Increased UAE was most prevalent in patients with metastatic disease. UAE may be a prognostic marker in metastatic breast cancer.

Vaglio A, Buzio I, Cravedi P.²⁰⁵ UAE is an independent prognostic factor that is related to disease stage in patients with renal cell carcinoma.

Christopher Glis, Robert D Levin, Joel L Granick, et al.⁴⁹
Baseline serum albumin level was a powerful prognostic variable in breast cancer.

Mellanen L, Sorsa T, Lahdevirta J, Helenius M, Kari K, Meurman JH.¹²⁹ A significant increase in the studied salivary proteins suggesting leakage of serum components into the mouth.

S. D. HEYS*, L. G. Walker, D. I. DEEHAN et al¹⁶⁷ pretreatment serum albumin concentration was found to be an independent prognostic indicator in patients with localized colorectal cancer

Berry WR, Laszlo J, Cox E, Walker A, Paulson D.²⁸ pretreatment serum albumin concentration was found to be an independent prognostic indicator in patients with prostate cancer

Sirott MN et al¹⁸⁰. pretreatment serum albumin concentration was found to be an independent prognostic indicator in malignant melanoma.

Keating KJ, Smith TL, Gehan EA, McRedie KB, Bodey GP, Freireich EJ.¹⁰⁶ . Pretreatment serum albumin concentration was found to be an independent prognostic indicator in acute leukemia **Falconer JS et al**⁶⁴. serum albumin concentration was found to be an independent prognostic indicator in advanced unresectable pancreatic cancer.

McFarlane H et al¹²⁷. serum albumin concentrations have been used previously as an index of nutrition status. A low serum albumin concentration has been reported in association with malnutrition in children.

SUMMARY AND CONCLUSION

During primate evolution, a major factor in lengthening life span and decreasing age-specific cancer rates may have been improved protective mechanisms against oxygen radicals⁶.

It has been widely reported that (**Chappell 1997, Battino et al 1999**) that free radicals or reactive oxygen species are often essential for biological processes and that tissue damage can easily take place when antioxidant defense systems do not efficiently counteract their action.

In this sense, the oral cavity is a critical site because there is evidence of something of this nature. Previous reports strongly suggest increase in lipid peroxidation and a reduction of the antioxidant defense systems in several human cancers.

Since saliva has found application as a diagnostic aid in an increasing number of clinical situations (**Mandel 1990**) and a systematic study of its antioxidant capacity is still lacking in oral cancer, the present study aimed to investigate the extent of lipid peroxidation and the antioxidant levels in the saliva of oral squamous cell carcinoma {OSCC} patients.

The results of the study were:

1. Increased lipid peroxidation in OSCC patients compared to controls.
2. Decreased glutathione levels in OSCC patients compared to controls.
3. Reduced uric acid levels in OSCC patients compared to controls.
4. Increased albumin levels in OSCC patients when compared to controls.
5. No significant differences in lipid peroxidation and antioxidant levels between OSCC patients at various stages and grades.

Hence, the present study demonstrates significant imbalance in the salivary oxidant-antioxidant status in oral squamous cell carcinoma. This is consistent with previous reports in serum of OSCC.

Therefore saliva is a potent diagnostic tool which can be efficiently employed to measure the oxidant-antioxidant status in OSCC.

The scope of the present study should extend to investigate the role of individual antioxidant.

Some individuals have more oxidized DNA or lipid than others. Exploration of the following possible reasons is yet to be started.

1. Variations in rates of production of reactive oxygen species.
2. Differences in the levels of antioxidant defense systems.
3. Differing efficiencies of repair of oxidative damage, especially DNA.

The usefulness of antioxidants as markers of disease progression, treatment outcome or monitors of chemo preventive or therapeutic strategies and modulation through gene therapy protocols is a future endeavor.

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