Review Article

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Antioxidants, Silent Soldiers in Health and Diseases: A Systematic Review

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ABSTRACT

Background: In the stressful modern life, there is another hidden stress within the human system creating more harm and injury. Such an oxidative stress induced secondary to activity of reactive oxygen species (free radicals) contribute to aging process along with development of several other diseases. Antioxidants especially endogenous are in constant defense with these free radicals to nullify them and protect our tissues. Analysis of the levels of the free radicals and antioxidants have been effective in prompting the disease state and its prognosis and serve as disease markers. Several studies report the use of antioxidants in supplementing the mechanism of healing in tissues, and hence have been considered as a part of treatment protocol in various disease conditions. This review article presents the details of free radicals and more importantly the antioxidants and their implications in health and various systemic and oral diseases and their application as a therapeutic agent.

Keywords: Free radicals, oxidation, reduction, antioxidants, systemic diseases, oral diseases, supplements.

INTRODUCTION

Oxygen is one of the basics for the life and as a source of energy derived from metabolism of fats, proteins and carbohydrates. Oxygen being a highly reactive atom can be associated with potentially damaging molecules known as “free radicals”. These free radicals cause the damage to the normal healthy cells causing changes in their structure and function.

The free radicals play a major role in contributing for the aging process and has been implicated in the pathogenesis of many diseases such as cardiovascular, immune system decline, cataracts, brain dysfunctions, cancers and several oral diseaes like lichen planus, oral submucous fibrosis and oral cancers. A lot of importance is gained by the antioxidants in the recent past as a disease marker, as a treatment modality or at least as a supplment in many general and oral diseases.

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This review article pertains to highlight the very significance of antioxidants as a whole, on the general and oral health and disease states.

MATERIALS AND METHODS

The data on the topic was collected from the printed literature from various articles, and from digital dental databases including the Research gate, Ovid, PubMed, PubGet, Index Copernicus and Cochrane. Research phrases used were free radicals, antioxidants, types, mechanism of action, in general health, in systemic diseases, in oral diseases, synthetic and herbal antioxidants.

Free radicals

Free radicals are highly reactive electrically charged chemical species, having an unpaired electron seeking to capture electrons from other substances so as to neutralize them. In the process, a new free radical is produced as a chain reaction generating thousands of free radicals. (1)

Free radicals can be classified according to their nature of derivation as, the oxygen derivatives known as reactive oxygen species (ROS) and nitrogen derived reactive nitrogen species (RNS).

Table 1: Classification of free radicals

ROS	Examples
Radical groups	Superoxide free radical anoin (O_2-), Hydroxyl radical (OH), Peroxyl radical (HOO)
Non-radicals	Hydrogen peroxides (H_2O_2), Singlet oxygen ($^1O_2$)
RNS	Examples
	Nitric oxide (NO), Peroxynitrite anion (ONOO)

REACTIVE OXYGEN SPECIES (2)

These are the highly reactive, oxygen containing species of molecules including free radicals. These cause the cellular damage by reacting with membrane lipids, nucleic acids, proteins and the enzymes.

The free radicals in a cell are produced in the following processes:

a) Endogenous mechanism

1. Normal aerobic metabolism where approximately 90% of the oxygen is utilized by mitochondria in electron transport system
2. Phagocytic mechanism – oxidative burst from phagocytes during phagocytosis of bacteria and viruses or foreign particles
3. Xenobiotic metabolism as a process of detoxification of toxic substances.
4. Cellular metabolism triggers: chronic inflammation, smoking, vigerous exercise, pollution, pesticides, insecticides.

b) Exogenous mechanism:

1. Environmental pollution
2. Radiations
3. Ozone
4. Chemical substances
5. Pathogenic microorganisms

ANTIOXIDANTS

Antioxidants are those molecules capable of deactivating and stabilizing the free radicals and hence are prime in maintenance of the optimum cellular and systemic health of an individual. According to US Food and Drug Administration (FDA), antioxidants are defined as substances used to preserve food by retarding deterioration, rancidity or discoloration due to oxidation. (3) Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the free radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc. (4)

Classification of antioxidants – Basically antioxidants can be of two types; Natural and synthetic antioxidants (5)

A) Natural antioxidants:

These are high or low molecular weight can differ in their composition, in their physical and chemical properties, mechanism and the site of action.

They are of following categories:

i) Enzymes:

- Superoxide dismutase (SOD),
- Catalase
- Glutathione peroxidase

Mechanism of action of antioxidants (6) – Enzymatic antioxidants reduce the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds.

a) **SOD** - catalyses the transformation of the superoxide radical into hydrogen peroxide, which can be transformed by enzyme catalase into water and molecular oxygen.

b) **Glutathione peroxidase (GPx)** - reduces lipid peroxides (ROOH), formed by the oxidation of polyunsaturated fatty acids (PUFA), to a stable, non toxic molecule - hydroxyl fatty acid (ROH). Together with phosholipase, GPx can also convert phospholipids hydro peroxide (PL-OOH) into phospholipids hydroxide (PL-OH)

ii) Low molecular weight antioxidants (7)

These are further subdivided into lipid-soluble antioxidants (tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). They delay or inhibit cellular damage by means of free radical scavenging system.

a) Lipids soluble antioxidants: These antioxidants are called so as they accumulate in lipid plasma lipoprotein (eg. LDL) ; upon supplementation. These act as highly efficient scavengers, such as against lipid peroxyl radical, which are formed as a consequence of free radical chain reaction of lipid peroxidation.

b) Water soluble antioxidants: These antioxidants are less efficient as these are principally unable to encounter most of these lyophilic radicals; yet, such a compound may well act in a synergistic manner with lipophilic antioxidants by regenerating them.

B) Synthetic antioxidants: (8)

These are synthetic chemicals and most effective antioxidants, added to the food items and is approved by Food and Drug Administration e.g., BHA (Butylated hydroxy anisole), BHT (butylated hydroxy toluene), TVHQ (tertiary butylated hydroxy quinone) etc The human has evolved a complex antioxidant system, both endogenous and exogenous in origin. These interact synergistically to neutralize the free radicals.

Table 2: List of antioxidants – (9)

1. Endogenous antioxidants	- Bilirubin - Thiols, e.g., lipoic acid, glutathione, N-acetyl cystine - NADPH and NADH - Uric acid - Enzymes like Superoxide dismutase, - Iron dependant kinases - Selenium dependant glutathione peroxidases
2. A. Exogenous antioxidants (Dietary Antioxidants)	- Vitamin C - Vitamin E - Beta carotenes, carotenoids and oxycarotenoids - Polyphenols like flavonoids, flavones, flavanols
B. Metal binding proteins –	- Albumin - Ceruloplasmin - Ferritin - Myoglobin - Transferrin

Oxidative stress –

It is a reasonable fact that our antioxidant defense system is extraordinary, but it may not always be adequate. The term "oxidative stress" has been coined to represent a shift towards the pro-oxidants In the pro-oxidant/antioxidant balance that can occur as a result of an increase in oxidative metabolism. Increased oxidative stress at the cellular level can come about as a consequence of many factors, including exposure to alcohol, medications, trauma, cold, infections, poor diet, toxins, radiation, or strenuous physical activity. Protection against all of these processes is dependent upon the adequacy of various antioxidant substances that are derived either directly or indirectly from the diet. Consequently, an inadequate intake of antioxidant nutrients may compromise antioxidant potential, thus compounding overall oxidative stress. (11)

Role of oxidative stress in human diseases:

Oxidative stress or damage to DNA, cellular proteins, and other macromolecules has been implicated in the pathogenesis of several diseases, most notably heart disease and cancer and with expanding list of diseases getting

Included in the recent times (5).

Cardiovascular Diseases (CVD):

CVD's are on a rise worldwide. Several causative factors, such as high cholesterol levels, hypertension, cigarette smoking, and diabetes, are believed to promote atherosclerosis, through oxidation of low-density lipoprotein (LDL) within the arterial wall. Studies show an inverse relationship between heart disease and plasma antioxidant levels.

Human studies found supplemental vitamin E increased vitamin E levels in LDL, increased the resistance of LDL oxidation, and decreased the rate of LDL oxidation.

Stampfer et al, in a retrospective study, found that nurses who consumed higher amounts of vitamin E on a regular basis had a 41% lower incidence of heart disease than nurses who consumed the lowest level of vitamin E.(20) It has been estimated that dietary increases in antioxidant vitamins may reduce the risk of heart disease by 20-30%. (11,12)

Diabetes Mellitus

Studies have observed that Type 2 diabetes patients are subjected to increased oxidative stress associated with depletion of total antioxidant capacity. Several studies show increased levels of ROS with oxidation of lipids like chylomicrons. (13) Increased free radical production is also associated with certain biomechanical pathways associated with hyperglycemia such as glucose oxidation, polyol - prostanoid biosynthesis and protein glycation. Nevertheless, the relationship between increased ROS species, reduced antioxidation capacity and glyce,ic control and development of complications in diabetics needs to be understood more. (14)

Rheumatic Diseases

Patients infected with Group A Beta hemolytic streptococci, either with rhuematic fever or rheumatoid arthrits have demonstrated low levels of serum Vitamin E and beta-carotines. Supplenetation of these vitamins along with penicillin therapy showed better response than penicillins alone. (15) Rheumatoid arthritis (RA) is an autoimmune disorder primarily affetcing the synovial joints resulting in inflmmation and periarticular erosion and juxtra articular osteopenia. Despite unclear etiolgy of rheumatoid arthritis, studies have highlightened the role of oxidative stresses and redox signalling in its pathogenesis. It is a consistant observation that RA is associated with different proinflammatory factors like cytokines (IL-1Beta, IL-6, tumor necrosis factor-alpha), prostaglandins along with higher values of ROS and RNS in the synovial joints as well as in blood cells of RA. Also note are very low levels of superoxid desmutase in the synovial fluid. Hence, vitamin antioxidant supplements are of significane in enhancing the treatment outcome. (16)

Osteoarthritis

Studies reveal that lipid peroxidation products like MDA is found in elevated levels in erythrocytes of osteoarthritis patients. This could be due to increased production of ROS caused by excessive oxidative damage formed in the

inflammatory process. Early in the disease an increase in the activities of SOD, glutathione peroxidase, glutathione-S-transferases and glutathione reductase is observed in patients with primary and secondry osteoarthritis. Also observed is a significant decrease in the levels of erythrocyte glutathione, ascorbic acid and plasma vitamin E in these patients. This decrease could be due to increased uptake of the antioxidants to counter the oxidative damage in osteoarthritis.(17)

Cancers

Cancers are on an up rise across the world. Epidemiological evidence suggest low antioxidant intake or low blood levels of antioxidants with increased cancer risk. Studies revel that, low dietary intake of fruits and vegetables doubles the risk of most types of cancers. (18)

ROS have ability to induce cell division, which is a critical factor in mutagenesis. Division of the mutated DNA causes derangement of cell metabolism and duplication, in turn resulting in carcinogenesis. Researchers are of opinion that antioxidants exert their protective effect by decreasing oxidative damage to DNA and thereby decreasing abnormal increases in cell division. Example, cigarette smoking and chronic inflammation–being the major causes of lung cancer and head and neck cancers, have strong free radical components as mutants. Many researches indicate that people who smoke tend to have lower antioxidant levels than nonsmokers and are at an increased risk of developing cancer and cardiovascular disease. (19)

Pulmonary Disorders

With the increasing levels of air pollution, a primary source of ROS the respiratory tract has been a soft target for oxidative damage. Such cellular damage is believed to be partly responsible for the bronchial inflammation in pulmonary disorders such as asthma. (20) It has been recommended that increasing antioxidant intake may help to reduce oxidant stress and hence prevent or minimize the development of asthmatic symptoms. Antioxidant supplements suggested are Vitamin C, vitamin E, and beta carotene. (21)

Neurological Disorders-

As the neural tissue receives a disproportionately large percentage of oxygen and contain a high concentration of polyunsaturated fatty acids which are highly prone to oxidation may be predominantly susceptible to oxidative damage as observed in cses of polyneuritis and Alzeimers disease. (22)

Cataracts

It is believed that cataract formation is a result of free radical induced damage to the lens proteins, with the loss of transparency of lens and hence reduced visual acuity. Some researchers beleive that cataract progression might be slowed with regular intake of supplemental antioxidants, especially vitamin E, vitamin C, and the carotenoids. Such regular intake of antioxidants in an estimation is believed to postpone the development of cataracts by 10 years and this woul account to even reduce the number of cataract surgeries to half the number conducted in US. (23)

Muscular Diseases

Studies have noted that the levels of ROS produced within the mitochondria and hence the oxidative damage are reported to increase with age. As a result, the energy produced by the cell decreases. It is observed that in certain chronic myalgias e.g., myofascial pain syndrome (MPS), fibromyalgia syndrome and chronic muscle fatigue diseases, e.g., chronic fatigue immunodeficiency syndrome (CFIDS), there is an aberration or dysfunction of mitochondrial energy production. (24) This dysfunction is related to damage caused by ROS produced as a result of increased oxidative stress and insufficient antioxidant defenses. (25)

Functionally, mitochondria is supported by a varied number of nutritional elements including antioxidants and its support systems. N-acetyl carnitine, which assists fatty acid transport into the mitochondria, and N-acetyl cysteine, which stimulates mitochondrial glutathione synthesis and acts as an antioxidant are the two important modulators that appear to clinically benefit mitochondrial function. (24,25)

Vitiligo

The recent studies show that patients with pigmentation disorders such as in vitiligo

accumulate increased levels of hydrogen peroxide in their epidermis, possibly by increased activity of epidermal monoamine oxidase A, NADPH-oxidases, nitric oxide synthetase, increased TNF-alpha and photo oxidation of epidermal 6-biopterin and sepiapterin and to certain extent in the blood. This excess hydrogen peroxide leads to inactivation of catalaze and glutathione peroxidase. (26)

With increased H_2O_2 levels and decreased levels of antioxidant enzymes leads to lipidperoxidation, protein oxidation and DNA damage in the skin and blood. Few studies also reported increased MDA (Melanodialdehyde) and Se (Selenium) levels in plasma of vitiligo patients. Reports claim that a cocktail of antioxidants along with selenomethionine is beneficial for repigmentation in vitligo patients. (27)

Aging

Aging is a natural process, but the tissue changes associated with aging is undesirable but an uncontrollable event. The mechanim of the changes associated with aging is related to effects on DNA on the accretion of cellular and functional damage induced by the free radicals. Increased levels of oxidative nucleotides like glycol, dTG, and 8-hydroxy-2-deoxyguanosine has been observed during oxidative damage to DNA because of free radicals or UV radiation.

Studies have reported that mitochondrial DNA are more susceptible to oxidative damage and has a significant role in aging as well as inducing cancers.Studies have observed that reducing free radicals and decreasing their rate of production might reduce the aging process. As it is known that oxidative stress increases with aging the regular and optmial intake of nutritional antioxidants would enhance the qulality of life and also better the life span. (28,29,30)

Anioxidants in Oral Diseases

Many of the oral diseases have utilized antioxidant estimation for assessment of the disease progression and as a marker in specific conditions and as a supplementary or a treatment modality in various oral disease conditions.

Oral leukoplakia

Free radical excess is a finding in the patients with oral leukoplakia especially the by-products of lipid peroxidation alongside with decreasing levels of antioxidant enzymes such as glutathiones, GPx, catalases and SOD. This combination of increased free radicals and depleting antioxidation enzymes in the plasma prepare the arena for oxidative stress in leukoplakia patients. (31, 32, 33) Several studies reveal that use of antioxidants in oral leukoplakia patients have shown reduction in the clinical symptoms. Commonly used antioxidants are 13cRA, beta-carotene, retinol, vitamin E either alone or in combination. Even though few had beneficial result, others found side effects with high doses of these agents. (34,35,36)

Oral Lichen Planus and Lichenoid Reactions

Studies report that serum and salivary MDA (melanodialdehyde) levels being significantly elevated in patients with lichen planus and lichenoid reaction suggesting, whereas serum and salivary TAOC (total antioxidation capacity) was reduced significantly oxidative stress in the etiology of the disease. (37, 38) Etretinate has found a major use in oral lichen planus, with several researchers finding reduction in the clinical features. Purslane has also found to be effective as well with studies showing partial to complete clinical improvement in these patients. (39,40)

Oral Sub Mucous Fibrosis

Several researchers have demonstrated reduced serum levels of vitamin E and plasma B-carotenes in patients with various grades of OSMF. Serum and salivary levels of oxidative markers like MDA, 8-OHdG in patients were found to be higher and lower levels of Vitamin C and E were noted. The same were reversed after intake of curcumin. (41) Increase in levels of MDA, lipid peroxidation product in OSF has been reported which may be due to the poor antioxidant system, excessive free radical formation due to various tissue abuse habits and decomposition of polyunsaturated fatty acids present in membranes. SOD and vitamin – A levels reduced indicating inefficient antioxidant system in these patients. (42, 43)

Clinical trials with multiple micronutrients such as Vitamin A, Vitamin E, Vitamin D and Vitamin

B complex have found significant clinical improvement in these patients. Some studies reveal the antioxidant effect of turmeric with DNA protecting and antimutagenic activity and improved the clinical state of the patients with OSMF. (42, 43, 44, 45)

Oral Cancers

Studies suggest that antioxidants produce cancer regression, inhibition of metastasis and prevention of carcinogenisis. Tumor cells are found to be low in superoxide dismutase (SOD) and catalase activity compared with normal tissue as a result of a higher level of aerobic metabolism, higher concentration of hydroxyl ion. (46) Plasma levels of antioxidant enzymes and the antioxidant such as SOD, catalase were negatively correlated with cancer mortality rates. (47) Antioxidants have the ability of destroying cancer cells through immuno enhancement, molecular genetics pathway and depression of tumor angiogenesis activity. (48) The studies reveal higher levels of lipid peroxidation products (malonaldehyde) in early and advanced cancers in comparison to controls. (49)

In a normal tissue, the hydrogen peroxide produced will be destroyed by the blood rich in antioxidants, whereas in the tumor because of reduced blood flow and antioxidant enzyme, the lipid peroxides remain active and cause cell injury. This is explained by Fenton chemistry by the function of ascorbate metal dependant production of hydroxyl radicals. Vitamin C is used as a dietary supplements in these patients would be exhibiting a dual role as a pro-oxidant and antioxidant.

Studies also suggest that ionizing radiation and chemotherapeutic agents like anthracyclines and bleomycin exert their anticancer effect by the producing free radicals. Increased intake of fruits and vegetables rich in antioxidants are found to be beneficial in cancers. (47)

Periodontal Diseases

In smokers periodontal problems along with effects on oral mucosa is due to the plenty of free radicals contained in the cigarette smoke. Also found are elevated levels of ROS generated by inflammatory cells (neutrophils) during periodontitis. This mechanism could be either directly, via oxidation of vital tissues as well as indirectly via the activation of redox-sensitive gene transcription factors like nuclear factor k-B, inturn leading to downstream pro-inflammatory cytokine and chemokine production. (50)

With increasing oxidative stress, antioxidant enzyme levels appear to be increased in periodontal disease as observed in the crevicular fluid. Salivary and serum 8-hydroxy-deoxyguanosine (8-OHdG) levels have been reported in patients with periodontal diseases. On the other hand reduced serum and crevicular fluid levels of TAOC has been observed. Other studies reveal increased MDA and TOS (total oxidation stress) values in serum, saliva and GCF (gingival crevicular fluid)

Vitamin C acts as a first line of defense against any free radicals, released as a result of interaction between the host immune response and the pathogenic bacteria. This vitamin acts by neutralizing the free radicals and prevent cellular damage. (51)

Conflicts over the use of antioxidants

Antioxidants may have been well researched of their application in various fields of medicine and oral health. Contrarily, there are studies which limit the use of antioxidants for the fact that their use has not been scientifically validated or have been associated with few adverse effects like administration of Vitamin A in pregnant females have shown embryotoxic effects as well as being teratogenic and increased doses of ascorbic acid in reproductive females have led to inhibition of steroid formation of ovary and so increased chances of abortion. (52, 53)

In the recent times a number of synthetic phenolic antioxidants such as butylated hydroxytoluene (BTH) and butylated hydroxyanisole (BHA) have been used as additives in food items, and in cosmetic and as therapeutics. But some of the harmful nature of these compounds, being carcinogenic properties has made them undesirable. Hence, a prompt effort is made to utilize the best of naturally available antioxidants. (54) Various studies report the antioxidant contents in several vegetables like

tomatoes, potatoes, spinach and legumes and in fruits such as berries and cherries. Recently green tea and black tea have gained the interest of the people around the world and set a trend towards habituating green tea as a part of daily life. (55)

Herbs have also been of greater importance as far as natural products are concerned. Herbs like cardamom, asparagus recemosus, aloe vera, cinnamon and fenugreek seeds are popular among common people. Studies do support supplementation of natural antioxidants to synthetic ones. (56)

SUMMARY

Free radicals are the byproducts of several integral physiological process of the human system and also been derived from external environment. These free radicals released as a result of oxidative stresses induce cell damage, which monitor the aging process along with establishment of various diseases either systemic or oral. The assessment of the levels of free radicals and antioxidant levels hence would act as disease markers and help in understanding the status of the disease. Antioxidants, on the other hand either endogenous or exogenous are much required elements for combating the deleterious effects of the free radicals. Low levels of the antioxidants make an individual vulnerable for disesases. As antioxidants acts as first line of defense in protecting the cells against oxidative stresses and reactive oxygen species, regular and optimum intake of exogenous antioxidants as micronutrients in the diet becomes very important. Consumption of antioxidants either through vegetables, fruits, herbs or as supplements has provided promising results in various diseases. Intake of particularly natural antioxidants should be encouraged for maintaining the integrity of the tissues in heath and disease states.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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