ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

REVIEW ARTICLE

Free Radicals, Antioxidants and Oxidative Stress

Sachin Sharma[1*], B. R. Shrivastav[2] and Archana Shrivastav[3]

1. Department of Biotechnology, College of Life Sciences, CHRI Campus, Gwalior, M.P. India.
2. Director, Cancer Hospital and Research Institute, Gwalior, M.P. India.
3. Department of Microbiology, College of Life Sciences, CHRI Campus, Gwalior, M.P. India.

Manuscript Info

Manuscript History:

Received: 12 October 2013
Final Accepted: 22 October 2013
Published Online: November 2013

Key words:
Free radicals, Enzymatic antioxidants, Non enzymatic antioxidants, Oxidative stress markers.

Corresponding Author

Sachin Sharma

Abstract

Free radicals are highly reactive molecule having one or more unpaired electrons, they can cause damage to some vital components of the cells such as lipid, protein and DNA. Free radicals are generated by various sources such as exposure to harmful radiations UV radiations, X-rays, gamma rays and microwave radiations, activity of neutrophils and macrophages, pollutants, electron transport chain in mitochondria, smoking etc. various diseases such as cancer, diabetes, Alzheimer's disease, Parkinson's disease, Atherosclerosis, AIDS etc. have the involvement of free radicals. The cells contain a defense system of antioxidants to neutralize the effect of free radicals, two types of antioxidants are present in cells, Enzymatic antioxidants (Superoxide dismutase (SOD), Catalase (CAT), and Glutathione peroxidase (GPx) and non enzymatic antioxidants (Vitamin E, Vitamin C, Uric acid, Reduced glutathione, Coenzyme Q10. Imbalance between free radicals and antioxidants give rise to a condition of oxidative stress which can be identify by different markers such as Lipid peroxidation, protein oxidation, Nitric oxide, DNA damage.

Introduction

1. Free radicals and Oxidative stress

Free radicals are highly reactive molecules or atoms which contain one or more unpaired electrons in their outer orbitals (Singal *et al*., 2000). Due to the presence of an unpaired electron, free radicals are highly unstable and tend to react with cellular elements (Halliwell, 1991). Recently, free radicals / reactive oxygen species (ROS), the oxidants are paid particular attention as they cause lipid peroxidation; damaging the compounds of all biochemical classes; including nucleic acid, lipids, proteins, lipoproteins, carbohydrates and connective tissue micro molecules (Carrol, 1987) also play an important role in the pathogenesis of tissue damage in many clinical disorders (Sinclair *et al*., 1991). Free radicals have been known to play an important role in the initiation and promotion of multistep carcinogenesis. The improper balance between ROS production and antioxidant defense results in oxidative stress which deregulates the cellular functions leading to various pathological conditions including cancer (Bandyopadhyay *et al*., 1999; Powell *et al*., 2005; Abidi and Ali, 1999). Oxidative stress caused by increased free radical generation and/or decreased antioxidant level in the target cells and tissues has been suggested to play an important role in carcinogenesis (Diplock, 1991; Halliwell and Gutteridge, 1999; Rajneesh *et al*., 2008; Cerutti, 1994, Lu, 2007). Oxidative damage has been implicated in carcinogenesis in human cancers and in cancer models for other animals 4. Free radicals are capable of altering all major classes of biomolecules, such as lipids, nucleic acids and proteins, with changes in their structure and function (Chen *et al*., 2000). Prime targets of free radicals are the polyunsaturated fatty acids in cell membranes and their interaction results in lipid peroxidation. The levels of free radical molecules are controlled by various cellular defense mechanisms, consisting of enzymatic catalase,

glutathione peroxidase, superoxide dismutase) and non-enzymatic (vit E, vit C and glutathione) components (Guyton and Kensler, 1993; Lopaczynski and Zeisel, 2001)

1.1 Sources of free radicals Generation

Free radicals can be produced endogenously or exogenously. In vivo free radicals are formed during normal aerobic respiration, by activation of phagocytosing cells, in peroxisomes where fatty acids are degraded, and by auto-oxidation of various molecules (Frei *et al*., 1989; Halliwell, 2007). Different sources of free radicals are UV radiations, X-rays, gamma rays and microwave radiation, metal-catalyzed reactions, Oxygen free radicals as pollutants in the atmosphere, Inflammation initiates production of ROS and RNS by neutrophils and macrophages, neutrophils stimualated by exposure to microbes, in mitochondria-catalyzed electron transport reactions, oxygen free radicals produced as by product. Several sources like mitochondrial cytochrome oxidase, xanthine oxidases, lipid peroxidation, metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells, interaction with chemicals, automobile exhausts fumes, smoking of cigarettes, cigars, beedie, Burning of organic matter during cooking, forest fires, volcanic activities, Industrial effluents, excess chemicals, alcoholic intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins and xenobiotics also increase generation of free radicals (Valko *et al*., 2006; Nagendrappa, 2005; Ali *et al*., 1996; Cadenas, 1989; Bagchi and Puri, 1998).

1.2 Involvement of free radicals in various diseases

Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases including neurodegenerative disorder like alzheimer's disease, parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, memory loss and depression. Cardiovascular disease like atherosclerosis, ischemic heart disease, cardiac hypertrophy, hypertension, shock and trauma. Pulmonary disorders like inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease. Diseases associated with premature infants, including bronchopulmonary, dysplasia, periventricular leukomalacia, intraventricular hemorrhage, retinopathy of prematurity and necrotizing enterocolitis. Autoimmune disease like rheumatoid arthritis. Renal disorders like glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia. Gastrointestinal diseases like peptic ulcer, inflammatory bowel disease and colitis. Tumors and cancer like lung cancer, leukemia, breast, ovary, rectum cancers etc. Eye diseases like cataract and age related of ratina, maculopathy. Ageing process, diabetes, skin lesions, immunodepression, liver disease, pancreatitis, AIDS, infertility. (Pham-Huy *et al*., 2008; Valko *et al*., 2007; Agarwal and Prabakaran, 2005; O'donovan and Fernandes, 20004, Gupta *et al*., 1997; Kehrer and Smith1994; Sen *et al*., 2009).

2. Antioxidant defense system

An antioxidant is a molecule that inhibits the oxidation of other molecules. The human body is equipped with certain enzymatic and non-enzymatic antioxidant systems (Faruk Tas *et al*., 2005, Portakal *et al*., 2000). Antioxidants are known to dispose, scavenge, and suppress the formation of free radicals or oppose their action and increase with the severity of the disease (Singh *et al*., 2003, Galleotti *et al*., 1991). The antioxidants neutralize the free radicals, stopping the chain of propagation and reducing their harmful effects on the body. They include a number of enzymes, notably superoxide dismutase, glutathione reductases and catalase, vitamin E and C, carotenoids, flavonoids, albumin, uric acid and glutathione (Fang *et al*., 2002). In the case of a weakening of such an antioxidant defence or excess production of free radicals, a state of oxidative stress occurs (Maritim *et al*., 2003; Sies, 1997). Antioxidants are classified into two broad divisions, depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation[9]. These compounds may be synthesized in the body or obtained from the diet (Silvia *et al*., 2004; Clarkson and Thompson, 2000). To control the influence of reactive oxygen species (ROS), aerobic cells have developed their own antioxidant defense system, which includes both enzymatic and non-enzymatic antioxidants (Bakan *et al*., 2003).

Types of Antioxidants

- **Enzymatic antioxidants**
- **Non Enzymatic antioxidants**

2.1 Enzymatic antioxidants

The three major antioxidant enzymes are Superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase (GPx or GSH-Px). SOD and CAT are enzymes form the first line defense against oxidants, e.g. superoxide anion and hydrogen peroxide (Gago-Dominguez and Castelao, 2006). The antioxidant enzyme superoxide dismutase (SOD) widely distributed in all cells is present in high amounts in erythrocytes (Speranza *et al*., 1997). In humans, three different forms of SOD are cytosolic-CuZn-SOD, mitochondrial Mn-SOD, and extracellular SOD (Halliwell, 2007). Superoxide dismutase, the antioxidant enzyme plays an important role in scavenging the superoxide radical. SOD protects cells against superoxide radical by dismutation of the highly reactivesuperoxide anion to oxygen and to a less reactive oxygen species, hydrogen peroxide (McCord, 2000). The generated H2O2 is removed mainly by peroxiredoxins, thioredoxin-dependent peroxidase enzymes (Halliwell, 2007). CAT neutralizes hydrogen peroxide. SOD and CAT also function as anticarcinogenes by inhibiting the initiation and promotion phases in carcinogenesis (Kumaraguruparan *et al*., 2005, Halliwell, 2007). Glutathione peroxidase (GPx) is a selenoprotein, and essential for the conversion of glutathione to oxidized glutathione. Glutathione peroxidase reduces lipidic or non-lipidic hydroperoxides as well as H_2O_2 while oxidizing glutathione (Michiels *et al*., 1994).Glutathione reductase is a flavoenzyme and an important predictor of general oxidant/antioxidant status (Halliwell and Gutteridge, 2007). In addition to SOD, CAT and GPx, there are numerous antioxidant enzymes reacting with and detoxifying compounds produced by oxidative offense (Sies, 1993).

2.2 Non enzymatic antioxidants

2.2.1 Antioxidant Vitamins

Vitamin E (tocopherol) is the major lipid-soluble antioxidant present in lipid membranes and human plasma lipoproteins (Abiaka *et al*., 2001). Vitamin E is a strong inhibitor of apoptosis and a stabilizer of biological membranes (Kolanjiappan *et al*., 2002, Gago-Dominquez and Castelao, 2006). Vitamin E functions in vivo as a strong protector against lipid peroxidation (Slater, 1984, Niki *et al*., 1991). Vitamin E is the only lipid-soluble chain-breaking antioxidant in human plasma, it is responsible for only 2–3% of the total peroxyl radical trapping capacity (TRAP) of plasma (Burton *et al*., 1983, Uotila *et al*., 1994). vitamin E inhibit of cell adhesion, proliferation and protein kinase activity as well as enhancement of immunity and modulation of gene expression (Brigelius-Flohé *et al*., 2002).Vitamin C or L-ascorbic acid, or simply ascorbate (the anion of ascorbic acid), is an essential nutrient for humans and certain other animal species. Ascorbic acid is well known for its antioxidant activity, acting as a reducing agent to reverse oxidation in liquids (McGregor and Biesalski 2006)

2.2.2 Uric acid

Urate (uric acid) is is an important intra and extracellular hydrophilic radical scavenger (Gil *et al*., 2006). Uric acid is the end product of purine metabolism the reaction of hypoxanthine to xanthine and xanthine to uric acid catalyses by xanthine oxidase reduce oxygen and superoxide anion and hydrogen peroxide, are formed respectively. Uric acid is the major constituent of adenosine triphosphate, DNA and RNA (Kumar *et al*., 2008). Uric acid is a powerful scavenger of singlet oxygen, peroxyl radicals and OH• radicals (Ames *et al*., 1981). It protects unsaturated fatty acids from oxidation and may act by preserving ascorbic acid (Davies *et al*., 1986).

2.2.3 Reduced Glutathione

Reduced glutathione (GSH) is a tripeptide with a free thiol group (-SH group) which consists of three amino acids joined together (glutamic acid, cysteine and glycine). Oxidized glutathione (GSSG) is formed by joining two reduced glutathione molecules by their -SH groups. Glutathione peroxidase catalyses the reduced form of glutathione (GSH) to 25 the oxidized form (GSSG). It is thought that only less than 0.2% of the total GSH exists as GSSG, as some GSH is bound to low-molecular-weight compounds and proteins (Meister, 1988). Glutathione modulates cell proliferation and plays a key role in protecting cells against oxidants (Kumaraguruparan *et al*., 2005).

2.2.4 Coenzyme Q_{10}

Oil-soluble, vitamin-like Coenzyme Q10 (CoQ10) is present in most eukaryotic cells, as an initial part of the electron transport chain (ETC) and involved in aerobic cellular respiration and generation of energy (ATP) in the mitochondria.. CoQ10 level decreases with age, and it is particularly low in patients with chronic diseases such as

heart conditions, muscular dystrophy, Parkinson's disease, cancer, diabetes, and HIV/AIDS (Lockwood *et al*., 1995). CoQ_{10} molecule continuously goes through oxidation-reduction cycle. As it accepts electrons, it becomes reduced. As it gives up electrons, it becomes oxidized, thus, act as an antioxidant CoQ_{10} inhibits lipid peroxidation by preventing the production of lipid peroxyl radicals (LOO). Some researchers have indicated that CoQ10 supplement diets can prevent age-related DNA double-strand breaks and can increase lifespan (Quiles *et al*., 2004).

3. Oxidative stress markers

3.1 Lipid peroxidation

Lipid peroxidation is a form of oxidative damage of cell membranes in which the free radicals react with polyunsaturated fatty acids (Lopaczynski and Zeisel, 2001). Lipid hydroperoxides LOOH are formed at the initiation of the process which further form malondialdehyde (MDA) and are the source of highly reactive aldehydes which modify DNA and protein in the cell (Urso and Clarkson, 2003; Lopaczynski and Zeisel, 2001). Levels of lipid peroxidation can be measure by measuring MDA levels in the plasma or serum (Morabito *et al*., 2004; Dormandy, 1983). Circulating inflammatory cells produce reactive oxygen species (ROS) in tumor tissues which can promote lipid peroxidation. (Seven *et al*., 1999).

3.2 Protein oxidation

protein oxidation can be identify by formation of protein carbonyl derivates, oxidized amino acid side chains, protein fragments, and formation of advanced glycation end products (Lopaczynski and Zeisel, 2001). Protein carbonyl derivates are formed due to the cleavage of peptide main chain and oxidation of some amino acid (arginine, lysine, proline, or threonine) side chains (Berlett and Stadtman, 1997, Dean *et al*., 1997, Lopaczynski and Zeisel, 2001, Morabito *et al*., 2004). Hodgkin´s lymphoma patients, patients have higher circulating protein carbonyl groups compared to healthy controls shows the increase in protein oxidation in Hodgkin´s lymphoma patients (Morabito *et al*., 2004).

3.3 Nitric oxide (NO•)

Nitric oxide (NO•)has an extremely short half-life, only a few seconds in an aqueous environment, and is highly reactive molecule which can either be oxidized or form complexes with other molecules, the blood NO• level does not necessarily reflect the NO• status of the tissues (Metzger *et al*., 2006). Nitric oxide has an important role in the initiation of apoptosis in various cell types (Ellis *et al*., 1998). It has been shown that increased production of nitric oxide may protect cells from oxidative stress (Gönenç *et al*., 2006) and the overproduction of reactive nitrogen species is referred as nitrosative stress (Klatt and Lamas, 2000).

3.4 DNA damage

Reactive oxygen species (ROS) are able to cause permanent structural changes in DNA, as base-pair mutations, deletions, insertions, rearrangements and sequence amplification (Cerutti, 1994). The tendency to mutate is increased with incremental ROS during DNA replication (Senturker and Dizdaroglu, 1999, Dizdaroglu *et al*., 2001). Continuous oxidative damage to DNA may lead to alterations in signaling cascades or gene expression, may induce or arrest transcription, and may cause replication errors and genomic instability (Powell et al., 2005). Point mutations is generally occurs at the guanine-cytosine (G-C) pair (Halliwell, 2007; Hirano *et al*., 1996). Urinary 8-oxoGua and 8-oxodG are generally use as markers of oxidative damage to DNA (Shigenaga *et al*., 1994; Loft and Poulsen 1999).

4. Summary

Free radicals formed by various sources such as exposure to harmful radiations and activities of neutrophils and macrophages, pollutants, electron transport chain in mitochondria, smoking etc are found to be involved in various diseases including Cancer, Diabetes, Alzheimer's disease, Parkinson's disease, Atherosclerosis, AIDS etc. Antioxidants in cell protect various components of cells such as lipid, proteins and DNA of from the damaging effects of free radicals. Any alterations in the balance between free radicals and antioxidants leads to a condition of oxidative stress. Oxidative stress can be identify by the levels lipid peroxidation, protein oxidation, nitric oxide and DNA damage.

References

- **Abiaka, C., Al-Awadi, F., Gulshan, S., Al-Sayer, H., Behbehani, A., Farghaly, M. and Simbeye, A. (2001):** Plasma concentrations of alpha-tocopherol and urate in patients with different types of cancer. J. Clin. Pharm. Ther., 26: 265–270.
- **Abidi, S. and Ali, A. (1999):** Role of oxygen free radicals in the pathogenesis and etiology of cancer. Cancer Letters, 142: 1-9.
- **Agarwal, A. and Prabakaran, S.A. (2005):** Mechanism, measurement and prevention of oxidative stress in male reproductive physiology. Indian J. Exp. Biol., 43: 963-974.
- **Ali, A.T.M.M., Al-Swayeh, O.A., Al-Rashed, R.S., Al- Mofleh, I.A., Al-Dohayan, A.D. and Al-Tuwaijri, A.S. (1996):** Role of oxygen-derived free radicals on gastric mucosal injury induced by ischemia-reperfusion. The Saudi J. of Gastroenterol., 2: 19-28.
- **Ames, B.N., Cathcart, R., Schwiers, E. and Hochstein, P. (1981):** Uric acid provides an antioxidant defense in humans against oxidant- and radical-caused aging and cancer: a hypothesis. Proc. Natl. Acad. Sci., 78: 6858–6862.
- **Bagchi, K. and Puri, S. (1998):** Free radicals and antioxidants in health and disease. Eastern Medit. Health J., 4: 350-360.
- **Bakan, N., Taysi, S., Yilmaz, O., Bakan, E., Kuşkay, S., Uzun, N. and Gündoğdu, M. (2003):** Glutathione peroxidase, glutathione reductase, Cu-Zn superoxide dismutase activities, glutathione, nitric oxide, and malondialdehyde concentrations in serum of patients with chronic lymphocytic leukemia. Clin. Chim. Acta. 338:143–149.
- **Bandyopadhyay, U., Das, D. and Banerjee, R.K. (1999):** Reactive oxygen species: oxidative damage and pathogenesis. Current Sci., 77: 658.
- **Berlett, B.S. and Stadtman, E.R. (1997):** Protein oxidation in aging, disease, and oxidative stress. J. Biol. Chem., 272: 20313–20316.
- **Brigelius-Flohé, R., Kelly, F.J., Salonen, J.T., Neuzil, J., Zingg, J.M. and Azzi, A. (2002):** The European perspective on vitamin E: current knowledge and future research. Am. J. Clin. Nutr., 76: 703–716.
- **Burton, G.W., Joyce, A. and Ingold, K.U. (1983):** Is vitamin E the only lipid-soluble, chain-breaking antioxidant in human blood plasma and erythrocyte membranes? Arch Biochem. Biophys., 221: 281–290.
- **Cadenas, E. (1989):** Biochemistry of oxygen toxicity. Annual Rev. Biochem., 58:79–110.
- **Carrol, C.E. (1991):** Oxygen free radicals and human disease. J. Ann. Int. Med., 107: 526-545.
- **Cerutti, P.A. (1994):** Oxy-radicals and cancer. Lancet, 344: 862–863.
- **Chen, X., Ding, Y.W., Yang, G., Bondoc, F., Lee, M.J. and Yang, C.S. (2000):** Oxidative damage in an esophageal adenocarcinoma model with rats. Carcinogenesis, 21(2): 257-263.
- **Clarkson, P.M. and Thompson, H.S. (2000):** Antioxidants: what role do they play in physical activity and health? Am. J. Clin. Nutr., 72: 637–646.
- **Davies, K.J., Sevanian, A., Muakkassah-Kelly, S.F. and Hochstein, P. (1986):** Uric acid-iron ion complexes. A new aspect of the antioxidant functions of uric acid. Biochem. J. 235:747–754.
- **Dean, R.T., Fu, S., Stocker, R. and Davies, M.J. (1997):** Biochemistry and pathology of radical-mediated protein oxidation. Biochem. J., 324: 1–18.
- **Diplock, A.T. (1991):** Antioxidant nutrients and disease prevention: an overview. Am. J. Clin. Nutr., 53: 189-193.
- **Dizdaroglu, M., Jaruga, P. and Rodriguez, H. (2001):** Measurement of 8-hydroxy-2' deoxyguanosine in DNA by high-performance liquid chromatography-mass spectrometry: comparison with measurement by gas chromatography-mass spectrometry. Nucleic Acids Res., 29: E12.
- **Dormandy, T.L. (1983):** An approach to free radicals. Lancet, 2: 1010-1014.
- **Ellis, A., Li, C.G. and Rand M.J. (1998):** Effect of xanthine oxidase inhibition on endothelium dependent and nitrergic relaxations. Eur. J. Pharmacol., 356: 41–47.
- **Fang, Y.Z., Yang, S. and Wu, G. (2002):** Free radicals, antioxidants and nutrition. Nutrition, 872-879.
- **Faruk, Tas., Hansel, H., Belce, A., Ilvan, S., Argon, A., Camlica, H. and Topuz, E. (2005):** Oxidative stress in breast cancer. Med Oncol., 22 (1): 11-15.
- **Frei, B., England, L. and Ames, B.N. (1989):** Ascorbate is an outstanding antioxidant in human blood plasma. Proc. Natl. Acad. Sci., 86: 6377–6381.
- **Gago-Dominguez, M. and Castelao, J.E. (2006):** Lipid peroxidation and renal cell carcinoma: further supportive evidence and new mechanistic insights. Free. Radic. Biol. Med. 40: 721–733.

- **Galleotti, T., Masotti, L. and Borrello, S. (1991):** Oxy-radical metabolism and control of tumour growth. Xenobiotica, 21: 1041-1051.
- **Gil, L., Siems, W., Mazurek, B., Gross, J., Schroeder, P., Voss, P, and Grune, T. (2006**): Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. Free Radic. Res. 40: 495–505.
- **Gönenç, A., Erten, D., Aslan, S., Akinci, M., Simşek, B. and Torun, M. (2006):** Lipid peroxidation and antioxidant status in blood and tissue of malignant breast tumor and benign breast disease. Cell Biol. Int., 30: 376–380.
- **Gupta, S.K., Joshi, S., Velpandian, T., Awor, L. and Prakash, J. (1997):** An update on pharmacological prospective for prevention and development of cataract. Indian J. Pharmacol., 23: 3-10.
- **Guyton, K.Z. and T.W. Kensler, 1993:** Oxidative mechanism in carcinogenesis. Br. Med. Bull., 49: 523-544.
- **Halliwell, B. (2007):** Oxidative stress and cancer: have we moved forward? Biochem. J., 401: 1–11.
- **Halliwell, B. and Gutteridge, J.M.C (2007):** Free radicals in biology and medicine, 4th ed. Oxford University Press, Oxford, UK.
- **Halliwell, B. And Gutteridge, J.M.C. (1999):** Free radicals in biology and medicine. 3rd ed. UK, Oxford Science Publications, pp: 192.
- **Halliwell, B., (1991):** Reactive oxygen species in living systems: source, biochemistry and role in human disease. Am. J. Med., 91: 14-21.
- **Hirano, T., Yamaguchi, R. and Asami, S. (1996):** 8-hydroxyguanine levels in nuclear DNA and its repair activity in rat organs associated with age. J. Gerontol. A Biol. Sci. Med. Sci., 51: 303-307.
- **Kehrer, J.P. and Smith, C.V. (1994):** Free radicals in biology: sources, reactivities, and roles in the etiology of human diseases In: Frei B. Natural Antioxidants in Human Health and Disease, Academic Press, San Diego: 25-62.
- **Klatt, P. and Lamas, S. (2000):** Regulation of protein function by S-glutathiolation in response to oxidative and nitrosative stress. Eur. J. Biochem., 267: 4928–4944.
- **Kolanjiappan, K., Manoharan, S. and Kayalvizhi, M. (2002):** Measurement of erythrocyte lipids, lipid peroxidation, antioxidants and osmotic fragility in cervical cancer patients. Clin. Chim. Acta., 326: 143–149.
- **Kumar, S., Dispenzieri, A., Lacy, M.Q., Hayman, S.R., Leung, N., Zeldenrust, S.R., Buadi, F.K., Kyle, R.A., Rajkumar, SV. and Gertz, M.A (2008):** Serum uric acid: novel prognostic factor in primary systemic amyloidosis. Mayo. Clin. Proc., 83: 297–303.
- **Kumaraguruparan, R., Kabalimoorthy, J. and Nagini, S. (2005):** Correlation of tissue lipid peroxidation and antioxidants with clinical stage and menopausal status in patients with adenocarcinoma of the breast. Clin. Biochem., 38:154–158.
- **Lockwood, K., Moesgaard, S. and Yamamoto, T (1995):** Progress on therapy of breast cancer with vitamin Q10 and the regression of metastases. Biochem. Biophys. Res. Commun., 212: 172-177.
- **Loft, S. and Poulsen, H.E. (1999):** Markers of oxidative damage to DNA: Antioxidants and moleculer damage. Methods Enzymol., 300: 167-184.
- **Lopaczynski, W. and Zeisel S.H. (2001):** Antioxidants, programmed cell death and cancer. Nutr. Res., 21: 295-307.
- **Lu, F. (2007):** Reactive oxygen species in cancer, too much or too little? Med. Hypotheses 69: 1293–1298.
- **Maritim, A.C., Sanders, R.A. and Watkins, J.B. (2003):** Diabetes, oxidative stress and antioxidants: a review. J. Biochem. Mol. Toxicol., 17: 24-38.
- **McCord, J.M. (2000):** The Evolution of Free Radicals and Oxidative Stress. Am. J. Med., 108: 652-659.
- **McGregor, G.P. and Biesalski, H.K. (2006):** "Rationale and impact of vitamin C in clinical nutrition". Curr. Opin. Clin. Nutr. Metab. Care, 9 (6): 697–703.
- **Meister, A. (1988):** Glutathione metabolism and its selective modification. J. Biol. Chem. 263: 17205–17208.
- **Metzger, I.F., Sertorio, J.T. and Tanus-Santos, J.E. (2006):** Relationship between systemic nitric oxide metabolites and cyclic GMP in healthy male volunteers. Acta Physiol., 188: 123–127.
- **Michiels, C., Raes, M., Toussaint, O. and Remacle, J. (1994):** Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic. Biol. Med. 17: 235–248.

- **Morabito, F., Cristani, M., Saija, A., Stelitano, C., Callea, V., Tomaino, A., Minciullo, P.L. and Gangemi, S. (2004):** Lipid peroxidation and protein oxidation in patients affected by Hodgkin's lymphoma. Mediators Inflamm., 13: 381–383.
- **Nagendrappa, C.G. (2005):** An appreciation of free radical chemistry- 3, free radicals in diseases and health. Resonance, 10: 65-73.
- **Niki, E., Yamamoto, Y., Komuro, E. and Sato, K. (1991):** Membrane damage due to lipid oxidation. Am. J. Clin. Nutr., 53: 201–205.
- **O'donovan, D.J. and Fernandes, C.J. (2004):** Free radicals and diseases in premature infants. Antioxid. Redox Signal., 6: 169-176.
- **Pham-Huy, L.A., He, H. and Pham-Huy, C. (2008):** Free radicals, antioxidants in disease and health. Int. J. Biomed. Sci., 4: 89-96.
- **Portakal, O., Ozkaya, O., Inal, M., Bozan, B., Kosan, M. and Sayek, I. (2000):** Coenzyme Q10 concentrations and antioxidant status in tissues of breast cancer patients. Clin. Biochem., 33(4): 279-84.
- **Powell, C.L., Swenberg, J.A. and Rusyn, I. (2005):** Expression of base excision DNA repair genes as a biomarker of oxidative DNA damage. Cancer Letters, 229: 1–11.
- **Quiles, J.L., Ochoa, J.J. and Huertas, J.R. (2004):** Coenzyme Q supplementation protects from age-related DNA double-strand breaks and increases lifespan in rats fed on a PUFA-rich diet. Exp. Gerontol., 39: 189-194.
- **Rajneesh, C.P., Manimaran, A., Sasikala, K.R. and Adaikappan, P. (2008):** Lipid peroxidation and Antioxidant status in patients with Breast cancer. Singapore. Med. J., 49(8): 640-643.
- **Sen, S., Chakraborty, R., De, B. and Mazumder, J. (2009):** Plants and phytochemicals for peptic ulcer: an overview. Pharmacognosy Rev., 3: 270-279.
- **Senturker, S. and Dizdaroglu, M. (1999):** The effect of experimental conditions on the levels of oxidatively modified bases in DNA as measured by gas chromatography-mass spectrometry: how many modified bases are involved? Prepurification or not? Free Radic. Biol. Med., 27: 370-380.
- **Seven, A., Civelek, S., Inci, E., Inci, F., Korkut, N. and Burçak, G. (1999):** Evaluation of oxidative stress parameters in blood of patients with laryngeal carcinoma. Clin. Biochem., 32: 369–373.
- **Shigenaga, M.K., Aboujaoude, E.N., Chen, Q. and Ames, B.N. (1994):** Assays of oxidative DNA damage biomarkers 8-oxo-2'-deoxyguanosine and 8-oxoguanine in nuclear DNA and biological fluids by high performance liquid chromatography with electrochemical detection. Methods Enzymol., 234: 16–33.
- **Sies, H. (1993):** Strategies of antioxidant defense. Eur. J. Biochem., 215: 213–219.
- **Sies, H. (1997):** Oxidative stress: Oxidants and antioxidants. Experimental physiol., 82 (2): 291–95.
- **Silvia, V., Angela, A. and Stefano, M. (2004):** The Antioxidants and Pro-Antioxidants Network: An Overview. Curr. Pharm. Des., 10 (14): 1677–1694.
- **Sinclair, A.J., Barnett, A.H. and Lunec, J. (1991):** Free radicals and antioxidant system in health and disease. JAMA, 7: 409-417.
- **Singal, P.K., Li, T., Kumar, D., Danelisen, I. and Iliskovic, N. (2000):** Adriamycin-induced heart failure: mechanism and modulation. Mol. Cell Biochemistry, 207: 77–86.
- **Singh, R., Singh, R.K., Mahdi, A.A., Singh, R.K., Kumar, A. and Tripathi, A.K. (2003):** Circadian periodicity of plasma lipid peroxides and other antioxidants as putative markers in gynecological malignancies. In Vivo, 17: 593-600.
- **Slater, T.F. (1984):** Free-radical mechanisms in tissue injury. Biochem. J., 222: 1–15.
- **Speranza, M.J., Bagley, A.C. and Lynch, R.E. (1993):** Cells Enriched for Catalase are Sensitized to the Toxicities of Bleomycin, Adriamycin, and Paraquat. J. Biol. Chem., 268 (25): 19039-19043.
- **Uotila, J.T., Kirkkola, A.L., Rorarius, M., Tuimala, R.J. and Metsä-Ketelä, T. (1994):** The total peroxyl radicaltrapping ability of plasma and cerebrospinal fluid in normal and preeclamptic parturients. Free Radic. Biol. Med., 16: 581–590.
- **Urso, M.L. and Clarkson, P.M. (2003):** Oxidative stress, exercise, and antioxidant supplementation. Toxicology, 189: 41–54.
- **Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M. and Telser, J. (2007):** Free radicals and antioxidants in normal physiological functions and human disease. The Int. J. Biochem. Cell Biol., 39: 44–84.
- **Valko. M., Rhodes, C.J., Moncola, J., Izakovic, M. and Mazur, M. (2006):** Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biol. Interactions, 160: 1–40.