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Review Article

A REVIEW ON FREE RADICALS, ROOT CAUSE OF DISEASES

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ABSTRACT

Free radicals can be defined as atoms or molecules containing one or more unpaired electrons in their orbital's. Their formation occurs continuously in the cells as a consequence of both enzymatic and nonenzymatic reactions. It has been estimated that the average person has around 10000–20000 free radicals attacking each body cell each day. Some free radicals are good in that they enable your body to fight inflammation, kill bacteria, and control the tone of smooth muscles, which regulate the working of internal organs and blood vessels. On the other hand increased or uncontrolled free radical activity might combine with other factors to cause some diseases such as neurodegenerative diseases, heart disease, cancers etc. The balance between the production of free radicals and the antioxidant defenses in the body has important health implications. Under the normal conditions the antioxidant defense system within the body can easily handle free radicals that are produced. If there are too many free radicals produced and too few antioxidants, this may cause chronic damage. The aim of this study is review the data on diseases which may be linked to free radicals in order to clarify the role of them in cause of the diseases.

KEY WORDS: *Free radicals, Biological effects, Pathological effect.*

INTRODUCTION

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbital's [1]. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. Radicals derived from oxygen represent the most important class of radical species generated in living systems [2]. The harmful effect of free radicals causing potential biological damage is termed oxidative stress and nitrosative stress.

Oxygen-free radicals (OFR), or more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism.

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ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems. It has been estimated that the average person has around 10000–20000 free radicals attacking each body cell each day [3]. Despite the cell's antioxidant defense system to counteract oxidative damage from OFR, radical-related damage of DNA and proteins have been proposed to play a key role in the development of degenerative processes including amyotrophic, lateral sclerosis, ischemic heart disease, Alzheimer disease, Parkinson disease, cancer, arthritis and aging. ROS are generated by mitochondria as the toxic by-products of oxidative phosphorylation, their energy generating pathway. Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, ischemia/reperfusion, other diseases and ageing [4]. These diseases fall into two

groups: (i) the first group involves diseases characterized by pro-oxidants shifting the thiol/disulphide redox state and impairing glucose tolerance the so-called "mitochondrial oxidative stress" conditions (cancer and diabetes mellitus); (ii) the second group involves disease characterized by inflammatory oxidative conditions" and enhanced "activity of either NAD(P)H oxidase (leading to atherosclerosis and chronic inflammation) or xanthine oxidase-induced formation of ROS (implicated in ischemia and reperfusion injury). The process of ageing is to a large extent due to the damaging consequence of free radical action (lipid peroxidation, DNA damage, protein oxidation) [5]. Convincing evidence for the association of oxidative/nitrosative stress and acute and chronic diseases lies on validated biomarkers of oxidative stress. Such biomarkers have to be objectively measured and evaluated on healthy and ill subjects for long periods. Table 1 summarises most representative biomarkers of oxidative damage associated with human diseases discussed below [4].

Cancer

Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared with normal cells; the redox imbalance thus may be related to oncogenic stimulation. Permanent modification of genetic material resulting from "oxidative damage" incidents represents the first step involved in mutagenesis, carcinogenesis, and ageing. DNA mutation is a critical step in carcinogenesis and elevated levels of oxidative DNA lesions have been noted in various tumors, strongly implicating such damage in the etiology of cancer. ROS-induced DNA damage involves single- or double-stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNAcross-links. DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which are associated with carcinogenesis [3]. DNA damage, mutations, and altered gene expression are thus all key players in the process of carcinogenesis. The involvement of oxidants appears to be the common denominator to all these events [3]. The role of oxidative stress at various stages of carcinogenic process and the process of apoptosis are outlined in the Figure 1.

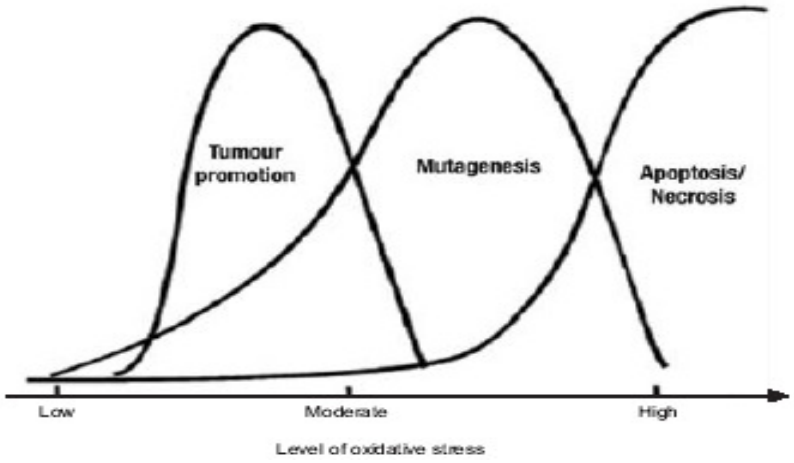

Figure 1 The dose depended effect of relationship between level of stress and tumor promotion process, process of mutagenesis and process of apoptosis/necrosis[6]

There are many different sources-induced free radicals which have been linked with different types of cancers: Hexavalent chromium is considered a potential lung carcinogen; Cr (VI)-induced cytotoxicity is associated with mitochondrial/lysosomal toxicity substantiated by the enhanced formation of free radicals [7]. Iron-induced oxidative stress is considered to be a principal determinant of human colorectal cancer. Occupational exposure to asbestos

containing about 30% (weight) of iron is related to increased risk of asbestosis the second most important cause of lung cancer. Occupational exposure to cadmium has been associated with occurence of increased oxidative stress and cancer. Cadmium itself is unable to generate free radicals directly, however, via indirect mechanism it can cause free radical-induced damage to the gene expression. It has been reported that cadmium can cause activation of cellular protein kinases (protein kinase C), which result in enhanced phosphorylation of transcription factors and consequently lead to the transcriptional activation of target gene expression. It has been suggested that cadmium might also be implicated in the pathogenesis of human pancreatic cancer and renal carcinoma. Arsenic compounds are well-established human carcinogens, capable of binding to–SH groups and thus inhibiting various enzymes, including glutathione reductase. Studies support the hypothesis that arsenic may act as a co-carcinogen -not by causing cancer directly, but by allowing other factors, such as cigarette smoke or UV radiation, to cause DNA mutations more effectively. It tobacco smoke, a well known carcinogenic source of ROS, increased the oxidative DNA damage rate by 35–50%, as estimated from the urinary excretion of 8-OH-G, or by 20–50%, estimated from the level of 8-OH-G in leukocytes. In addition to the extensive studies devoted to the role of oxidative nuclear DNA damage in neoplasia, there exists evidence about the involvement of mitochondrial oxidative DNA damage in the carcinogenesis process. Mutations and altered expression in mitochondrial genes encoding for complexes I, III, IV and V, and in the hyper variable regions of mitochondrial DNA, have been identified in various human cancers. Hydrogen peroxide and other reactive oxygen species have been implicated in the activation of nuclear genes that are involved in mitochondrial biogenesis, transcription, and replication of the mitochondrial genome. Although the region of tumor cells that possess mutated mitochondrial DNA and the extent to which mitochondrial DNA alterations participate in the cancer process have not been satisfactorily established, a significant amount of information supporting the involvement of the mitochondria in carcinogenesis exists [3]. This connection supports the fact that fragments of mitochondrial DNA have been found to be inserted into nuclear DNA, suggesting a possible mechanism for activation of oncogenes Apart from DNA damage, the lipid peroxidation process has been implicated in the mechanism of carcinogenesis. Once formed, lipoperoxyl radicals(ROO•) can be rearranged via a cyclisation reaction to endoperoxides with the final product of the peroxidation process being malondialdehyde (MDA).

Table 1. Biomarkers of oxidative damage associated with some human diseases[4]

S.No	*Disease*	*Biomarkers*
1	Cancer	MDA,GSH/GSSG Ratio,NO_2 – Tyr,8-OH-dG
2	Parkinson's disease	HNE,GSH/GSSG Ratio,Carbonylated protein, Iron level
3	Cardiovascular disease	HNE,GSH/GSSG Ratio,Acrolein, NO_2 – Tyr,F_2-Isoprostanes
4	Rheumatoid arthritis	GSH/GSSG Ratio, F_2-Isoprostanes
5	Alzheimer's disease	MDA, GSH/GSSG Ratio,NO_2– Tyr, HNE, F_2-Isoprostanes,AGE
6	Ischemia/Reperfusion	F_2-Isoprostanes, GSH/GSSG Ratio
7	Atherosclerosis	MDA, NO_2 – Tyr, HNE, Acrolein, F_2-Isoprostanes
8	Diabetes mellitus	MDA, NO_2 – Tyr, GSH/GSSG Ratio,AGE,S-Gluthationylated protein

Abbreviations: MDA, malondialdehyde; HNE, 4-hydroxy-2-nonenal; AGE, advanced glycation end products; 8-OH-dG, 8-hydroxy-20- Deoxyguanosine; GSH, reduced glutathione; GSSG, oxidized glutathione; NO2-Tyr, 3-nitro-tyrosine.

MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. 4-hydroxy -2-nonenal (HNE) is weakly mutagenic but appears to be the major toxic product of lipid peroxidation. There are also other exocyclic DNA adducts that arise from lipid peroxidation. For example etheno-dA,

etheno-dC and etheno-dG have been detected by both P-post-labeling and GC–MS.

Antioxidant Status and Cancer

Many of the biological effects of antioxidants appear to be related to their ability not only to neutralize deleterious free radicals but also modulate cellsignalling pathways. Thus the modulation of cell signaling pathways by antioxidants could help prevent cancer by (i) preserving normal cell cycle regulation; (ii) inhibiting proliferation and inducing apoptosis; (iii) inhibiting tumor invasion and angiogenesis; (iv) suppressing inflammation; (v) stimulating phase II detoxification enzyme activity and other effects. It has been demonstrated that activation of NFÊB by nearly all stimuli can be blocked by antioxidants, including l-cysteine, N-acetyl cysteine (NAC), thiols, green tea polyphenols, and Vitamin E [6]. A large number of studies have established an association between cancer incidence and various disorders of GSH-related enzyme functions, alterations of glutathione S transferases (GSTs) being most frequently reported [8]. GSTs are a family of enzymes that utilize glutathione in reactions contributing to the transformation of a wide range of compounds, including carcinogens, therapeutic drugs, and products of oxidative stress. The GSH/GSSG ratio measured in the blood of patients with colon and breast cancer has been found to be significantly decreased compared to the control [8]. There exists significant experimental and clinical evidence connecting thioredoxin to cancer : (i) elevated levels of TRX have been reported in a wide range of human cancers including cervical carcinoma hepatoma, gastric tumors, lung, and colorectal carcinomas; (ii) many cancer cells have been shown to secrete TRX; (iii) TRX is able to stimulate the growth of a wide variety of human leukemia and solid tumour cell lines; (iv) overexpression of TRX protected cells from oxidative-stress induced apoptosis and provided a survival as well as a growth advantage to tumors; (v) the elevated levels of thioredoxin in human tumors may cause resistance to chemotherapy (e.g. doxorubicin, cisplatin and others). As it is well known, own molecular weight antioxidants are involved directly in the conversion of ROS to less reactive species However, antioxidant protection therapy in cancer patients should be used only with caution since its effects depend on the stage at which it is introduced [9].

Cardiovascular Disease

Reactive oxygen or oxidant species (ROS) participate in normal cell signaling as mediators that regulate vascular function.[10] In the vascular wall, ROS are produced by all layers, including endothelium, smooth muscle, and adventitia.[11] ROS include free radicals such as superoxide anion (O2.-), hydroxyl radical (HO˙), lipid radicals (ROO-) and nitric oxide (NO). Other reactive oxygen species, hydrogen peroxide (H2O2), peroxynitrite (ONOO-) and hypochlorous acid (HOCl), although are not free radicals but they have oxidizing effects that contribute to oxidative stress. ROS has been implicated in cell damage, necrosis and cell apoptosis due to its direct oxidizing effects on macromolecules such as lipids, proteins and DNA. Production of one free radical can lead to further formation of radicals via sequential chain reactions.[3] Under physiological conditions, ROS are produced in low concentrations and act as a signaling molecule that regulate vascular smooth muscle cell (VSMC) contraction and relaxation, and participate in VSMC growth.[10] Under pathophysiological conditions, these free radicals play important roles in various cardiovascular disease conditions.

ROS in Pathophysiology of Heart Disease

One of the strategies used to assess the role of oxidative stress in the pathogenesis of cardiac dysfunction has been to expose isolated cardiac tissues to a defined oxidation stress condition and study the resulting effects. Further in vivo and ex vivo studies have provided precious evidence supporting the role of oxidative stress in a number of conditions (atherosclerosis, ischemia-reperfusion injury, hypertension, catecholamine-induced cardiomyopathy, diabetic cardiomyopathy, cardiac hypertrophy and congestive heart failure etc.) leading to severe cardiovascular dysfunctions. In this review the role of ROS in atherosclerosis is being emphasized as, besides

being considered as the major cause of morbidity and mortality [12] its outcome is also linked to other conditions leading to cardiovascular disorders. The role of ROS in other above-mentioned conditions has been extensively reviewed and the reader is referred to a number of excellent reports.Most cardiovascular events are secondary to atherosclerosis, a disease of the arteries involving a local thickening of the vessel wall. A stroke or myocardial infarction occurs when the lumen of the vessel becomes completely occluded, usually by a thrombus forming at the site of a plaque. Atherosclerotic lesions are thought to be initiated by emigration of monocytes into the arterial inner core (tunica intima), recruited by adhesion molecules, possibly in response to arterial endothelium injury [13]. A variety of factors have been implicated in causing this initial injury, including mechanical damage from flow stress worsened by high blood pressure, viral infection (herpes viruses and cytomegalovirus), exposure to bloodborne toxins such as xenobiotics from cigarette smoke and elevated levels of normal metabolites such as glucose, homocysteine or cholesterol**.** Although a high level of plasma cholesterol is considered to trigger atherosclerosis, the oxidation of cholesterol seems to be a necessary step. In fact, uptake of oxidized low-density lipoprotein (oxLDL) was shown to be an early event leading to the development of atherosclerosis. OxLDL and oxidized lipoproteins have been reported to stimulate O_2•- formation leading to apoptosis of cells in the umbilical vascular wall; this was prevented by treatment with antioxidants SOD and catalase. In cultured human coronary artery smooth muscle cells, low levels of oxLDL stimulate the extracellular matrix synthesis indicating the involvement of oxidative stress in the pathogenesis of atherosclerosis. High levels of oxLDL were apoptotic implicating the additive role of ROS in increased plaque vulnerability this effect was reduced by probucol and catalase. Patients with atherosclerosis and hypercholesterolemia showed higher susceptibility of LDL to oxidation in comparison to patients treated with lipid-lowering agents such as lovastatin and probucol.

In the atherosclerotic lesion produced in the rabbit aorta, significant increases in the iron content were observed suggesting that ironcatalysed free radical reactions may be associated with the development of atherosclerosis. The occurrence of intracellular Ca^{2+}overload has been proposed as a mechanism of injury due to oxidative stress because human endothelial cells subjected to oxidative stress showed an increase in the level of intracellular Ca^{2+} and plasma membrane blebbing. Endothelial dysfunction may play an important role in the atherosclerotic process because in patients with atherosclerosis, the antioxidants, probucol and ascorbic acid, improved the endothelium-dependent relaxation suggesting the involvement of ROS in endothelial dysfunction**[14]**. Increased production of O_2•- has been implicated in the impaired endothelium-dependent relaxation in cholesterol fed rabbits and was suggested to be an early event in the hypercholesterolemic atherosclerotic process. Oxidative inactivation of NO• by superoxide has been proposed as a plausible explanation for endothelial dysfunction. When exposed together, O_2•- and NO• react with each other three times faster than the reaction rate of O_2•- with either Mn2+ and Cu^{2+}/Zn^{2+}-SOD. Therefore, O_2• would preferentially react with NO• rather than SOD and cause inactivation of NOd. In human atherosclerotic arteries, the production of endothelial nitric oxide synthase (the enzyme catalysing NO• formation) as well as NO• has been depressed. SOD protect the inactivation of NO• in the canine coronary artery. The generation of O_2•- was thought to be due to the activation of the vascular and endothelial enzyme NADH/NADPH oxidase**[14]**. Moreover, an increase in NADH/NADPH oxidase-dependent vascular O_2•- was reported in hypercholesterolaemic rabbits**[15]**. Oxidation of NO• by O_2•- results in the formation of peroxynitrite which could initiate lipid peroxidation or play a role in the oxidation of lipoproteins. Both of the above may be important steps in the development of atherosclerosis.

Rheumatoid Arthritis Oxidative Stress:

Rheumatoid arthritis is an autoimmune disease characterized by chronic inflammation of the joints and tissue around the joints with infiltration of macrophages and activated T cells [16]. The pathogenesis of this disease is due to the generation of ROS and RNS at the site of inflammation. Oxidative damage and inflammation in various rheumatic diseases were proved by increased levels of isoprostanes and prostaglandins in serum and synovial fluid compared to controls [16].

Pulmonary Disease and Oxidative Stress:

There is now substantial evidence that inflammatory lung diseases such as asthma and chronic obstructive pulmonary disease (COPD) are characterized by systemic and local chronic inflammation and oxidative stress [17]. Oxidants may play a role in enhancing inflammation through the activation of different kinases and redox transcription factors such as NF-kappa B and AP-1 [17].

Ocular Disease and Oxidative Stress:

Oxidative stress is implicated in age-related macular degeneration and cataracts by altering various cell types in the eye either photo chemically or nonphotochemically. Under the action of free radicals, the crystalline proteins in the lens can cross-link and aggregate, leading to the formation of cataracts. In the retina, long-term exposure to radiation can inhibit mitosis in the retinal pigment epithelium and choroids, damage the photoreceptor outer segments, and has been associated with lipid peroxidation [18].

Nephropathy and Oxidative Stress:

Oxidative stress plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis, chronic renal failure, proteinuria, uremia [19]. The nephrotoxicity of certain drugs such as cyclosporine, tacrolimus (FK506), gentamycin, bleomycin, vinblastine, is mainly due to oxidative stress via lipid peroxidation [19]. Heavy metals (Cd, Hg, Pb, As) and transition metals (Fe, Cu, Co, Cr)-induced different forms of nephropathy and carcinogenicity are strong free radical inducers in the body.

Diabetes

A relatively small amount (10%) of patients suffering from diabetes mellitus has type 1, or insulin dependent diabetes [20, 21]. However, the majority of diabetes patients are non-insulin-dependent and capable at least initially of producing insulin, but are deficient in their cellular response. This type of diabetes is called as the type 2 diabetes mellitus. Decreased uptake of glucose into muscle and adipose tissue leads to chronic extracellular hyperglycemia resulting in tissue damage and pathophysiological complications, involving heart disease, atherosclerosis, cataract formation, peripheral nerve damage, retinopathy and others [20]. Increased oxidative stress has been proposed to be one of the major causes of the hyperglycemia-induced trigger of diabetic complications. Hyperglycemia in an organism stimulates ROS formation from a variety of sources. These sources include oxidative phosphorylation, glucose autooxidation, NADPH oxidase, lipooxygenase, cytochrome P-450 monooxygenases, and nitric oxide synthase (NOS).

NEUROLOGICAL DISEASE:

The brain is particularly vulnerable to oxidative damage because of its high oxygen utilisation, its high content of oxidisable polyunsaturated fatty acids, and the presence of redox-active metals (Cu, Fe). Oxidative stress increases with age and therefore it can be considered as an important causative factor in several neurodegenerative diseases, typical for older individuals.

Alzheimer's Disease:

The brains of patients with Alzheimer's disease (AD) show a significant extent of oxidative damage associated with a marked accumulation of amyloid á peptide, the main constituent of senile plaques in brain, as well as deposition of neurofibrillary tangles and neurophil threads [22]. The direct evidence supporting increased oxidative stress in AD brain include (i) increased Cu, Fe, Al, and Hg content; (ii) increased lipid peroxidation and

decreased polyunsaturated fatty acid content, and an increase in 4-hydroxynonenal, an aldehyde product of lipid peroxidation in AD ventricular fluid; (iii) increased protein and DNA oxidation; (iv) diminished energy metabolism and decreased cytochrome C oxidase content; (v) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase 1, and SOD-1 in neurofibrillary tangles, (vi) the presence in activated microglia surrounding most senile plaques of nitrotyrosine, formed from peroxynitrite (ONOO•). As mentioned above, elevated production of Aá, as a preventive antioxidant for brain lipoproteins under the action of increased oxidative stress and neurotoxicity in ageing, is postulated to represent a major event in the development of Alzheimer's disease [22].

Parkinson's Disease:

Parkinson's disease (PD) involves a selective loss of neurons in an area of the midbrain called the substantia nigra. The cells of the substantia nigra use dopamine (a neurotransmitter-chemical messenger between brain and nerve cells) to communicate with the cells in another region of the brain called the stratium. Thus, a reduction in nigral dopamine levels results in a decrease in stratial dopamine that is believed to cause PD symptoms. Neuronal loss and Lewy bodies, the pathological hallmarks of PD, have been found in cerebral cortex, anterior thalamus, hypothalamus, amygdala and basal forebrain. The major component of intracytoplasmic Lewy bodies are filaments consisting of á-synuclein. Two recently identified point mutations in á-synuclein are the genetic causes of PD [23]. A majority of studies explored the effect of oxidative stress that contributes to the cascade of events leading to dopamine cell degeneration in PD [24]. The occurrence of oxidative stress in PD is supported by both postmortem studies and by studies demonstrating the capacity of oxidative stress to induce nigral cell degeneration. There is evidence that there are high levels of basal oxidative stress in the substantia nigra pars compacta (SNc) in the normal brain, but that this increases in PD patients. However, other factors involving inflammation, excitotoxic mechanisms, toxic action of nitric oxide, and mitochondrial dysfunction play roles in the etiology of PD.

CONCLUSION

Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, Alzheimer's disease and Parkinson's disease etc. Free radicals generate in body through different source and attack at cellular level and make them inactive.

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