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Free Radicals and Its Role in Different Diseases

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ABSTRACT

Free radicals can be defined as atoms or molecules containing one or more unpaired electrons in their orbitals. Their formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. Mainly two types of free radicals are produced in the body which are responsible for the damage i.e. ROS (reactive oxygen species) and RNS (reactive nitrogen species). Free radicals are very unstable and react quickly with other compounds, and try to capture the needed electron to gain stability. A chain reaction thus gets started. Once the process is started, it can cascade, and finally results in the disruption of a living cell. Generally, harmful effects of reactive oxygen species on the cell are most often like damage of DNA, oxidations of polyunsaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivate specific enzymes by oxidation of co-factors.The free radicals are combated by antioxidants that safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in situ, or externally supplied through foods and/or supplements. Apart from the destructive effects of free radical, they are also responsible for some vital actions like destroy the bacteria and other cells of foreign matter, kill cancer cells, turning on and off of genes and fight infection, to keep our brain alert and in focus. Free radicals and oxidants play a dual role as both toxic and beneficial compounds since they can be either harmful or helpful to the body. They are produced either from normal cell metabolisms in situ or from external sources (pollution, cigarette smoke, radiation, medication). When an overload of free radicals cannot gradually be destroyed, their accumulation in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases.

INTRODUCTION

Free radicals are unstable, especially reactive atom or group of atoms that has one or more unpaired electrons especially one that is produced in the body by natural biological processes or introduced from an outside source (such as tobacco smoke, toxins, or pollutants) and that can damage cells, proteins, and DNA by altering their chemical structure sometimes resulting in the mutations that can lead to cancer. Antioxidants in the foods we eat can neutralize the unstable molecules, reducing the risk of damage. [1]

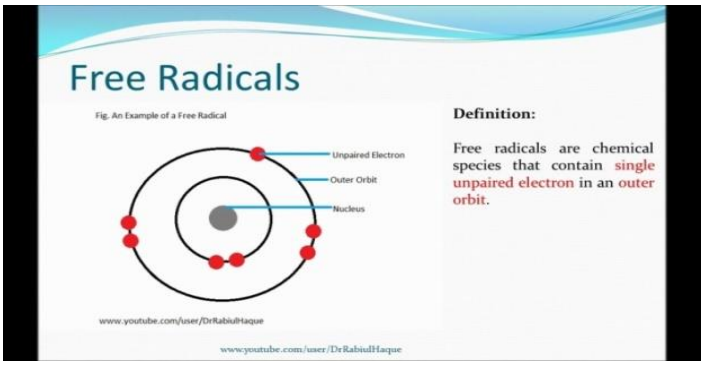

Figure no.1 An Example of free radical

Oxygen in the body splits into single atoms with unpaired electrons. Electrons like to be in pairs, so these atoms, called free radicals, scavenge the body to seek out other electrons so they can become a pair. Free radicals are toxic molecules of oxygen that damage most tissues of our body. They are nothing but natural by-products of continuous biochemical reactions in the body, which include routine metabolic processes and immune system responses. [5]

The most common free radicals are reactive oxygen species. For example, superoxide radical, hydroxyl radical and peroxyl radical. These free radicals are produced in the body by cellular metabolism or inflammation by immune cells. They are generated externally also because our body is exposed routinely to radiation, toxic chemicals, alcohol, smoke and other pollutants, pharmaceutical products, hydrogen peroxide etc. They are also generated by oxidized polyunsaturated fats and cooked food. Free radicals causes various diseases including atherosclerosis, cancer, cataracts, Alzheimer's disease and weakening of immune system. Oxidative stress occurs when there are too many free radicals and too much cellular damage. Oxidative stress is associated with damage of proteins, lipids and nucleic acids. [2]

The human body has several mechanisms to counteract oxidative stress by producing antioxidants, which are either naturally produced in-situ, or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants act as “free radical scavengers” by preventing and repairing damages caused by ROS and RNS, and therefore can enhance the immune defense and lower the risk of cancer and degenerative diseases. Substances that generate free radicals can be found in the food we eat, the medicines we take, the air we breathe and the water we drink, these substances include fried foods, alcohol, tobacco smoke, pesticides and air pollutants. [1]

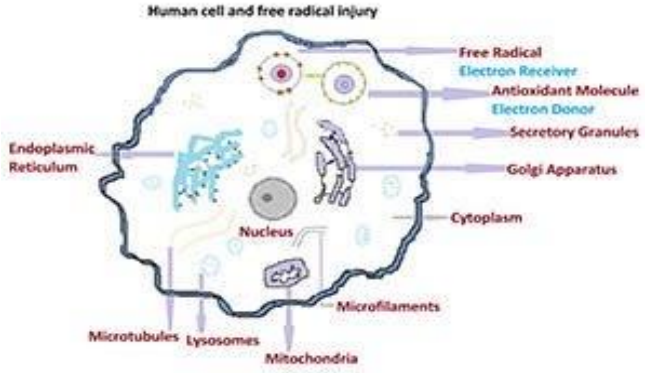

Figure no.2 Human cell and free radical injury

Types of Free Radicals:

There are many types of free radicals, in humans, the most significant are oxygen free radicals (reactive oxygen species). Examples include singlet oxygen, hydrogen peroxide, superoxide, and hydroxyl anions.[1]

Broadly, free radicals grouped into two broad categories:

1. Reactive oxygen species (ROS) 2.Reactive nitrogen Species (RNS).

Reactive oxygen species comprises peroxides, hydroxyl radical, superoxide, oxybenzone and hypochlorous molecules. Reactive oxygen species so far are the most powerful free radicals that directly attack cellular structures and DNA.

Reactive oxygen species (ROS) are very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROS is formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. During environmental

stress, ROS levels can increase dramatically, which can result in significant damage to cell structures. Harmful effects of ROS on the cell are most often like -Damage of DNA, oxidations of polyunsaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors.[20]**The ROS and the RNS** perform the dual role as both beneficial and detrimental compounds to the processes of life inside of us. At low and moderate levels, involved in significant functions such as immune function, in cellular signal and in regulating the conditions of the cell. But, at high concentrations, both ROS/RON exert reactive stress on our cells which can lead to the damage of cellular components. Hence, ROS and RNS levels in the cells must be healthy and any shift in their concentrations can alter the balance and have consequences for our health. [4]

Superoxide ($O_2^{\bullet)}$ ion is an oxygen molecule with an extra electron. Adding one electron to the oxygen molecule makes Superoxide, which is generally a poor reactive radical. Some superoxide is made by "accidents of chemistry", in that many molecules in the body react directly with oxygen to make superoxide. This free radical can cause damage to mitochondria, DNA and other molecules. Our body can neutralize superoxide ions by producing superoxide dismutase. [21]

Hydrogen peroxide (H_2O_2) is the most stable reactive oxygen metabolites. It is involved in the production of many reactive oxygen species. Hydrogen peroxide is a byproduct of oxygen metabolism and is neutralized by peroxidases.

Hydroxyl radical is formed by the reduction of an oxygen molecule in the electron transport chain. It is a neutral form of the hydroxide ion. Radiation can split water in the body to generate the hydroxyl radical. Because of its high reactivity, it will damage most organic molecules such as carbohydrates, DNA, lipids and proteins. [4]

Singlet oxygen ($^1O_2$): It is also not a free radical and is formed as a result of spin reversal of electron in outer orbital of oxygen molecule. It is considered highly potent oxidant with short half-life causing tissue damage. **Singlet oxygen** is formed by our immune system, causes oxidation of LDL cholesterol.

Nitric oxide ($NO^{\bullet}$) which is produced bythe vascular endothelium as a relaxing factor and also by phagocytes and in the brain.Nitric oxide has many physiological functions, but excess nitric oxide can be toxic.

Hydroperoxyl radical also known as the perhydroxyl radical HO2, is the protonated form of superoxide. It is formed through the transfer of a proton to an oxygen atom, can act as an oxididant in a number of biologically important reactions, such as the abstraction of hydrogen atoms from tocopherol and polyunsaturated fatty acids in the lipid bilayer. It may be an important initiator of lipid peroxidation.

Mechanism for the formation of free radicals: Free radicals can be formed by three ways.

• By homolytic cleavage of covalent bond of normal molecule, with each fragment retaining one of paired electrons. $X: Y \rightarrow X^* + Y^*$

• By the loss of single electron from normal molecule. $X : Y \rightarrow X^+ + Y^-$

• By addition of single electron to normal molecule. $X + e^- \rightarrow X^-$

A radical might donate its unpaired electron to other molecule. It might take electron from other molecule in order to pair or it might simply join to the molecule. When radical gives one electron or takes one electron or simply adds on to the anion to become a radical.[1]

Characteristics of Free Radicals:

ROS and RNS are the terms collectively describing free radicals and other non-radical reactive derivatives also called oxidants. Radicals are less stable than non-radical species, although their reactivity is generally stronger. [5]Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron, by cleavage of a radical to give another radical and, also via redox reactions. Free radicals include hydroxyl ($OH^\bullet$), superoxide ($O_2^\bullet$), nitric oxide ($NO^\bullet$), nitrogen dioxide ($NO_2^\bullet$), peroxyl ($ROO^\bullet$) and lipid peroxyl ($LOO^\bullet$). Also, hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH), are not free radicals and generally called oxidants, but can easily lead to free radical reactions in living organisms.[10]

Steps involving free radical generation

Free radicals take part in radical addition and radical substitution as reactive intermediates. Chain reactions involving free radicals can usually be divided into three distinct processes: initiation, propagation, and termination. Initiation reactions, which result in a net increase in

the number of free radicals which involve the formation of free radicals from stable species or they may involve reactions of free radicals with stable species to form more free radicals. Propagation reactions involve free radicals in which the total number of free radicals remains the same. Termination reactions are reactions resulting in a net decrease in the number of free radicals. Typically two free radicals combine to form a more stable species, for example: $2Cl\cdot \rightarrow Cl2$. [1]The formation of radicals may involve breaking of covalent bonds homolytically, a process that requires significant amounts of energy. For example, splitting H2 into 2H· has a $\Delta H°$ of +435 kJ/mol, and Cl2 into 2Cl· has a $\Delta H°$ of +243 kJ/mol. This is known as the homolytic bond dissociation energy and is usually abbreviated as the symbol DH°. The bond energy between two covalently bonded atoms is affected by the structure of the molecule. Homolytic bond cleavage most often happens between two atoms of similar electronegativity. However, propagation is a very exothermic reaction. Radicals may also be formed by single electron oxidation or reduction of an atom or molecule. An example is the production of superoxide by the electron transport chain. [5]

Free radical-targets: Free radicals attack three main cellular components

Lipids: Peroxidation of lipids in cell membranes can damage cell membranes by disrupting fluidity and permeability. Lipid peroxidation can also adversely affect the function of membrane bound proteins such as enzymes and receptors. [1,3]

Proteins: Direct damage to proteins can be caused by free radicals. This can affect many kinds of protein, interfering with enzyme activity and the function of structural proteins.

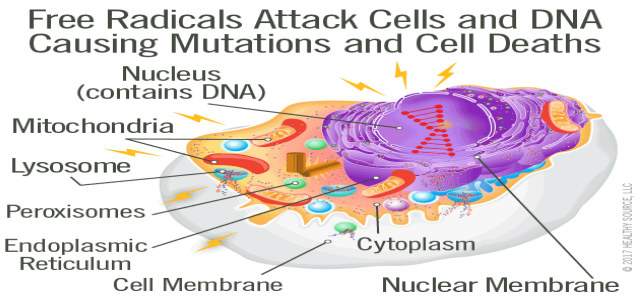

Figure no.3: free radicals attack cells and DNA causing mutations and cell deaths

DNA: Fragmentation of DNA caused by free radical attack causes activation of the poly (ADP-ribose)synthetase enzyme. This splits NAD+ to aid the repair of DNA. However, if the damage is extensive, NAD+ levels may become depleted to the extent that the cell may no

longer be able to function and dies. The site of tissue damage by free radicals is dependent on the tissue and the reactive species involved. Extensive damage can lead to death of the cell; this may be by necrosis or apoptosis depending on the type of cellular damage. [3]

Damaging effects:

Cells normally defend themselves against ROS damage through the use of enzymes such as superoxide dismutase and catalase. Small molecule antioxidants such as ascorbic acid,uric acid and glutathione also play important roles as cellular antioxidants. Similarly, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals. The negative effects of ROS on cell metabolism include roles in programmed cell death and apoptosis, whereas positive effects include induction of host defense genes and mobilization of ion transport systems. In particular, platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury.

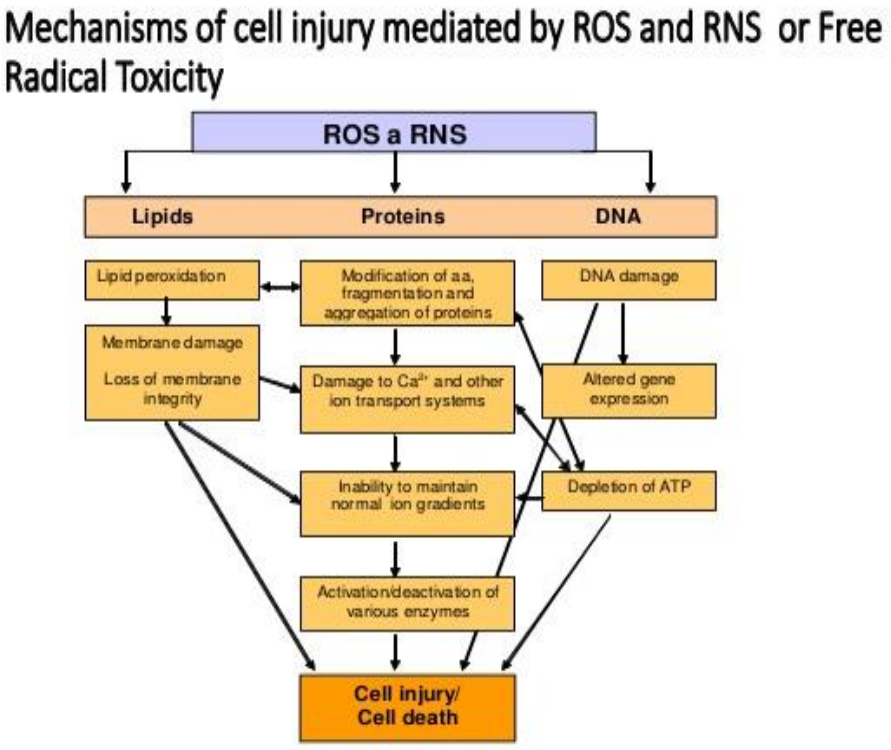

Figure no.4: Mechanisms of cell injury mediated by ROS and RNS

The harmful effects of reactive oxygen species on the cell are often:[4]

- Damage of DNA

- Oxidations of polyunsaturated fatty acids in lipids.

- Oxidations of amino acids in proteins.

Figure no. 5: Oxidative stress

Sources of free radicals

In the living organisms, different kinds of free radicals being produced continuously and spontaneously inside all the metabolically active cells. Free radicals are resulting end products in response to active enzymatic cellular metabolism. They are regularly created inside lysosomes, mitochondria, peroxisomes and other enzymatically dynamic structures inside the cells. Reactive oxygen species lead by hydroxyl radical is the most powerful endogenous oxidant. Sources cause free radicals formation. such as solar radiation, air pollutants such as carbon monoxide, ozone, benzene, chlorine, toluene, consumption of medicines, heavy metals in food and water, Inhalation of chemical solvents such as paints, pesticides, food color agents, trans fats, etc. Free radicals are generated when the human body expose to various stress conditions which resulting in splitting up of a water molecule to Hydroxyl and hydrogen ions.[1] $H_2O = OH^+ + H^+$ This OH^+ (Hydroxyl) molecule is the most powerful of ROS.

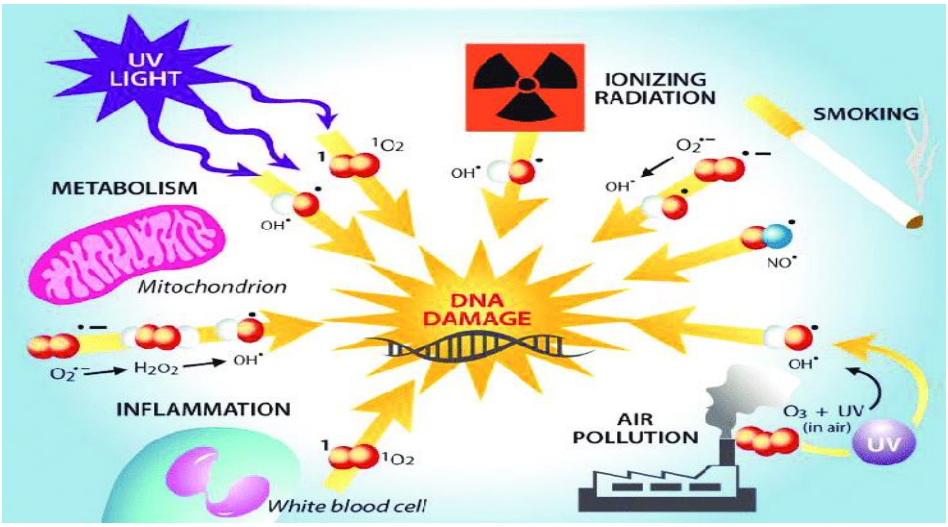

Figure no.6: Formation of free radicals

Some internally generated sources of free radicals are: Mitochondria, Phagocytosis, Xanthine oxidase, Inflammation, Arachidonate pathways, Exercise, Ischemia/reperfusion injury, Peroxisomes.

Some externally generated sources of free radicals are: Cigarette smoke, Environmental pollutants, Radiation, Certain drugs, pesticides, Industrial solvents.

Free Radicals Due to Normal Metabolic Processes: The production of free radicals in normal metabolic processes such as this is one of the reasons that the risk of cancer increases with age, even when people have few exposures to cancer-causing substances.[4]

Free Radicals Due to Exposure to Carcinogens: Exposure to carcinogens in our environment can also produce free radicals. Examples of some carcinogens include:

- Tobacco smoke, Ultraviolet radiation, Radon in the home, Environmental and occupational substances and chemicals such as asbestos and vinyl chloride, some viruses, Medical radiation, Air pollution.

Production route of free radicals: Production of free radicals in the body is continuous and inescapable. The basic causes include the following:

- **The immune system:** Immune system cells deliberately create oxy-radicals and ROS (Reactive oxygen species) as weapons.

- **Energy production:** During energy-producing cell generates continuously and abundantly oxy-radicals and ROS as toxic waste. The cell includes a number of metabolic processes, each of which can produce different free radicals.[5]

- **Pollution and other external substances:** Air pollutants such as asbestos, benzene, carbon monoxide, chlorine, formaldehyde, ozone and toluene, Chemical solvents i.e. cleaning products paints, Perfumes, Water pollutants such as chloroform and trihalomethanes caused by chlorination, Cosmic radiation, Radon gas, food containing farm chemicals, like fertilizers and pesticides, processed foods containing high levels of lipid peroxides, are all generator of free radicals.

- **General factors:** Aging, Metabolism,

- **Stress Dietary factors:** Additives, alcohol, coffee, foods of animal origin, broiled, fried, grilled, or otherwise cooked at high, temperatures, foods that have been browned or burned, herbicides, hydrogenated vegetable oils, pesticides, sugar. [4]

- **Toxins:** Carbon tetrachloride, Paraquat, Benzo (a) pyrene, Aniline dyes, Toluene.

- **Drugs**: Adriamycin, Bleomycin, Mitomycin C, Nitrofurantoin, Chlorpromazine.[1]

Beneficial role of Free radicals

➢ Free radicals perform many critical functions in our bodies in controlling the flow of blood through our arteries, to fight infection, to keep our brain alert and in focus.

➢ Phagocytic cells involved in body defense produce and mobilize oxygen free radicals to destroy the bacteria and other cells of foreign matter which they ingest. Some free radicals such as nitric oxide and superoxide are produced in very high amount by immune cells to poison viruses and bacteria.

➢ Some free radicals kill cancer cells. In fact, certain cancer drugs aim in increasing the free radical amount in body. [4]

Conditions responsible to promote formation of free radicals [1,4]

1. Pesticides: If pesticides and herbicides are used on the crops, consumption of foods prepared from such crops can cause increase in the existing free radical load in the body. It is therefore important to consume food prepared from organically grown crops.

2. Tobacco smoke: All smokers including passive smokers are exposed to enhanced amounts of chemicals generating free radicals in the body.

3. X-rays and other ionizing radiation: ionizing radiation (x-rays, gamma rays, alpha, beta particles, neutrons and cosmic rays) also cause generation of free radicals.

4. Stress: Stress related to urban lifestyles and competitive job environments is also a contributing factor to excess free radical generation in your body.

5. Sunlight: Consistent exposure to sunlight, especially to ultra-violet radiation, can boost the generation of free radicals in the body. Avoid excessive exposure to sunlight or expose only during early morning or late evening sunlight for the benefit of vitamin D.

6. Food additives like preservatives, essences and colors are added to improve the appearance, aroma, shelf life and stability of processed foods, but these potentially boost the formation of free radicals in the body. Consume food that is freshly prepared, and has minimal additives. [1]

7. Food preparation: Cooking, especially frying and deep frying definitely create substances that after consumption enhance the formation of free radicals in your body.

Antioxidants:

Antioxidants are substances, usually vitamins, minerals, enzymes or phytonutrients that protect the body from damage caused by oxidation. Oxidation is a process that causes damage to our body tissues through the work of free radicals. Chemically antioxidants donate an electron to the free radical and convert it to a harmless molecule.[2]The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention. They bind to the free radicals and then transform them into non-damaging substances. Ithelp to foil the progression of heart disease by preventing oxidation. Antioxidants fight against chronic inflammation.

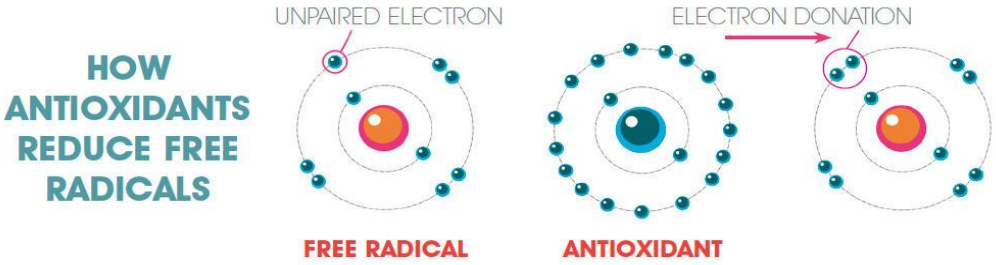

Figure no.7: Role of Antioxidants

Antioxidants classification:

Endogenous compounds in cells can be classified as **enzymatic antioxidants and non-enzymatic antioxidants**. [11]

The major **antioxidant enzymes** directly involved in the neutralization of ROS and RNS are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx).SOD, the first line of defense against free radicals, catalyzes the dismutation of superoxide anion radical ($O_2^{\bullet}$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is transformed into water and oxygen (O_2) by catalase (CAT) or glutathione peroxidase (GPx). The selenoprotein GPx enzyme removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG). Glutathione reductase, a flavoprotein enzyme, regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH).

The **non-enzymatic antioxidants** are also divided into metabolic antioxidants and nutrient antioxidants. **Metabolic antioxidants** belonging to endogenous antioxidants are produced by metabolism in the body, such as lipoic acid, glutathione, L-arginine, coenzyme Q10, melatonin, Uric acid, bilirubin, metal-chelating proteins, transferrin, etc. While **nutrient antioxidants** belonging to exogenous antioxidants are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals, flavonoids, omega-3 and omega-6 fatty acids etc. [7]

Nutrient antioxidants: Antioxidants from our diet play an important role in helping endogenous antioxidants for the neutralization of oxidative stress. The nutrient antioxidant deficiency is one of the causes of numerous chronic and degenerative pathologies. Each nutrient is unique in terms of its structure and antioxidant function. [11]

Anthocyanins: Found most abundantly in berries, eggplant, red cabbage, red grapes, and other richly-colored food plants, anthocyanins are purple-colored pigments common to all plants. They're what make blueberries blue and raspberries red. Anthocyanins provide a broad range of health benefits.

Polyphenols are a group of several thousand phytochemicals with antioxidant properties. You often hear about the polyphenols in chocolate, but scientists are pursuing and publishing more and more research on the polyphenol called curcumin, the active curcuminoid compound in turmeric.

Curcuminoids protect and promote health by activating the immune system, protecting the brain, and influencing gene expression among other beneficial effects.

Vitamin E is a fat-soluble vitamin with high antioxidant potency, is a chiral compound with eight stereoisomers: α, β, γ, δ tocopherol and α, β, γ, δ tocotrienol. Only α-tocopherol is the most bioactive form in humans and it also safeguards cell membranes from damage by free radicals. Its antioxidant function mainly resides in the protection against lipid peroxidation. Sources are vegetable oils, wheat germ oil, whole grains, nuts, cereals, fruits, eggs, poultry and meat. It is used for the prevention against colon, prostate and breast cancers, some cardiovascular diseases, ischemia, cataract, arthritis and certain neurological disorders.

Vitamin C also known as ascorbic acid is a water-soluble vitamin. It is essential for collagen, carnitine and neurotransmitters biosynthesis.Benefits of vitamin C are antioxidant, anti-atherogenic, anti-carcinogenic, immunomodulator and preventing stomach cancer, lung and colorectal cancer. Sources are acid fruits, green vegetables, tomatoes. Vitamin C works synergistically with vitamin E to quench free radicals.

Beta-carotene, is a fat soluble member of the carotenoids, converted to retinol, which is essential for vision. It is a strong antioxidant and is the best quencher of singlet oxygen. Beta-carotene is present in many fruits, grains, oil and vegetables (carrots, green plants, spinach).

Lycopene: Lycopene is a bright red pigment found in tomatoes, watermelons, and papayas. Itis a carotenoid — a type of phytochemicals with antioxidant properties. Lycopene contributes to a lower risk of prostate cancer, blood clots, and stroke.

Selenium is a trace mineral found in soil, water, vegetables (garlic, onion, grains, nuts, and soybean), seafood, meat, liver, yeast. It forms the active site of several antioxidant enzymes including glutathione peroxidase. At low dose, health benefits of Se are antioxidant, anti-carcinogenic and immunomodulators. Selenium is also necessary for thyroid function.

Flavonoids are polyphenolic compounds which are present in most plants. Beneficial effects of flavonoids on human health mainly reside in their potent antioxidant activity and are to prevent chronic and degenerative ailments such as cancer, cardiovascular diseases, arthritis, aging, cataract, memory loss, stroke, Alzheimer's disease, inflammation, infection. Sources are green tea, grapes, apple, soybean, curcuma, berries, onion, broccoli, etc.

Role of free radicals in different diseases

Inside the human cells, there is an effective antioxidant defense system to counter damaging actions of reactive oxygen. Free radicals attack all the main cellular components. The extent of tissue damage depends on the type of reactive species involved. Extensive damage can lead to the death of the cell and part or whole of the tissue.[6]Peroxidation of lipids in cell membranes can cause the collapse of cell membranes and alter the function of membrane-bound proteins such as enzymes and receptors. Direct damage to structural proteins and DNA inside the cells may result in loss of cell architecture and lack of its ability to restore.

Effect of Free Radicals on the Body: Oxidative Stress

Once free radicals are generated, whether through exposure to a carcinogen or doing the normal processes of body metabolism, they are free to do damage. The availability of free radicals creates what is known as **oxidative stress** in the body.[7]When one free radical "steals" an electron from a molecule, that molecule is then missing an electron (becomes a free radical), and so on. Free radicals can damage DNA, proteins, lipids, cell membranes, and more in the body. [1]

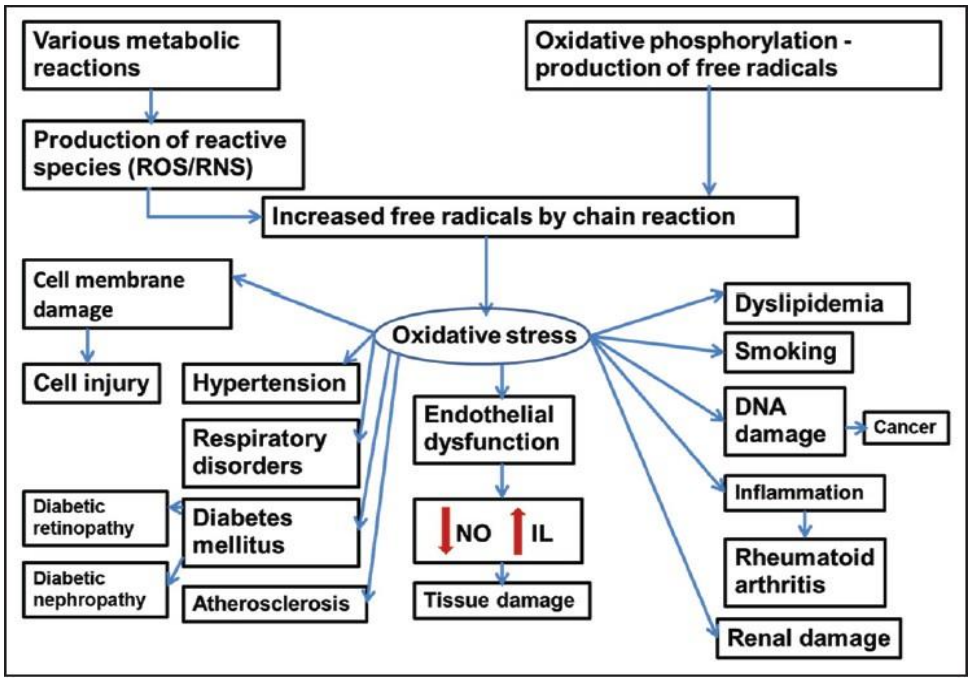

Figure no.8: Oxidative stress: Effect of free radicals on body to cause diseases

Free radicals cause diseases in humans:

Oxidative damage to various structural components inside the cell is the most common reason for the development of different disease manifestations in humans. Direct attack on chromosomes and DNA can cause a mutation which when continue unregulated may lead to the development of cancers. Endothelial damage inside the vessels can cause atherosclerosis, vascular blockage, loss of vessel integrity, bleeding, etc., can cause various cardiovascular diseases. Free radicals have also been attributed for cataracts, Alzheimer's disease, neuro-degenerative diseases, kidney tubular dysfunction, connective tissue diseases, age-related eye disease, etc.

Figure no. 9: Role of free radicals in diseases

Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by elevated blood glucose level in which the beta cells of the pancreas produce insufficient insulin or the body cells are not responding to the insulin produced. [2] The three main types of diabetes are: **Type I DM** occurs when the pancreas fails to produce enough insulin. **Type IIDM** initiates from insulin

resistance, in which the cells fail to respond to the insulin properly. **Gestational diabetes** third main form of DM occurs in pregnant women having no previous history of diabetes and develops high blood sugar level in pregnancy.

Free Radicals in Diabetes Mellitus:

Oxidative stress is a condition in which there is an imbalance between the systemic manifestation of ROS and biological system's ability to detoxify the reactive intermediates or repairing of the resulting damage that is antioxidant defense. People with diabetes tend to have an increased generation of ROS and decreased antioxidant protection and hence there is increased oxidative damage. Hyperglycemia, a condition with high blood glucose levels has been shown to increase the ROS and the end products of oxidative damage in cell cultures. There are studies indicated that oxidative damage is greater in people with type II DM compared to people with typeI DM.[20]Diabetes mellitus is a diverse group of long-standing disorder where the patients exhibit characteristic enhanced blood glucose levels resulting from defective insulin secretion (in type I diabetes), resistance to insulin action (in type II diabetes), or both. The enhanced blood levels (known as hyperglycemia) in diabetes is associated with increased production of free radicals or decreased activity of the antioxidant system present in our body. And this induces increased free radicals in our cellular system which can have adverse effects on our body and health.[14]

Diabetes and Oxidative stress:

In the diabetes chronic hyperglycemia was occurred. In the presence of high blood glucose levels reactive oxygen species was produced by various processes called,

- **Glucose Oxidation**
- **Glucose Toxicity**
- **Oxidative Phosphorylation**

The production of reactive oxygen species leads to elevation of free radicals. These free radicals will involve in process called oxidative stress and prone to beta cell dysfunction and destruction. This leads to impairment of insulin action.

Persistent hyperglycemia in diabetic patients leads to generation of oxidative stress due to a) auto oxidation of glucose; b) non-enzymatic glycosylation and c) polyol pathway. Auto-oxidation of glucose involves reduction of molecular oxygen to superoxide and hydroxyl radicals, which are highly reactive and interact with all biomolecules. They also accelerate formation of advanced glycation end products (AGEs). AGEs such as pyrroles and imidazoles tend to accumulate in the tissue. Crosslinking AGE-protein with other macromolecules in tissues results in abnormalities in the cell and tissue function.

Neurodegenerative Diseases:

The central nervous system has been reported to be more vulnerable to free radicals due to the high consumption of oxygen, lower levels of antioxidant enzymes and its natural high lipid content the regions of brain such as hippocampus, substantia nigra, and the striatum has been particularly observed to be more susceptible to stress by free radicals. The oxidative stress brought about by free radicals has been implicated in many neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's and multiple sclerosis(MS).[8]

Alzheimer's disease is a chronic neurodegenerative disease that usually starts slowly and gradually worsens over time. It is the cause of 60–70% of cases of dementia. The most common symptom is difficulty in remembering recent events. As the disease advances, symptoms can include problems with language, disorientation, mood swings, loss of motivation, not managing self-care, and behavioural issues.[8]The brain in Alzheimer's disease (AD) is under increased oxidative stress and this may have a role in the pathogenesis of neuron degeneration and death in this disorder. The direct evidence supporting increased oxidative stress in AD is: (1) increased brain Fe, Al, and Hg in AD, capable of stimulating free radical generation; (2) increased lipid peroxidation and decreased polyunsaturated fatty acids in the AD brain, and increased 4-hydroxynonenal, an aldehyde product of lipid peroxidation in AD ventricular fluid; (3) increased protein and DNA oxidation in the ad brain; (4) diminished energy metabolism and decreased cytochrome c oxidase in the brain in AD; (5) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1 and SOD-1 in neurofibrillary tangles and AGE, heme oxygenase-1, SOD-1 in senile plaques; and (6) that amyloid beta peptide is capable of generating free radicals. So free radicals are possibly involved in the pathogenesis of neuron death in Alzheimer's.The disease is characterized by typical neuropathology, impaired synaptic function and massive cell loss. The pathobiochemistry of this disorder involves oxidative stress, which accumulates free radicals

leading to excessive lipid peroxidation and neuronal degeneration in certain brain regions. Radical induced disturbances of DNA, proteins and lipid membranes have been measured. The hypothesis has been proposed that cellular events involving oxidative stress may be one basic pathway leading to neurodegeneration in Alzheimer's disease. In this work we report evidence for increased oxidative stress and disturbed defense mechanisms in Alzheimer's disease, which may result in a self-propagating cascade of neurodegenerative events. Furthermore it is evident from experimental data, that aggregation of beta-amyloid and beta-amyloid toxicity is favourably caused by oxidative stress. Therefore, oxidative stress plays a key role in the conversion of soluble to unsoluble beta-amyloid, suggesting that oxidative stress is primary to the beta-amyloid cascade. [9]

Parkinson's disease (PD):

Parkinson's disease is a long-term degenerative disorder of the central nervous system that mainly affects the motor systemthe symptoms usually emerge slowly.The most obvious symptoms are shaking, rigidity, slowness of movement, and difficulty with walking.Thinking and behavioral problems may also occur. Dementia becomes common in the advanced stages of the disease. Depression and anxiety are also common,other symptoms include sensory, sleep, and emotional problems. The main motor symptoms are called "parkinsonism". Parkinson's disease is characterized by the loss of dopaminergic neurons (involve in learning, memory and motor control), especially in the midbrain area called the substantia nigra, accompanied by deposition of inclusion bodies (Lewy bodies) of α-synuclein. The redox imbalance causes oxidative damage to these neurons and begins to alter the synthesis and metabolic pathway of dopamine leads to a further increase in oxidative stress because of quinine formation. The characteristic clinical symptoms of PD include jerky movements, trembling of the hands and lips, and tremors. Dopamine, a neurotransmitter, can also act as a metal chelator, has the ability to generate H_2O_2 via Fenton reaction. Ceruloplasmin (an extracellular ferroxidase required for regulating cellular iron load and transport) oxidation results in the decreased ferroxidase activity followed by the accumulation of intracellular iron in neurons in PD. The increased levels of Fe^{+3} mediate the production of hydroxyl radicals, results in the damage of dopaminergic neurons in PD. [8]

In the pathology of neurodegenerative disorders, the generation of free radicals, particularly ROS and RNS, are harmful, as they affect proteins, lipids, and nucleic acids.Oxidative damage

includes mitochondrial dysfunction, dopamine auto-oxidation, α-synuclein aggregation, glial cell activation, alterations in calcium signaling, and excess free iron. [22]

Kidney:

Mitochondrial free radical production induces lipid peroxidation during myohemoglobinuria. Iron catalyzed free radical formation and lipid peroxidation are accepted mechanisms of heme protein-induced acute renal failure. However, the sources of those free radicals which trigger lipid peroxidation in proximal tubular cells remains unknown. In conclusion, the terminal mitochondrial respiratory chain is the dominant source of free radical. [6]

Cancer

It is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer is one of the leading causes of death in humans free-radical oxidation breaks strands of DNA. The breaks are repaired, but some mistakes occurs leading mutations. These genetic mutations can cause cancers. The age-related increase in cancer rates might have something to do with an age-related rise in oxidative damage to DNA. [3]

Cancer cells when compared to normal cells, show elevated levels of oxidative stress partly due to the activation of oncogenes (cancer-promoting genes) and loss of tumor suppressor (anti-tumor) genes. ROS can alter growth signals and the expression of genes to help the continuous growth of cancer cells. ROS has the ability to damage DNA by inducing a change in the DNA chemistry of the cell and can lead to the proliferation of cancer cells. The role of free radicals in colorectal cancer, breast cancer, bladder cancer, prostate cancer, and lung cancer has been well studied and documented. [3]

Causes:

The majority of cancers are due to genetic mutations from environmental and lifestyle factors. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), lack of physical activity, Infection, Hormones and pollution. [2]

Free Radicals Can Cause Cancer

Cancer is caused by damage to DNA (Deoxyribonucleic acid) by free radicals.

➢ The development of cancer in humans is a complex process including cellular and molecular changes mediated by diverse endogenous and exogenous stimuli. Cancer initiation and promotion are associated with chromosomal defects and oncogene activation induced by free radicals.[3]

➢ A common form of damage is the formation of hydroxyled bases of DNA, which are considered an important event in chemical carcinogenesis. This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Oxidative DNA damage also produces a multiplicity of modifications in the DNA structure including base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites.

➢ For example, tobacco smoking and chronic inflammation resulting from noninfectious diseases like asbestos are sources of oxidative DNA damage that can contribute to the development of lung cancer and other tumors. The highly significant correlation between consumption of fats and death rates from leukemia and breast, ovary, rectum cancers among elderly people may be a reflection of greater lipid peroxidation.

➢ Damage done to genes in the DNA may result in genes that produce ineffective proteins.

Some of these mutations may involve genes known as tumor suppressor genes. These genes code for proteins that function to repair damages in DNA or cause cells that are damaged beyond salvage to be removed through a process of apoptosis (programmed cell death).

➢ Oncogenes are genes that code for proteins that promote the growth of cells. Normal genes in the body called "protooncogenes" are important in promoting the growth of a baby during pregnancy and transiently produce proteins that aid in tissue repair. Mutations in these genes (which are then oncogenes) result in the continuous production of proteins that promote the growth of a cell.

➢ Most often, it is a series of mutations in both tumor suppressor genes and oncogenes that leads to cancer. Damage (mutations) to tumor suppressor genes allows a damaged cell to survive unrepaired (abnormal) and damaged oncogenes promote the growth of that damaged cell. The result is the formation of a cancer cell.

➢ Antioxidants thwart cancer induction by neutralizing free radicals.

Cardiovascular diseases:

This is a class of diseases involving the blood vessels (arteries, capillaries, and veins) and the heart. They include cardiac diseases and the diseases pertaining to the circulatory system in the brain, kidney, etc. For example, in atherosclerosis which refers to a condition leading to the hardening of the arteries, there is a significant imbalance between oxidants and antioxidants resulting in oxidative stress.[14]Cardiovascular disease (CVD) is associated with a variety of risk factors for its development including hypercholesterolaemia, hypertension, smoking, diabetes, poor diet, stress and physical inactivity amongst others. Further *in vivo* and *ex vivo* studies have provided precious evidence supporting the role of oxidative stress in a number of CVDs such as atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy and congestive heart failure. [2]

Free radicals can cause CVD:

Cardiovascular disease is used for a range of diseases, which include ischemic heart disease (IHD), cerebrovascular disease (CVD), and other related diseases, for example, myocardial infarction (MI). Excess free radicals are thought to initiate atherosclerosis by damaging blood vessel walls. LDL-cholesterol has long been implicated in the development of heart disease and many clinicians report that lowering blood cholesterol is the most effective means of combating heart disease. However, LDL only poses a threat after oxidation by free radicals, as it is reported to migrate across the endothelial membrane into the arterial wall. These oxidized components attract macrophages, which absorb and deposit cholesterol within the cell to form what has been referred to as “foam cells”. These foam cells may initiate the formation of an atherosclerotic lesion, which can result in blockage of blood vessels. Interruption of the blood supply causes severe pain, known as angina pectoris, and may eventually cause death of the cardiac tissue. [14]

Hypertension also known as **high blood pressure** is a long-term medical condition in which the blood pressure in the arteries is persistently elevated. Long-term high blood pressure is a major risk factor for coronary artery disease, stroke, heart failure, atrial fibrillation, peripheral arterial disease, vision loss, chronic kidney disease, and dementia. Free radicals have been implicated in contributing the change in the physiology of the vascular cells and also leading to hypertension.[2]

Free radicals in hypertension:

Free radical induced oxidative stress in part contributes to endothelial dysfunction and development of hypertension. Increased ROS generation eliminates $NO^{\bullet}$ by forming $ONOO^{-}$, thus reducing $NO^{\bullet}$ bioavailability which leads to decreased endothelium-dependent vasodilation resulting in hypertension. A decrease in NO bioavailability and an increase in oxidative stress are present in human hypertension. Oxidation-induced impairment of NO also results in reduced opposition to the vasoconstrictive and hypertensive effects of angiotensin II. Angiotensin II decreases NO bioavailability by promoting oxidative stress.[14]Reactive oxygen species and oxidized low density lipoproteins (oxLDL) may play a critical role in the pathobiology of hypertension, in other conditions such as atherosclerosis, reperfusion injury and myocardial infarction. Essential hypertension (EH) is associated with increased superoxide anion and hydrogen peroxide production as well as decreased antioxidant capacity. The involvement of reactive oxygen intermediates in EH is also suggested by the observation of increased level of lipid peroxides and decreased concentrations of antioxidant vitamin E in plasma of EH patients. The patients with EH have plasma concentrations of free radical scavengers lower than healthy normotensive subjects The elevated consumption of plasma antioxidants was accompanied by increased activity of extracellular antioxidant enzymes (glutathione peroxidase and superoxide dismutase). Free radical production in EH overwhelmed antioxidant defense capacity. Oxidative stress in EH patients is accompanied with the decreased red blood cells and neutrophils superoxide dismutase and glutathione peroxidase activity. The oxidative process is a mere consequence of increased blood pressure. Reactive oxygen radicals may play a dual role in essential hypertension.

On one hand, they may inactivate nitric oxide converting them in peroxynitrite in reaction with superoxide anion, thereby causing arteriolar vasoconstriction and elevation of peripheral hemodynamic resistance.[20]On the other hand, enhanced production of free radicals may serve as trigger mechanism for oxidative damage of numerous macromolecules (par example low-density lipoprotein). The enhanced LDL oxidation has been observed in patients with essential hypertension. This conclusion was based on findings obtained in isolated LDL (which appeared more prone to oxidation triggered by exogenous stimuli) and on demonstration of autoantibodies directed against epitopes generated during oxidative modification of apoprotein B 100. Little is known, however, about the molecular processes underlying LDL oxidation in essential hypertension. To understand the mechanism for oxygen free radical formation in

hypertension, the cellular source must be identified. The endothelial cell, which is recognized as a source of NO, has been identified as a potential site of oxygen free radical production. Superoxide radicals in and around vascular endothelial cells were found to play a critical role in the pathogenesis of hypertension. The essential hypertension is associated with increased superoxide anion and hydrogen peroxide production by circulating leukocytes.

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. 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.[15]

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. The nephrotoxicity of certain drugs such as cyclosporine, tacrolimus (FK506), gentamycin, bleomycin, vinblastine, is mainly due to oxidative stress via lipid peroxidation. 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. [7]

Cataract:

Cataract is one of the most common causes of visual impairment, affecting almost 25 million people globally. Cataract is characterized by the opacity of the eye lens which results in visual impairment. Even though multiple factors such as smoking, drugs, genetic factors, diabetes, radiation, and malnutrition has been implicated in its incidence, free radical-induced oxidative stress has been considered one of the major cause for cataract disorder. Oxidation of DNA, lipids, and proteins has been observed in cataract lenses and this has been shown to induce the opacity of the eye lens seen in cataract patients.[6]

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 photochemically or non- photochemically. 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.[19]

Rheumatoid arthritis and 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. 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]

Atherosclerosis:

Atherosclerosis is a condition referred to as hardening of the arteries. Hyperlipidemia is a major risk factor for atherosclerosis. Elevated levels of oxidized low density lipoprotein (LDL), glucose and free fatty acids are found in patients with atherosclerosis, T2D, and obesity. The imbalance of oxidants and antioxidants resulting in oxidative stress is observed in atherosclerosis. In the vessel wall, endothelial cells, smooth muscle cells and macrophages are sources of free radicals. Endothelial dysfunction leads to increased endothelial permeability, up regulation of endothelial adhesion molecules, and inflammatory cell infiltration into the arterial wall. ROS are involved in endothelial injury, dysfunction, and lesion progression. The ROS dependent activation of the MMPs results in the degradation of intimal extracellular matrices and promotes smooth muscle cell migration. Cigarette smoking contain large amount of free radicals and may down-regulate key exogenous and endogenous antioxidants such as vitamin-D, carotenes, GPx and SOD and can lead to the dysfunction of monocytes and vascular smooth muscle cells. The pro-atherogenic agents such as oxidised lipids, high glucose and cigarette constituents give rise to increased free radical production.[2]

Fetus and oxidative stress:

Oxidative stress is involved in many mechanisms in the development of fetal growth restriction and pre-eclampsia in prenatal medicine. Some reports indicate that blood levels of lipid peroxidation products (F2-isoprostanes, MDA) are elevated in pre-eclamptic pregnancy and

intrauterine growth retardation and it has been suggested that ROS/RNS play a role in the etiology of these diseases. In pregnancies complicated by pre-eclampsia, increased expression of NADPH oxidase 1 and 5 isoforms which are the major enzymatic sources of superoxide in the placenta is seen. [17]

Asthma:

Asthma is common diseases affecting the airways of the lungs. Many studies have suggested that oxidative stress brought about by the build-up of free radicals contributes to tissue damage in Asthma patients. Free radicals have been implicated in various respiratory diseases such as respiratory distress syndrome, chronic bronchitis, asthma, and chronic obstructive pulmonary disease. [15]It is characterized by chronic inflammation of the airways involving variable and recurrent airflow obstruction and bronchial hyperreactivity associated with airway remodelling. Airway remodeling is a dynamic process involving mucous hypersecretion, collagen deposition, wall thickening, myocyte hypertrophy and hyperplasia, myofibroblast hyperplasia, vascular proliferation and alterations in airway elastic fibers, all of which culminate in persistent structural alterations of the airway. NO is endogenously produced in mammalian airways by NOS and is known to regulate many aspects of human asthma, including modulation of airway and vascular smooth muscle tone and the inflammation. Increased production of airway NO is a key factor in the development of airway hyperresponsiveness. [23]ROS are produced both intracellularly by lung parenchymal cells and extracellularly by lung macrophages. Increased generation of oxidants have been reported in asthma patients than in healthy individuals which provoked airway inflammation by inducing diverse pro-inflammatory mediators including macrophages, neutrophils and eosinophils. Oxidative stress is caused by overproduction of various free radicals or by an insufficient antioxidant defense system in asthma and thus it contributes to the tissue damage which is induced by inflammatory cells. Elevated levels of oxidative stress markers such as H_2O_2, 8-isoprostane, nitric oxide, and carbon monoxide were reported in exhaled air of asthmatic patients. Increased MDA levels, and Protein carbonyls; decreased protein sulfhydryl and antioxidant activity were observed in plasma, bronchoalveolar lavage fluid and exhaled air of asthmatic patients.

Free radical and aging:

The human body is in constant battle to keep from aging. Free radical damage to cells leads to the pathological changes associated with aging. An increasing number of diseases as well as aging process itself, demonstrate link either directly or indirectly to these reactive and potentially destructive molecules. The major mechanism of aging attributes to DNA or the accumulation of cellular and functional damage. Reduction of free radicals or decreasing rate of production may delay aging. Some of the nutritional antioxidants will retard the aging process and prevent disease. Based on these studies, it appears that increased oxidative stress commonly occurs during the aging process, and antioxidant status may significantly influence the effects of oxidative damage associated with advancing age. Free radicals have a significant influence on aging, that free radical damage can be controlled with adequate antioxidant defense, and that optimal intake of antioxidant nutrient may contribute to enhanced quality of life. [18]

CONCLUSION

Free radicals are a result of normal metabolic processes in our body which are involved in many bodily processes and disorders. When there is an imbalance between the antioxidants and the oxidants, free radicals accumulate inside of our cells leading to the damage of proteins, lipids and nucleic acids in our body. This can lead to tissue degeneration and other cellular abnormalities resulting in the many human disorders. We need to strive to keep the balance of free radicals in our body so as to attain an ability to promote good health.

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