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Histopathology Review of Free Radicals

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ABSTRACT

Histopathology is a branch of medicine that deals with the study of disease tissues. These diseases have an underlying cause which is the reaction of free radicals and other reactive species. Free radicals are highly reactive and unstable chemical molecules that are produced from normal metabolic and biochemical reactions in the body. Free radicals cause oxidative stress when their reactions overwhelm the counteraction of antioxidants in the body. Examples of free radicals are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric oxide (NO), peroxyl (ROO) and lipid peroxyl (LOO). Oxidative stress then leads to disease by provoking pathologic reactions in specific tissues of the organ based on the exposure of the tissues to free radicals. Oxidative stress is implicated in the pathogenesis of many diseases like diabetes mellitus, atherosclerosis, liver cancer etc. Antioxidants are molecules that can stabilize or deactivate free radical before they cause tissue damage. This review is focused on establishing the effects free radicals have in the pathogenesis of histopathology, in addition, the roles of antioxidant in deactivating these free radicals reaction. Free radicals cause disease when oxidation exceeds antioxidation or even when the generation of free radicals exceeds its removal.

Keywords: Histopathology, Free radicals, Oxidative Stress, ROS, Antioxidants, Tissue

INTRODUCTION

Histopathology is examination or study of tissues from the human body to spot the characteristics of a disease[1]. It is basically defined as the study of abnormal or diseased tissues and could also be defined as the tissue changes that affect a

part or accompany a disease[2]. Histopathology slides provide a more comprehensive view of disease and its effect on tissues, since the preparation process preserves the underlying tissue architecture. As such, some disease characteristics, e.g., lymphocytic infiltration of cancer, may be deduced only from a histopathology image. Additionally, the diagnosis from a histopathology image remains the 'gold standard' in diagnosing considerable number of diseases[3].

The cells of the body always adapt to physiological demands of the body in order to maintain homeostatic steady state. The cellular adaptations include atrophy, hypertrophy, hyperplasia and metaplasia. Cell injury occurs when the capacity for physiological adaptation is exceeded. This is when the stimulus is excessive or when the cell is unable to adapt without suffering some form of damage. Cellular injury can be reversible or irreversible. When it is reversible it means a non-lethal damage that occurred can be corrected by the removal of the stimulus but when it is irreversible the lethal damage cannot be corrected. Cells generate reactive oxygen as byproducts of metabolic reactions that reduce molecular oxygen to water. The cells of the body have antioxidant effect but when the actions of the free radicals over power the antioxidant it leads to oxidative stress. Oxidative stress is the underlying factor in the pathogenesis of most cellular diseases. It could be caused by inflammation, reperfusion injury, chemical injury and radiation damage[4]. Oxidative stress has been implicated in the pathogenesis of a variety of human diseases such as atherosclerosis, cancer, diabetes, liver damage, AIDS, Parkinson's disease and health complications associated with premature birth[5].

Free radicals are the products of normal cellular metabolism. A free radical can be defined as any atom or molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaving as oxidants or reductants[6]. The most important oxygen-containing free radicals in many disease states are hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical. These are highly reactive species, capable in the nucleus, and in the membranes of cells of damaging biologically relevant molecules such as DNA, proteins, carbohydrates, and lipids[8]. Free radicals attack important macromolecules leading to cell damage and homeostatic disruption. Targets of free radicals include all kinds of molecules in the body. Among them, lipids, nucleic acids, and proteins are the major targets[8].

Tissues are exposed to free radicals by metabolism, irradiation, environmental agents like drugs capable of redox cycling, xenobotics etc. Free radicals oxidize the important components of the cell and they lose their abilities to function normally resulting in cellular and cell death. Examples of free radicals are superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), nitric oxide (NO^*), peroxyl (ROO^*) and lipid peroxyl (LOO^*). Other oxidants that can generate free radicals include nitrogen dioxide (NO_2^*), ozone (O_3), nitrous acid (HNO_2), dinitrogen trioxide (N_2O_3), lipid peroxide ($LOOH$), hypochlorus acid ($HOCl$) and peroxynitrite ($ONOO^-$)[9].

Table 1: Presentation of Free Radicals and their Characteristics[10]

Name	Formula	Characteristics	Classification
Superoxide	$O_2^{\cdot -}$	Highly unstable	ROS
Hydrogen peroxide	H_2O_2	Cell toxicity, generation of other ROS	ROS
Hydroxyl radical	$OH^{\cdot}$	Free radical, highly unstable, very reactive agent	ROS
Alkoxyl radical	$RO^{\cdot}$	Free radical, reaction product of lipids	ROS
Peroxyl radical	$ROO^{\cdot}$	Free radical, reaction production of lipids	ROS
Hypochlorite anion	OCl^-	Reactive oxygen species, reactive chlorine species	ROS
Singlet oxygen	$^1O_2$	Induced/ excited oxygen molecule	ROS
Ozone	O_3	Environmental toxin	ROS
Nitric oxide	$NO^{\cdot}$	Environmental toxin	RNS
Peroxynitrite	$ONOO^-$	Highly reactive reaction intermediate of O_2 and NO	RNS
Nitrogen dioxide	$NO_2^{\cdot}$	Highly reactive radical, environmental toxin	RNS
Nitrogen oxides	NO_x	Environmental toxins, including NO and $^xNO_{2x}$ derived from the combustion process.	RNS

SOURCES OF FREE RADICALS

Free radicals are generated by normal biochemical reactions that take place in the body. They are also gotten from metabolic processes and immune system responses. Free radical-generating substances can be found in food, drugs, medicine, and oxygen. The free radicals, both the reactive oxygen species (ROS) and reactive nitrogen species (RNS), are derived from both endogenous sources (mitochondria, peroxisomes, endoplasmic reticulum, phagocytic cells etc.) and exogenous sources (pollution, alcohol, tobacco smoke, heavy metals, transition metals, industrial solvents, pesticides, certain drugs like halothane, paracetamol, and radiation)[11].

The mitochondria are major source of free radicals. The Tumor Necrosis Factor-alpha (TNF-a) stimulates the mitochondria by causing oxidative stress which then compromises cardiac function by altering mitochondrial redox state and membrane permeability transition pore to produce free radicals[12]. The other mitochondrial components which contribute to the formation of ROS include monoamino oxidase, a-ketoglutarae dehydrogenase, glycerol phosphate dehydrogenase and p66shc[13].

The lysosomal enzyme myeloperoxidase catalyzes the production of bacteriocidal hypochlorite from hydrogen peroxide and chloride ions. Free radicals can also be generated by eicosanoids from arachidonic acid during ischemia-reperfusion injuries. The enzymes of endoplasmic reticulum such as cytochrome p-450 and b5 enzymes and diamine oxidase contribute to the formation of ROS. The other endogenous sources of ROS include prostaglandin synthesis, auto-oxidation of adrenalin, phagocytic cells, reduced riboflavin, $FMNH_2$,

$FADH_2$, cytochrome P_{450}, immune cell activation, inflammation, mental stress, excessive exercise, infection, cancer, aging, ischemia etc[14].

Free radicals are also produced upon exposure to stress. The stress response creates a lot of free radicals by racing the body's energy apparatus. Cortisol and catecholamines mediate the stress reactions in the body, thereby degenerating to form free radicals. Exposure to radiation or sunlight also causes free radicals to be generated. These radicals formed, age the skin, causes wrinkles and skin toughness. When there is prolonged exposure it causes skin cancer. Other sources include alcohol, viruses, germs or fungi, exposure to ozone or nitric oxide, drug induced liver toxicity. Drugs like Nitrofuraton, Chlorpromazine, Adriamycin etc[15].

CHARACTERISTICS AND FORMATION OF FREE RADICALS

Free radicals are majorly divided into the reactive oxygen species and the reactive nitrogen species as shown in Table 1 above. The radicals are less stable than the non-radical species even though their reactivity is much stronger. Free radicals are highly unstable molecules that have available electrons that are able to react with various organic substrate such as lipids, proteins and DNA[9]. Atoms are most stable in the ground state. An atom is in the 'ground' state when every electron in the outermost shell has a complimentary electron that spuns in opposite direction. Free radicals like nitrogen and oxygen have at least one unpaired electron (valence electron) in their outermost shell that is why it is capable of existing independently[16]. According to Karlsson[16], free radicals are easily formed when a covalent bond between entities is broken

and one electron remains with each newly formed atom. Oxygen centered free radicals have two unpaired electrons in the outermost shell. When free radicals steal an electron from a surrounding molecule, a new radical is then formed in its place. The newly formed radical now tries to return to its ground state by stealing electrons with anti-parallel spins from cellular molecules[17].

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

$$O_2 \longrightarrow H_2O_2 \longrightarrow OH \longrightarrow H_2O$$

The electron transport chain found in the mitochondrial membrane uses oxygen to generate energy in form of adenosine triphosphate (ATP). Studies have suggested that anywhere from 2 to 5% of the total oxygen intake during rest and exercise have the ability to form highly damaging superoxide radical via electron escape. Oxygen consumption during exercise increases 10 to 20 fold in 35-70ml/kg/min. The electron escape from the electron transport chain is then enhanced[16].

EXAMPLES OF FREE RADICALS

Reactive Oxygen Species (ROS)

These are oxygen-free radicals that damage biological systems. They are the main byproducts formed in the cell of aerobic organisms during metabolism and homeostasis. The ROS can be generated during metal catalyzed reactions, present in the atmosphere as pollutants, generated during UV light irradiation and are by products of mitochondrial catalyzed electron transport reactions[18]. The electron transport reaction takes place in the mitochondria to generate ATP. The ATP generated is essential for life as it generates the energy the body needs for its metabolic activities. The reactive oxygen species (ROS) produced by the mitochondria is very

important since it underlines the oxidative damages done in the body. Superoxide anion that arises through metabolic processes or the process after oxygen activation by irradiation is termed 'primary' ROS; it then further interacts to form 'secondary' ROS[19]. The common ROS are superoxide, hydrogen peroxide and the hydroxyl ion. The superoxide is the proximal ROS. Superoxide (O_2) is produced when there is a leak of a small number of electrons leak to oxygen prematurely during transduction[20]. Some measurements suggest that sub-mitochondrial particles like the electrons in the electron transport chain leak to generate superoxide instead of aiding in the reduction of oxygen to water. Three major complexes are involved in the production of ROS in the electron transport chain in complex 1, where there is premature leakage of oxygen. This makes complex 1 the main site for the production of superoxide[21].

The hydrogen peroxide, (H_2O_2) is sometimes produced by the dismutation of superoxide by the enzyme super oxide dismutase[22]. Recent works have shown that an extra mitochondrial disease can lead to a leak even when the mitochondria lack Zn-SOD and Cu[23]. Often times not all the hydrogen peroxide produced by the mitochondria survive to the efflux of the mitochondria as a result of the peroxidases of the mitochondria and even catalases and peroxiredosins[24]. The neutral form of the hydroxide ion is the hydroxyl radical (OH^-). This radical has very high reactivity, thus making it a very dangerous radical. It has a short half-life *in vivo*. OH^- produced *in vivo* reacts close to the site of its formation. At the site of formation copper and iron are required to be present in trace amounts[25].

ROS production by mitochondria can lead to oxidative damage to mitochondrial proteins, membranes and DNA, impairing the ability of mitochondria to synthesize ATP and to carry out their wide range of metabolic functions, including the tricarboxylic acid cycle, fatty acid oxidation, the urea cycle, amino acid metabolism, haem synthesis that are common to the normal activities of most cells of most cells. Mitochondrial oxidative damage can also increase the tendency of mitochondria to release inter-membrane space proteins such as cytochrome c to the cytosol by mitochondrial outer membrane permeabilization and thereby activate the cell's apoptotic machinery. In addition, mitochondrial ROS production leads to induction of the mitochondrial permeability transition pore, which renders the inner membrane permeable to small molecules in situations such as ischaemia and hypoxia induced oxidative stress injury. Consequently, it is unsurprising that mitochondrial oxidative damage contributes to a wide range of pathologies. In addition, mitochondrial ROS may act as a modulatable redox signal, reversibly affecting the activity of a range of functions in the mitochondria, cytosol and nucleus[24].

Apoptosis affects the production of free radicals as a result of too much damage to the mitochondria. Super oxide dismutase is the enzyme that destroys the super oxide radical and then converts the super oxide to peroxide. By doing this, it minimizes the production of hydroxyl radical. The peroxides that are produced by the super oxide dismutase are also very toxic[26]. It contains three transitional metals iron, copper and manganese.

Iron

Iron is very abundant in the body because of its role in electron transport and oxygen transport[27]. As a result of these roles the human body had to develop physiological means of controlling iron in the body. Hence, its regulation ensures that there is no free intracellular iron. When the body is under stress, there is an excess of superoxide which then release 'free iron' from iron containing molecules[28]. The released iron Fe^{2+} then participates in the Fenton reaction generating highly reactive hydroxyl radical.

$Fe^{2+} + H_2O_2 \dashrightarrow Fe^{3+} + OH^{+} + OH^{-}$

$Fe^{3+} + H_2O_2 \dashrightarrow Fe^{3+} + O^{----}_2 + OH^{+}$

$Fe^{3+} + O^{+}_2 \dashrightarrow Fe^{3+} + O_2$

$Fe^{3+} + H_2O_2 \dashrightarrow Fe^{3+} + OH^{+} + OH^{-}$

Fenton Reaction

Under stress, superoxide acts as the oxidant for [4Fe-4S] cluster-containing enzymes and helps hydroxyl ion production from hydrogen peroxide by making the free iron available for the Fenton reaction[29]. Free irons are like loose bombs chemically, they can cause the most devastating actions an example is lipid peroxidation. Lipid peroxidation is a process where oxidants like free radicals attack lipids containing carbon-carbon double bonds on the cell membrane. This then leads to the dysfunctional ability of the membranes and then cell death. Organisms that suffer from iron overload like in the cases of hemochromatosis, β-thalassemia and even hemodialysis have a high amount of 'free iron' and this has a very harmful effect. The free iron is then transported into a 'labile iron pool', which represents a steady state exchangeable and readily chelatable iron compartment[30].

Copper

The major role of copper in the body is in the development of healthy nerves, bones, collagen and the skin melanin pigment. Copper is normally absorbed from food, and excess is excreted through bile. In Wilson's disease, copper is not eliminated well and then copper tends to accumulate leading to a life – threatening level. This disease can be treated if it's diagnosed early and then the patients live normal lives later. Symptoms typically begin between the ages of 12 and 23 years. Hydroxyl ions are generated by a phosphate-buffered copper-hydrogen peroxide system. In each system the buffer, Herpes was seen to stimulate radical generation significantly. The main reasons for this could be, Herpes increases Cu^{2+} solubility in phosphate-buffered systems. It then forms a complex with that is effective in generating OH from hydrogen peroxide. Cu^{2+} chelators are also capable of generating OH radicals. Once Cu^{2+} radicals are present, OH radicals would be generated[31].

Manganese

The chemical and biological behoviour of manganese which is a transition metal with the OH radical still remains unknown. Inorganic complexes of Manganese, unlike iron and copper complexes are known to exist in high concentration in certain cells. The methods used to detect OH were also used to investigate the activity of biologically relevant inorganic manganese complexes[32]. The direct and indirect effects of manganese on the OH flux were compared by attempting to distinguish the effects of hydrogen peroxide, superoxide, and hydroxyl through the use of selective scavengers and generators. Hydroxyl generation is mainly dependent on the presence of superoxide and hydrogen peroxide. Therefore, with hydroxyl as with hydrogen peroxide and superoxide,

manganese complexes appear to behave in a fundamentally different pattern from copper and iron[32].

Reactive Nitrogen Species (Nitric oxide)

Reactive nitrogen species are highly reactive radicals. These radicals only last for few seconds in the body. They are made from the amino acid arginine by the enzyme called nitric oxide synthase. There are three forms of this radical namely: neuronal, endothelial and inducible. The neuronal form makes nitric oxide that serve strictly to signal the neurons. The endothelial form is found in the blood vessels and it produces nitric oxide that causes their dilation. The inducible form is found in the macrophages and certain other immunological cells and this form plays a major role in phagocytosis. It has a half-life of only a few seconds in aqueous environment. It has a greater stability in an oxygen-low environment with a half life greater than 15 seconds[33]. When there is an overproduction of reactive nitrogen species it is called nitrosative stress. Nitrosative stress could lead to nitrosylation reactions that tend to alter the protein structures and inhibit normal functions[34].

Nitric oxide which is produced by macrophages and used to attack microbes, then reacts with superoxide to form more toxic molecules like peoxynitrite. Nitric oxide synthase belongs to the family of heme-containing mono-oxygenases that are capable of producing free radicals[35]. Nitric oxide depends on the binding of L-arginine, while nitric oxide synthase produces super oxide and hydrogen peroxide only when the substrate concentration is low. Free radicals here are generated by the binding of the substrate which helps to determine the pathways. By knowing the role of substrate binding, the

substrate binds within the distal heme pocket near the sixth coordination position of the nitric oxide synthase heme iron. L-arginine then alters the coordination properties of the heme iron which promotes the formation of perferryl complex NOS-$[Fe^{5+}O]^{3+}$. The ability of the nitric oxide synthase to generate free radicals during enzymatic cyclic shows that NOS-$[Fe^{5+}O]^{3+}$acts like an analogous iron-oxo complex of cytochrome P-450 during aliphatic hydroxylation[35]. Nitric oxide is a cellular messenger that acts by a lot of mechanisms like the activation of soluble guanylatecyclase, nitrosylation of thiols and the formation of peroxynitrite. Nitric oxide actions generally depend on the oxidative condition of the cell. Nitric oxide will induce motor neuron apoptosis when it reacts with super oxide and forms peroxynitrite[35].

ADVANTAGES OF FREE RADICALS

The most beneficial free radical is hydrogen peroxide that is generated by super oxide dismutase which helps to activate white blood cells. Hydrogen peroxide is also produced by some normal body flora to kill bacteria and fungi. Natural killer cells which are white blood cells produce peroxide to destroy pathogens and cancer cells. The reactive oxygen species and reactive nitrogen species are very important for the maturation process of cellular structures and can act as weapons for the host defense system at low or moderate concentrations. Phagocytes also release free radicals to destroy foreign pathogenic microbes which is the body's major defense mechanism[36-37]. Patients with granulomatous disease are the perfect examples to show the benefits of free radicals, as they have defective membrane bound NADPH oxidase system which then makes them unable to produce the superoxide anion radical (O_2), which now results in multiple and

persistent infection[29]. Reactive oxygen species and reactive nitrogen species also play a lot of physiological roles in the functioning of cellular signaling systems[38-39]. Nitric oxide for example is an intracellular messenger for modulating blood flow, thrombosis, and neutral activity[29]. It is also important for non-specific host defense and also to kill intracellular pathogens and tumors. Free radicals also help in the induction of mitogenic response[38]. Studies have shown that free radicals like superoxide, hydrogen peroxide etc. act as signal substances that cause the heart to beat with the correct force. Summarily, these free radicals are very vital to basic human health.

DELETERIOUS ACTIVITIES OF FREE RADICALS

Free radicals cause damage to parts of cells such as proteins, DNA, and cell membranes by stealing their electrons through a process called oxidation. The radical damage is also known as oxidative damage[40]. The most dangerous free radicals are the small, mobile, and highly reactive oxy radicals. Free radicals are usually oxygen molecules that have lost an electron hence their instability. They begin to covet the neighbouring molecules to electrons[41] which makes them unstable and this continues in a chain, a chain which leads to stealing an electron, which acta as "terrorists" disrupting normal cell function. They can attack DNA, leading to dysfunction, mutation and cancer. They also attack enzymes and proteins, producing a chain reaction of destruction. Such membrane damage in cells that line the blood vessels hardens and thickens the blood vessels which lead to heart attacks and strokes. When free radicals attack collagen fibers it can cause cross-linking of protein molecules which now leads to stiffness of that tissue[40].

In conditions where free radicals and oxidants are produced in excess it leads to a phenomenon called "Oxidative Stress". This will then alter the cell membranes and structures like proteins, lipids, lipoproteins and deoxyribonucleic acid[37]. Oxidative stress is defined as a state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them[42]. Oxidative stress can arise when cells cannot properly destroy the excess of free radicals formed[9]. When there is an imbalance in the formation and neutralization of these free radicals, oxidative stress occurs. When hydroxyl radicals and peroxynitrite are in excess in a cell it causes cell membrane damage and also damage lipoproteins by lipid peroxidation. This then leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. However, oxidative stress is really useful in some cases. For example, oxidative stress induces apoptosis to prepare the birth canal for delivery. Also, biological defense mechanisms are strengthened by oxidative stress during appropriate physical exercise and ischemia. Therefore, a more useful definition of oxidative stress may be a "state where oxidation exceeds the antioxidant systems because the balance between them has been lost"[42].

Lipid peroxidation basically occurs by radical chain reaction so therefore, once this process starts, it spreads rapidly and affects a lot of lipid molecules[43]. Oxidative stress damage to proteins could also lead to structural changes and loss of enzyme activity[39,43]. Mutations are caused when there has been an oxidative damage to DNA which now leads to the formation of different oxidative DNA lesions. Antioxidants and DNA repair are the body's major mechanism to reverse the actions of these free radicals[44]. Oxidative stress can induce

a lot of chronic and degenerative diseases if not properly managed. It could also cause some acute pathologies and even the aging process.

DISEASES LINKED TO OXY RADICALS AND REACTIVE OXYGEN SPECIES

Atherosclerosis

This is the leading cause of mortality and morbidity in the world[45]. Atherosclerosis is a disease that is caused by factors such as genetic and environmental factors. The risk of atherosclerosis is higher in men than in women as a result of lifestyle, habits and dietary control. It is also very common in white men than in black men.[46] Many evidences have suggested the involvement of free radicals in the pathogenesis of atherosclerosis. According to Steinberg *et al.*[47], oxidation of low density lipoproteins causes atherosclerosis. Ample evidence has supported this hypothesis that the oxidation of low density lipoproteins (LDL) is a major key to the progression of atherosclerosis[47]. When LDLs are oxidized it leads to the simulation of endothelial cells to produce inflammatory markers, which are now involved in the formation of foam cells, which also has cytotoxic effects on the endothelial cells, inhibits the motility of tissue macrophages and then inhibits nitric oxide-induced vasodilation[48].

Pathogenesis

The reactive oxygen species are generated by extracellular and intracellular mechanisms and are responsible for the oxidation of low density lipoproteins. When peroxynitrite and nitric oxide is generated by the action of angiotensin II on the macrophages and endothelial cells it is said to be extracellular production. ROS production is increased in the endothelial cells

after the stimulation of the endothelial cells by Tumor Necrotic Factor-alpha and other cytokines like Interleukin 1. The intracellular production is through the membrane-bound NAD(P)H oxidases, xanthine oxidase and uncoupled NO synthase, which accounts for more than 90% of ROS production[49]. When there is an increase in the vascular superoxide radical production in association with NO produced by the endothelium, it enhances the availability of the harmful peroxynitrite[50]. These radicals cause lipid peroxidation, DNA damage and protein changes[51].

LDL may be oxidized within arterial walls and also in peripheral sites of inflammation[52]. Lipids are very vulnerable to free radical attack and they are readily oxidized to produce aldehydes, which now reacts with lysines and tyrosines in apo-lipoprotein B-100 resulting in modification and functional loss. During the oxidation of the LDL, the apolipoproteins, cholesterol and the unsaturated fatty acids which are now esterified in phospholipids are usually modified. ROS elicit *in vivo* and *in vitro* oxidative decomposition of omega-3 and omega-6 polyunsaturated fatty acids of membrane phospsolipids, a process called lipid peroxidation[52].

Lipid peroxidation involves the beta-cleavage reaction of lipid hydroperoxides and eventually leads to the aldehydic end products like 4-hydroxy-2,3, 4-hydroxy-2,3 nonenal alkenals. These producs are said to be putative and very toxic messengers which can then mediate oxidative stress injury at a molecular level. These messengers are said to be in the subendothelial space of the human aorta[53].

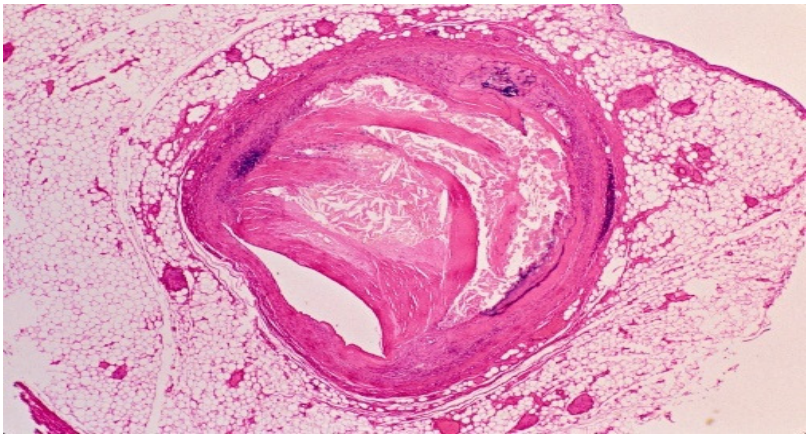

Plate 1: Light micrograph of Atherosclerosis (showing a gap in the lumen of the blood vessel as a result of the amyloid plaque)

Plate 2: Light micrograph of a normal Blood Vessel

Nephropathy: Diabetes Mellitus

Diabetic nephropathy is the common complication of type 1 and type 2 diabetes leading to end-stage of renal disease[54]. This disease is defined as a "progressive and irreversible renal disease characterized by the accumulation of extracellular matrix in glomerular mesangium and kidney interstitial tissue that eventually leads to renal failure[55]. It is simply characterized by elevated levels of blood glucose and insufficiency in the production or action of insulin which is

produced by the pancreas[56]. Diabetic nephropathy starts with endothelial cell dysfunction (ECD) and then ends with end-stage renal failure. Endothelial cell dysfunction is preceded by glomerular hyperperfusion and hyperfiltration. High glucose causes ECD and increases oxidative stress in glomerular mesangial cells, a target cell of diabetic nepheropathy. Oxidative stress caused by free radicals is a major key in the development of vascular complications in diabetes particularly type 2 diabetes.[9] Oxidative stress induces mRNA expression of NFkB genes which then produce pro-inflammatory proteins: TGF-B, fibronection, laminin, elastin, IL-1, IL-6 and PGDF. When ROS level is high it is as a result of the decrease in the destruction or increase in the production of catalase (CAT-enzymatic/non-enzymatic), glutathione peroxidise, superoxide dismutase antioxidants. As a result of these variations, the tissues are then susceptible to oxidative stress thus leading to diabetic complications[57].

Pathophysiology

Free radical formation in diabetes by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to the damage of enzymes, cellular machinery and then increased insulin resistance as a result of oxidative stress[56]. Oxidative stress plays a very important role in different renal diseases like glomerulonephritis, uremia, proteinuria, tubulointerstitial nephritis and chronic renal failure[36]. Transition metals like copper, iron, carbon-monoxide, chromate and heavy metals like cadmium, mercury, lead and arsenic are strong free radicals inducers that induce different forms of nephropathy and carcinogenicity[19]. In diabetes mellitus, the main source of oxidative stress is the mitochondria. When the mitochondria undergoes oxidative metabolism, a component of

the utilized oxygen is reduced to water, then the remaining oxygen is transformed to oxygen (O*) and then it is converted to $ONOO^-$, OH and H_2O_2[58]. ROS/RNS modulate insulin by two methods: (i) ROS and RNS are produced to exert full physiological function in response to insulin; (ii) ROS and RNS have negative regulation on insulin signaling, which then interprets them to develop insulin resistance which is a risk factor for diabetes type 2.

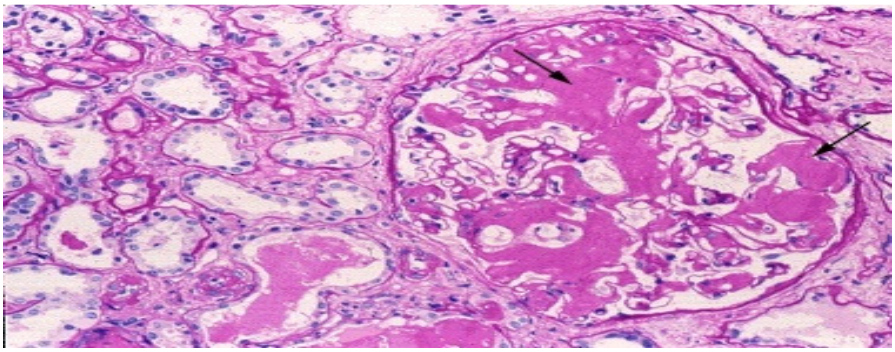

Plate 3: Light micrograph of Diabetic nephropathy (arrows indicating the nodular and amorphous materials)

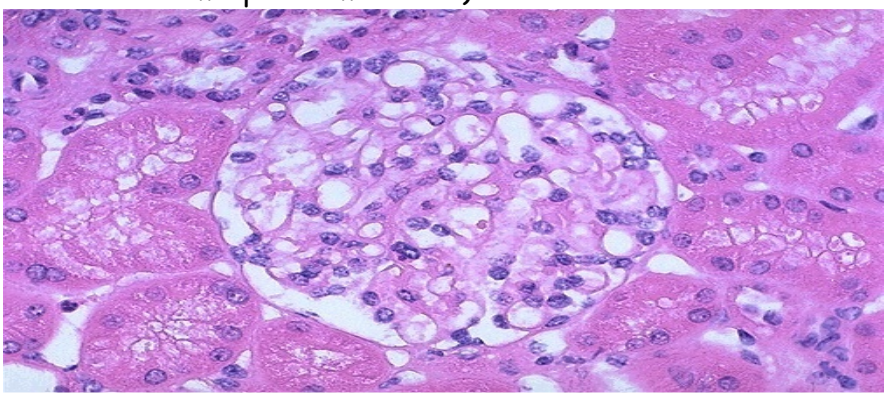

Plate 4: Light micrograph of a normal kidney

Ocular Disease: Cataract

Cataract is a disease of the eye that causes opacity and unless treated it can lead to blindness. Free radicals have been associated with various eye and systemic diseases and also the aging process. Oxidative stress is seen is age-related macular

degenerations and cataracts by altering the various cell types in the eye. When free radicals act in the eyes, crystalline proteins in the lens of the eyes crosslink and aggregate leading to the formation of cataracts[59]. In the retina, long-term exposure to radiation can inhibit mitosis in the retinal pigment epithelium and choroids, which damages the photoreceptor outer segments and it has been associated with lipid peroxidation[60].

Pathophysiology

Oxidative stress has been said to play a major role in the pathogenesis of several eye defects like cataract and macular degeneration. When ROS is produced and there is reduction of endogenous antioxidants, there tends to be cataract formation[61]. When the crystalline lens is subjected to oxidative stress as a result of frequent radiation it leads to the damage of crystalline proteins, lipids, polysaccharides and nucleic acids[62]. Aging also increases the chances of cataracts as a result of the decreased efficiency of repair mechanisms and the accumulation of oxidized lens components. Furthermore, lipid peroxidation has been proposed to be a factor that causes cataract[63]. An increased concentration of primary molecular lipid peroxidation products have been found in people with cataracts than in people without cataract. Several studies have associated the formation cataract with oxidative stress[63].

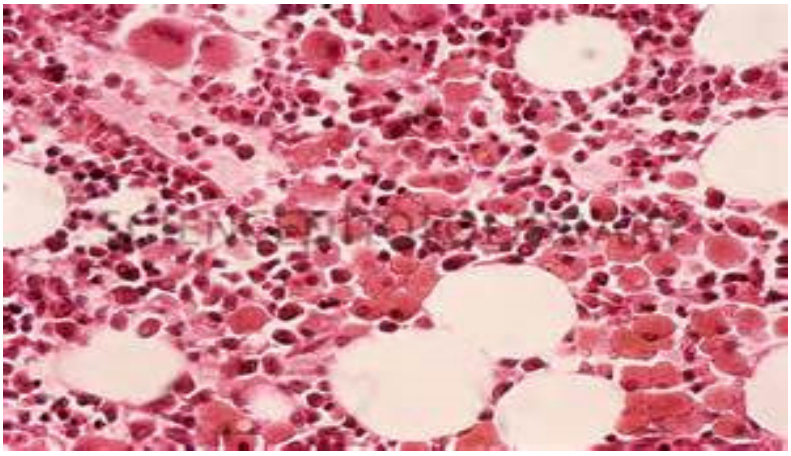

Plate 5: Light micrograph of Ocular disease

Neurological Disease: alzheimer's disease (AD)

Alzheimer's disease is the most common neurodegenerative disease that causes dementia in the elderly. It is characterized by the gradual deterioration of memory and cognitive functions. It leads to complete incapacity of the brain and death of the patient within 3-9 years of diagnosis[64]. Overtime evidences have showed that oxidative stress is a very important factor that contributes to the initiation and progression of Alzheimer's disease. In a diseased condition like Alzheimer's, a lot of research has been done to prove that oxidative damage plays a major role in the loss of neurons and progression to dementia[65]. B-amyloid which is a toxic peptide produced and often seen in Alzheimer's patients, is due to oxidative stress[66]. The oxidation of the mitochondrial DNA and also a part of the nuclear DNA have been observed in the parietal cortex of Alzheimer's disease[67].

Lipid peroxidation has also been increased in the brains of AD patients especially in the temporal lobe where histopathological changes are very noticeable[68]. Brain membrane phospholipids are usually made up of poly unsaturated fatty acids which

accompany lipid peroxidation in AD. When polyunsaturated fatty acids are oxidized, they produce aldehydes like 4-hydroxynonenal which is a highly reactive cytotoxic substance that can inhibit glycolysis, nucleic acid and protein synthesis and degrade proteins. There are lots of free radicals involved in AD one of which is the hydroxyl radical because of its high toxicity and its chemical reactions like Fenton's reaction which involves iron. Other free radicals are superoxide and hydrogen peroxide which is seen in amyloid neurotoxicity and peroxynitrite, which is formed by the combination of super oxide and nitric oxide[68].

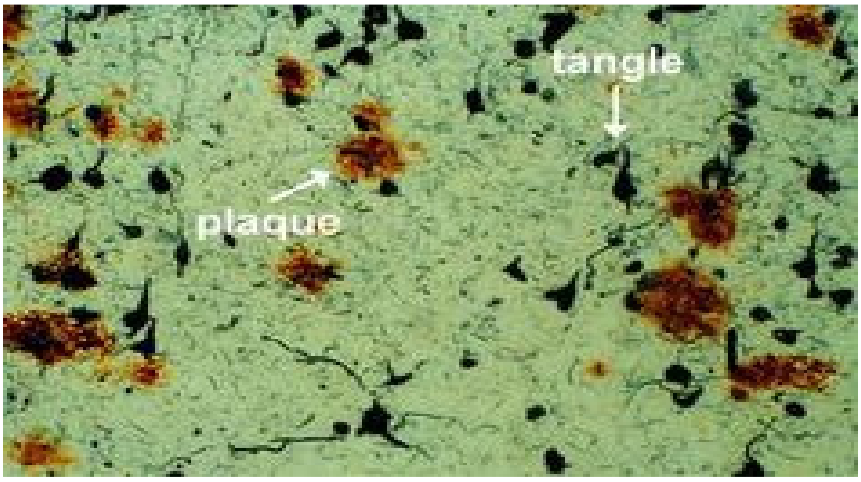

Plate 6: Light micrograph of Alzheimer's disease stained with silver stain (showing neuritic plaques)

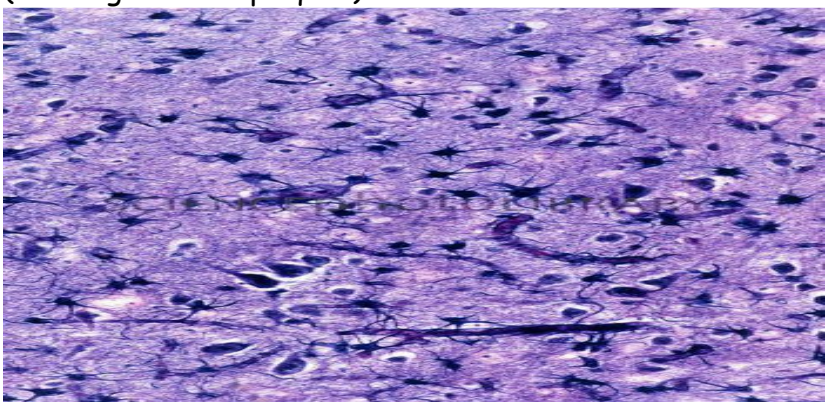

Plate 7: Light micrograph of normal Brain Glial Cells

Rheumatoid Arthritis

Rheumatoid arthritis is a systemic disease that is characterized by progressive, erosive and chronic polyarthritis. There is cellular proliferation of the synoviocytes and noe-angiogenesis which leads to the formation of pannus which destroys the articular cartilage and the bone[69]. This disease is a type of disease that is characterized by chronic inflammation of the joints and tissues around the joints with infiltration of macrophages and activated T-cells[70]. The pathogenesis of this disease is the generation of reactive oxygen species and reactive nitrogen species at the site of inflammation. Oxidative damage and rheumatic diseases have been proven to be increased by high levels of isoprostanes and prostaglandins in serum and synovial fluid[70]. Free radicals play an important role in inflammation. Nitric oxide plays a role in the regulation of vascular tone, which contributes to the cardinal signs of inflammation, superoxide free radical also has a role in fibroblast proliferation while hydrogen peroxide helps in the activation of transcription factors like Nuclear factor kappa B (NF-$_k$B). NF-$_k$B plays an important role as it is a major factor that controls the transcription of some cytokine genes like IL-2 and TNF-alpha[71].

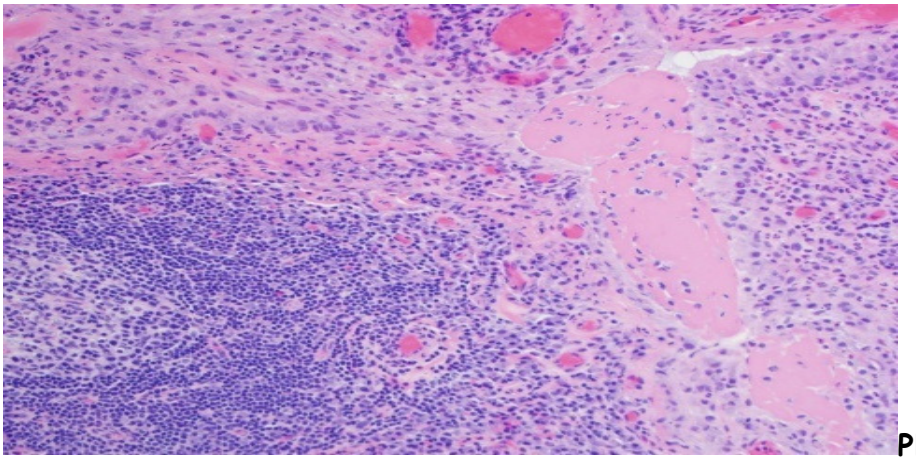

Plate 8: Light micrograph of Rheumatoid arthritis (showing the congestion of inflamed cells in the bone)

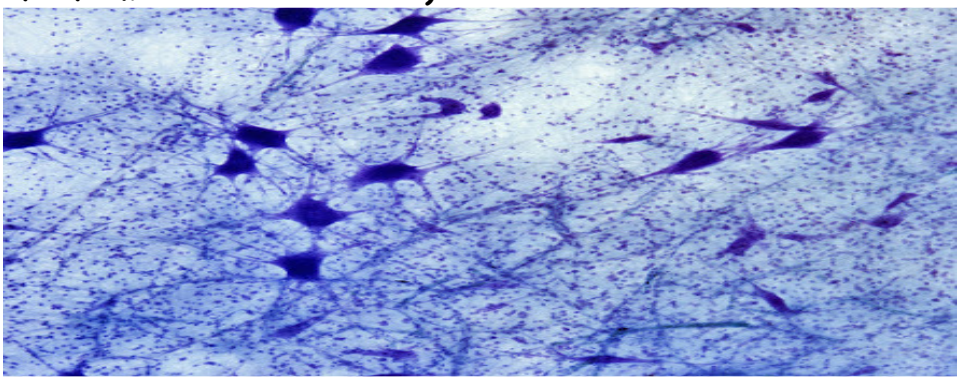

Plate 9: Light Micrograph of Normal Bone

Pulmonary Disease (Chronic Obstructive Pulmonary Disease)

Chronic Obstructive Pulmonary Disease is an incurable but preventable respiratory disease with high prevalence that is on the way to becoming the third most common cause of death worldwide[72]. This disease is characterized by persistent progressive airflow limitation and hyperinflation, with both systemic inflammation and chronic inflammation of the airways and lung parenchyma thai is mainly caused by the smoke of tobacco and airborne particulate matter. They are both responsible for the endogenous generation and release of oxidative stressors in the airways[73]. When there is an increase in the oxidative stress it leads to the starting of a lot of

pathologic processes like inactivation of antiproteases and enhancing bronchial inflammation by the activation of redox-sensitive transcription factors. The ROS causes an imbalance of proteases and antiproteases which then leads to the inactivation of antiproteases. Also an upregulation of gene transcription by the ROS leads to an increased gene expression of inflammatory mediators and cytokines (IL-1, TNFalpha, IL-8)[74].

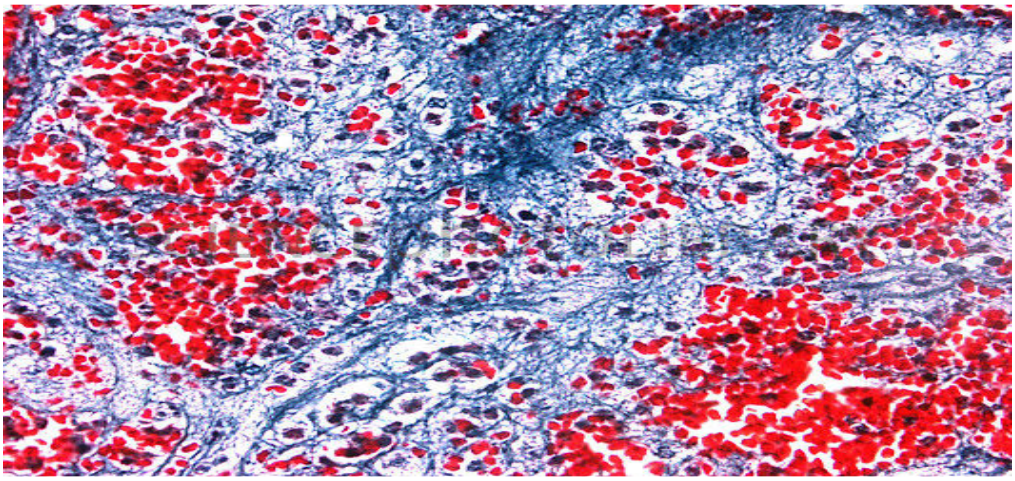

Plate 10: Light micrograph of Chronic Obstructive Bleeding Emphysema: Internal bleeding in lung tissue due to pulmonary emphysema. The bleeding is seen as numerous red blood cells (red). This bleeding resulted from the breakdown of the lung's smallest blood vessels (the pulmonary capillaries) during emphysema.

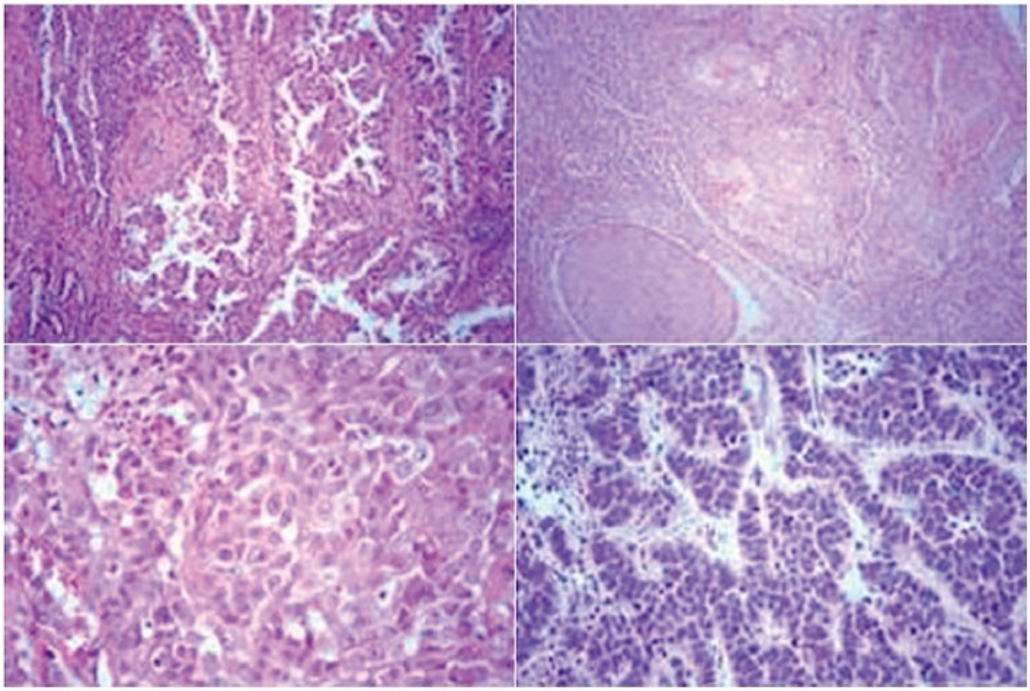

Plate 11: Light micrograph of Chronic Obstructive Pulmonary Disease

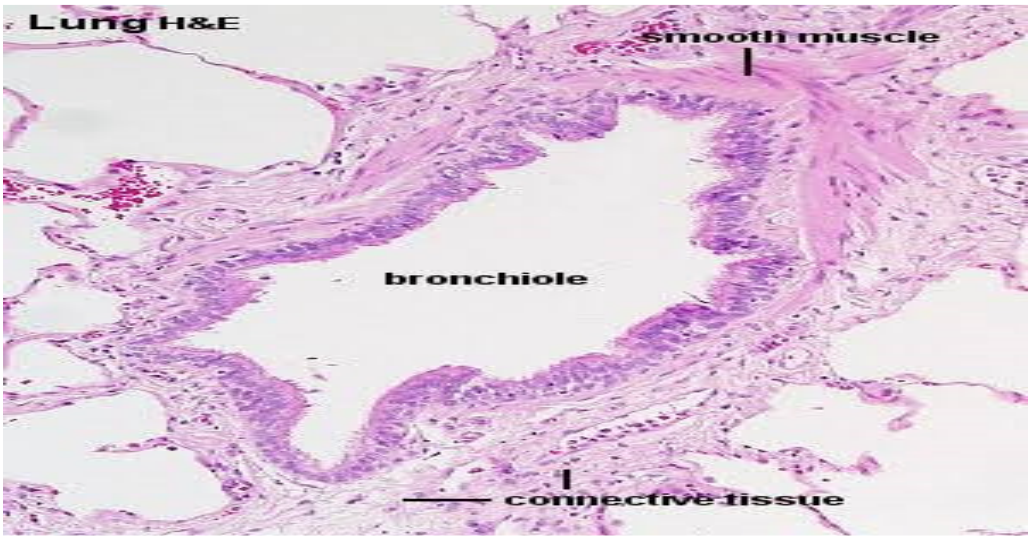

Plate 12: Light micrograph of Normal Lung Tissue

Cancer: Liver Cancer

Hepatocellular disease is the third most common cause of death in the world today and it is the most frequent worldwide spreading disease[75-76]. In aerobic life, oxidative stress arises

from both endogenous and exogenous sources. Despite antioxidant defense mechanisms, cell damage from oxygen free radicals (OFR) is ubiquitous. OFR-related lesions that do not cause cell death can stimulate the development of cancer. It is a general knowledge that oxidative DNA damage is responsible for cancer development[19]. A large body of evidence suggests important roles of OFR in the expansion of tumour clones and the acquisition of malignant properties. Chromosomal defects and oncogene activation induced by free radicals is associated in inducing and promoting cancer. In view of these facts, OFR may be considered as an important class of carcinogens. The formation of hydrolyzed bases of DNA, which is a very important event in chemical carcinogenesis, is a common form of DNA damage[19,39].

Oxidative damage to DNA 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, tobacco smoking and chronic inflammation from noninfectious diseases like asbestos are major sources of oxidative DNA damage that can contribute to the development of lung cancer[44]. The ineffectiveness of preventive antioxidant treatments in several recent clinical trials is surprising. Thus, reducing the avoidable endogenous and exogenous causes of oxidative stress is, for the present, the safest option. In the near future, new insights in the action of tumour suppressor genes and the DNA repair mechanisms may lead the way to additional tools against carcinogenesis from OFR[77].

Hepatocellular carcinoma is a very common type of hepatic malignant tumor worldwide. The main risk factor associated

with hepatocellular carcinoma is liver cirrhosis[78]. When oxidative stress is triggered, a lot of liver cells are affected[79]. ROS often react directly with the DNA hence damaging specific genes that are in charge of cell growth and differentiation[80]. During the progression phase of carcinogenesis, ROS easily stimulate the growth of cancer cells. The hydroxyl radical is a very dangerous radical that is responsible for base modifications that induce a point mutation in the daughter DNA[81].

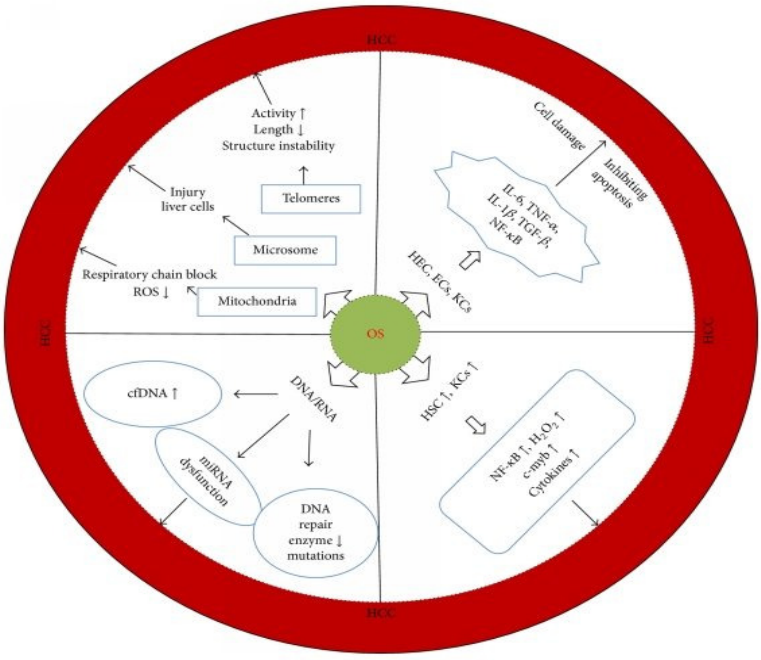

Figure 1: Mechanisms of oxidative stress on the regulation of liver cells[82]

Other factors associated with liver carcinogenesis is Hepatitis B virus, Hepatitis C virus, non-alcoholic fatty liver disease, obesity, dietary habits and even iron accumulation[83]. HBV and HCV related chronic inflammation are most times induced by oxidative stress and it contributes solely to the pathogenesis of hepatocellular carcinogenesis. During an HBV infection,

macrophages are usually activated to produce several pro-inflammatory cytokines like IL-1B, IL-6, CXCL-8 and TNF-alpha[84]. When there is an uncontrollable production of these cytokines, they tend to produce a lot of ROS which then takes part in hepatocarcinogenesis. HCV related chronic infections are generally associated with high level of serological markers and iron accumulation in the liver cells especially in the lysosmes. As a result of the induction of Fenton's reaction, there is an excess of bivalent iron which is very toxic. It is a general knowledge that iron accumulation influences liver cancer[85].

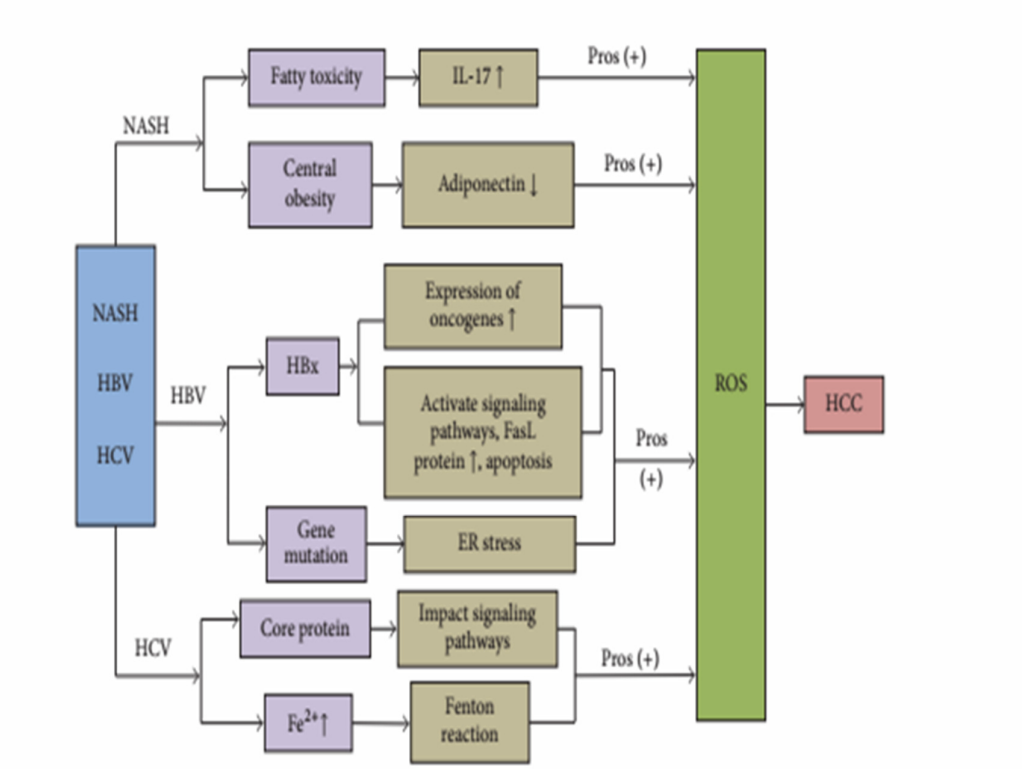

Figure 2: The mechanisms of oxidative stress on HBV-, HCV-, and NASH-related HCC[82]

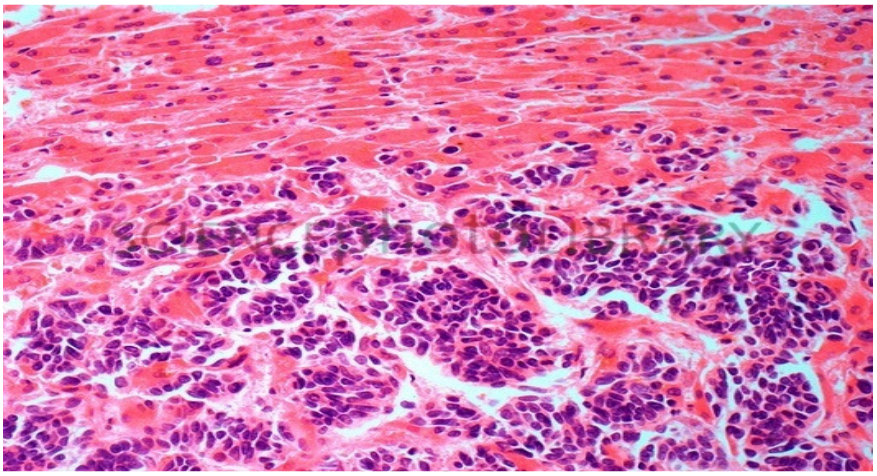

Plate 13: Light Micrograph of Liver Cancer

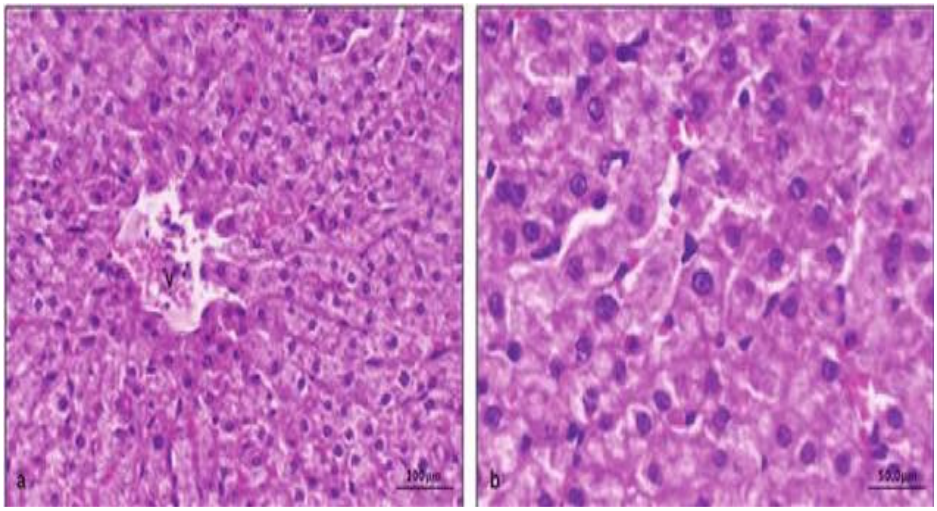

Plate 14: Light Micrograph of Normal Liver

CONCLUSION

To every biochemical or metabolic action there is an equal and opposite reaction. Free radicals damage contributes to the etiology of many chronic health problems such as cardiovascular and inflammatory disease, cataract, cancer etc. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. Continuous consumption of

foods rich in antioxidants in the right nutritional amount will help individuals live healthy and prevent oxidative stress. The implication of oxidative stress in the etiology of several chronic and degenerative diseases suggests that antioxidant therapy represents a promising avenue for treatment. In the future, a therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long term effective treatment.

Conflict of Interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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