Reactive Oxygen Species (ROS), Oxidative Stress and its role In HIV Infection

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ABSTRACT

Throughout several regular cell cycles, reactive oxygen species (ROS) play a critical role. When ROS values are high, and when the defence mechanism (antioxidants) cannot neutralise, they harm and modify the part of biological molecules. They also act as signalling molecules which generate a spectrum of disease. In this study, we reviewed existing oxidants, oxidative stress, and their relationship with infection by human immunodeficiency virus in patients, and the effects of oxidative stress in patients with HIV. Our prospect is to do a clinical study on HIV patients and estimate oxidative parameters like nitric oxide, total antioxidant level and correlate them with CD4 count and viral load which may be helpful during monitoring and giving efficient ART to the HIV patients. And also the importance of ROS in infection has been established through clinical and in vitro studies. Here we review the role of oxidative stress in HIV pathogenesis, the impact of ROS on immune responses in HIV patients, and ROS-mediated regulation of HIV infection. Future studies on the interplay between ROS and HIV infection may offer a new strategy for prevention and treatment.

INTRODUCTION

Oxidants are commonly classified as biological or exogenous compounds that are capable of oxidising the target molecules, either directly through electron abstraction or indirectly through the creation of highly reactive intermediate chemical entities. Free radicals are chemical species which contain one or more unpaired electron in their atomic structure which is responsible for its reactivity (Fakoya et al., 1998) and are capable of independent existence for very brief intervals of time. ROS involves all reactive oxygen sources, both radical and non-radical species involved in the initiation and/or transmission of extreme chain reactions. In addition to the immediate needs of cells, aggregation of ROS results in oxidative destruction of essential molecules, including DNA, proteins, and lipids (Schaller, 2005). Oxidative stress is responsible for inducing a variety of chronic and degenerative diseases, affecting a wide range of physiological functions. Recently there are many studies done to find the correlation of oxidants, antioxidants in HIV patients with CD4 and viral loads which may help us in prognosis and line of treatment of the HIV disease.

**Endogenous sources of oxidant radicals**

**Along with Electron transport chain (ECT)**

\[
O_2^{1-e} \rightarrow O_2^{-+1-e} \rightarrow H_2O_2^{++1-e} \rightarrow OH^{-+1-e} \rightarrow H_2O
\]

During oxidative phosphorylation in mitochondria
Types of ROS

The Most common ROS includes superoxide anion (O_2^-). Hydrogen peroxide(H_2O_2), hydroxyl radical (OH^-), hypochlorous acid (HOCl), peroxyl radicals, nitric oxide. Peroxynitrite (ONOO^-).

Superoxide anion (O_2^-)

The superoxide (much less reactive) is an anionic radical formed by reducing molecular oxygen by accepting a single electron. They are generated during oxidative phosphorylation via ECT during electron transport in mitochondria, typically with the escape of 1–2% of electrons that are caught by molecular oxygen (Figueira et al., 2013). The enzyme required to convert oxygen to superoxide is NADPH oxidases (NOX/DUOX). NADPH enzyme contains 7 isomers DUOX1-DUOX2 andNOX1-NOX5. Activation of NOX family protein will produce ROS (Nisimoto et al., 2014). Cytochromes P450, generates superoxide anion that superoxide Anions also provide catabolise specific endogenous compounds and xenobiotics. In reaction to bacterial infection, the activated phagocytes do have a metabolic pathway to generate superoxide radicals. Superoxide the anion may also induce the metabolism of arachidonic acid to produce more superoxide and release the Fe2 + from the stores of ferritin (Yoshida, 1995). Because of a negative charge, the superoxide anion is less active, but its protonation produces a higher oxidising per hydroxyl radical (HO_2^-).

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide dismutase (SOD) transforms superoxide into hydrogen peroxide and molecular oxygen, but superoxide naturally can also produce hydrogen peroxide, as well as singlet oxygen by a redox reaction (Witztum, 1994). Hydrogen peroxide’s ability to react is feeble. Still, with an improved oxidation power, it is converted into hydroxyl-radical. H_2O_2 in the presence of myeloperoxidase (neutrophil-derived enzyme). It is further converted to hypochlorous acid having reactive potential. H_2O_2 has a unique ability to transduce biological tissues that transforms it into a classical signalling molecule. (DAutreaux and Toledo, 2007)

Hydroxyl radical (OH^-)

The most reactive free radical hydrogen peroxide in vivo is formed in the presence of Fe_2+ or Cu^+ (catalyst) by the reaction of O_2^- with H_2O_2. (Fenton reaction) (Genestra, 2007) Haber-Weiss cycle result which involves the reduction by superoxide anions of ferric ions (Fe_3^+) into ferric ions, accompanied by Fenton reaction. Owing to its low half-life and the ability to oxidise the close vicinity to almost every molecule, including DNA,
phospholipids and proteins, Hydroxyl Radical is the most volatile ROS Ayala et al. (2014). The oxidation of these molecules contributes to the aggregation of a natural lipid peroxidation products like eight-oxoguanine (8-oxoG) and other oxidised nucleic bases, malondialdehyde (MDA), and 4-hydroxynonenale (HNE) and protein damages manifest by a protein carbonyl content. (Mayne, 2003)

Peroxy radicals

During the peroxidation of lipids, Peroxy radicals are predominantly generated. Though lipid peroxidation has proved useful in specific cellular processes, membrane peroxidation, i.e. Hydroxyl radical can react with polysaturated fatty acid (removes one hydrogen) not only to change the structural integrity and functionality of that fatty acid but also to produce multiple fatty acid radical, which spontaneously react with other lipids, proteins or core acids, thus spreading an electron transmission cascade and oxidating those substances. Cell damage caused by cell membrane lipid peroxidation can impact membrane fluidity, increase permeability and alter the electrical potential, which can contribute to cell lysis (Halliwell and Gutteridge, 1989). These free radicals also denature proteins, both structural and enzymatic (Imlay and Linn, 1988). Toxic oxygen metabolites can also target nuclear acids directly causing simple hydroxylation, cross-linkage and/or breaking of DNA strands which can lead to apoptosis and transformation (Aust et al., 1985).

Lipid peroxidation steps

The lipid oxidation by ROS includes three steps

Initiation step

The hydroxyl radical removes an H-atom forming a carbon-focused lipid radical from a methylene molecule. The fat radical can react with molecular oxygen and create a radical peroxyl.

Propagation step

By removing H-atom from a methylene carbon peroxyl radical is transformed into a lipid hydroperoxide to make a new lipid radicals, thereby causing a propagating sequence of the peroxidation lipid. Furthermore, hydroperoxides may be decayed to hydrocarbons, epoxides, alcohols, aldehydes, and ethanol. Malondialdehyde and the hydroxynonenal of these ingredients are additionally capable of producing cross-linking between these molecules to inactivate phospholipids, proteins and DNA.

Termination step

The contact with the radicals themselves or radicals and antioxidants, which form non-radical or unreac-tive radicals, prevents the chain reaction. The chain is usually completed as lipid radicals associate with vitamin E that forms tocopherol lipid hydroperoxide. (Esterbauer et al., 1990)

Singlet oxygen(1O2)

Singlet oxygen is a highly reactive species with high energy. There is no free radical, so it doesn’t carry any valence electron. Photochemical reactions such as transferring energy due to type II photosensitivity, thermal decomposition of endoperoxides and dioxetans are primarily involved in it (SIES, 1993). When mixed with the hydrogen peroxide, hypochlorous acid forms water which creates singlet oxygen during the reaction. Single oxygen is a highly reactive ROS which induces specific carcinogenic, genotoxic and mutagenic effects by acting on the DNA and polysaturated fatty acids. (Mascio et al., 1994)

Nitric Oxide (NO), Peroxynitrite (ONOO-) (Reactive nitrogen species)

Nitric oxide radical (NO••) in the biological structure is produced by nitric oxide synthase (NOSs) from L-arginine oxidation to citrulline (Pacher et al., 2007). The superoxide radicals and nitric oxide contributing to peroxynitrite are among the most critical reactions in physiological conditions.

\[ NO + O_2^- \rightarrow ONOO^- \]

Peroxynitrite

This reaction is essential in redox control and aids to preserve the equilibrium of ROS and superoxideradicals.

The protonated form of peroxynitrite (ONOOH) is a robust oxidising agent that may result in sulphydryl (SH) depletion and oxidation of several molecules which causes damage similar to OH. (Kohen and Nyska, 2002)

Antioxidants

Halliwell and Gutteridge (1989) have identified antioxidants to be substances that are capable of competing with other oxidisable substrates at fairly low concentrations and further slowing and significantly inhibiting their oxidation.

Their creation and removal (redox state) are stable. Cells guard against the toxicity of excess ROS / RNS in different forms, enzyme and non-enzyme antioxidants to preserve an oxido / redox equilibria.

Enzymatic antioxidants (Endogenous)

Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPX), Glutathione reductase (GRX)
Non-enzymatic antioxidants

1. Metabolic antioxidants-Lipoid acid, Glutathione, L-arginine, uric acid, bilirubin.
2. Nutrient antioxidants (exogenous)- Vitamine E, Vitamine C (ascorbic acid), Carotenoids, Trace elements (Se, Cu, Zn, Mn)

Mode of action of antioxidants

Preventive antioxidants

They work by joining and sequestering oxidation boosters and metals like iron and copper that include unpaired electrons and speed up free radical creation. An example involves haptoglobin (binding haemoglobin), hemopexin (binding heme), transferrin and lactoferrin (binding ferric ions), ceruloplasmin (binding Cu, catalysing ferrous ions oxidation to ferric because of its ferroxidase activity and increasing iron to transferrin bindings), and albumin, (binding copper and heme) (Cui et al., 2004)

Enzyme antioxidants

The endogenous enzymes are produced and degraded to less hazardous goods by specific ROS. Examples include glutathione (GSH), catalase (CAT), and superoxide dismutase (SOD). SODs transform the superoxide radicals to hydrogen peroxide, which is not an actual free radical but a forerunner of the very reactive radical hydroxyl. The primary intracellular enzyme antioxidants are SODs, catalase and GHS.

Scavenging or chain-breaking antioxidants

Chain breakers antioxidants work by oxidising in the free radical chain reaction and producing low-energy materials that are unable to proceed to spread the chain.

In cellular conditions, β-carotene, vitamin E and coenzyme Q (CoQ) as lipid-soluble scavengers (Murthy, 2001) and ascorbic acid, uric acid and bilirubin as a water-soluble scavenger; function as chain breakers.

Important antioxidants

Enzyme antioxidants

Superoxide Dismutase(SOD)

Superoxide dismutase destroys the free radical superoxide by converting it into peroxide that is further marred by the GHPX reaction catalase. The superoxide radicalturn into the less-reactive H₂O₂ by SOD (Whittaker and Whittaker, 1998).

Three forms of SODs are, Cytosolic-Copper-zinc superoxide dismutase (Cu, Zn-SOD) (SOD-I), (active site is made up of a copper and a zinc atom bridged by the common ligand) Mn-SOD (SOD-II), and extra-cellular SOD (EC-SOD).

In general, SOD catalyses the dismutation of O₂⁻ by successive oxidation and reductions in a ping-pong-type process at an active site of a transition metal, with fast reaction speeds. (Hsieh et al., 1998)

Catalase(CAT)

Is a hem-enzyme of tetrameric comprised of 4 similar tetrahedral subunits. Catalase reacts through H₂O₂ to form a nonpoisonous substance, i.e., water and oxygen. It is hugely structured that H₂O₂ at any concentration is hard to saturate. (Lledias et al., 1998)

Glutathione system

A primary protection against H₂O₂ and other peroxides is the glutathione mechanism (glutathione, glutathione reductase, glutathione peroxidase, and glutathione transferase). Glutathione is a tripeptide of both reduced and dimeric forms of L-gamma-glutamyl-L-cysteinyllglycine. The selenoprotein Glutathione peroxidase (GPx) enzyme removes H₂O₂ using H₂O₂ to oxidised glutathione (GSH) into oxidised Glutathione (GSSG). The flavoprotein enzyme, glutathione reductase regenerates GSH from GSSG with NADPH as a reduced power source. GPx also reduces lipid or non lipid hydroperoxides in addition to hydrogen peroxide during glutathione-oxidation (GSH). (Young and Woodside, 2001)

Non-enzyme antioxidants

Nutrient antioxidants

Vitamin E

Vitamin E is a fat-soluble vitamin, commonly known as a lipid-soluble antioxidant breaking chain for its biological function. Vitamin E is an eight-male stereoisomer chiral compound: α, β, γ, δ tocotrienol and α, β, γ, δ tocopherol. Most bioactive type in humans is α-tocopherol alone. α-tocopherol protects cell membranes by shielding them from lipid peroxidation and free radicals since it is fat-soluble. It functions by shielding LDL-Cholesterol against antioxidants. Vitamin C reduces LDL susceptibility to oxidation by reprocessing vitamin E and phenolic antioxidants in lipoprotein particles (Hannigan, 1994). Low consumption of vitamin E, C, A and beta carotene is related to inadequate immune responses and raise cancer risk. Vitamin E’s dietary inflences include edible oils, sprout butter, whole grain, nuts, cereals, berries, milk, meat, poultry and beef. Stor-
be taken when using the long-term vitamin E supplementation with high dose.

**Vitamin C**

Vitamin C is a water-soluble vitamin, also called ascorbic acid (Asch-). Conclusively, superoxide, peroxide, hypochlorite, hydroxyl radical, peroxyl radicals, and O2 have been shown to scavenge with vitamin C. It gives a free hydrogen atom to free radical. It neutrally converts it into an ascorbic radical, which is very stable. Ascorbic acid can also shield membranes from peroxidation by increasing the activity of the primary lipid-soluble antioxidant, tocopherol. Acid fruit, green vegetables, tomatoes are natural sources of vitamin C. Ascorbic acid is liable to be destroyed during the cooking (Naidu, 2003).

**β-carotene and vitamin A**

The fats-soluble carotenoid member, Betacarotenesare called as provitamins as they are converted into active vitamin A (retinol). β-carotene is a potent antioxidant and is the best quencher of singlet oxygen (Fukuzawa et al., 1998). The development and activity of T-cells, B-lymphocytes and natural killer cells modulated by vitamin A. So acts as an antioxidant and an immune stimulant. Beta-carotene ha/ been found in many fruits, nuts, fats, and vegetables (carrots, green seeds, beans, spinach). Carotenoid lycopene (in tomato) has an antioxidant and anti-proliferative effect.

**Flavonoids**

Polyphenolic compounds, which are found in most plants, are flavonoids. About 4000 flavonoids were known as flavanones, flavonols, flavones, proanthocyanidins, catechins, anthocyaninsand isoflavones and categorised through them. Efficient antioxidant behaviours are found in flavones and catechins[green tea] (Miller, 1996). A variety of chronic and degenerative diseases such as cancer, memory loss, asthma, stroke, cataract, heart disease, ageing, Alzheimer’s disease, inflammation, infection have been prevented by them or delayed the disorders. Soybean, broccoli, grapes (red wine), berries, cocoa, ginkgo Biloba, green tea, curcumin,apple, onion, etc. are the primary available source of flavonoids.

**Metals**

Zinc a metallic divalent cation bound to proteins in cell and cell membranes. Zinc present in many zinc metallo enzymes in the biological system. Zinc maintains the integrity of biological membranes by stabilising thiol groups and phospholipids and protects against oxidative injury.

Zinc finger which acts as transcript factors to interact with DNA and regulate gene activity (Sergio, 1988). Selenium constitutes a functioning site of many antioxidant enzymes, including GPx. Se, has antioxidant, anti-cancerous and immunomodulatory safety effects at low consumption (Pham-Huy et al., 2001). This mineral is present in vegetable products (garlic, cereals, seeds, soybean), seafood, meat, liver and yeast.

**Transitional metal-binding proteins**

Ceruloplasmin for copper and metal-binding protein ferritin for iron serves as the vital component of the antioxidant protection mechanism by the sequestration of iron and coppers to prevent the creation of hydroxyl radical.

**Oxidative stress**

If free radicals and oxidants are overproduced, they induce a phenomenon known as oxidative stress, a mechanism harmful to cell membranes as well as other materials such as deoxyribonucleic acid (DNA), lipoproteins, lipids, and proteins to be adversely affected. The formation and neutralisation of ROS / RNS are imbalanced. For instance, excess hydroxyl radical and peroxynitrites can destroy the lipoproteins and cell membranes using a process known as lipid peroxidation, which is an extreme chain reaction. This reaction contributes to the production of cytotoxic and mutagenic malondialdehyde (MDA) and conjugated diene compounds. Proteins may be weakened as well, causing changes in composition and lack of enzyme function. DNA disruption induces mutations (Pham-Huy and He, 2008). If not adequately controlled, oxidative stress can lead to a host of chronic, degenerative diseases rheumatoid arthritis Pancreatitis atherosclerosis, inflammatory diseases, neurological diseases and diabetes-the cumulative effects of ROS on various biological macromolecules responsible for ageing.

**Human Immunodeficiency Virus (HIV) and Oxidative Stress**

HIV-infected humans are under chronic oxidative stress. Owing to this, the use of specific signalling mechanisms and viral protein, including Gp120, Tat, Nef and Vpr, and reverse transcriptase, the HIV infection creates oxidative stress. It triggers mitochondrial dysfunction both in laboratory models and clinical environments (Ivanov et al., 2016a). HIV infection also activates the immune system to develop more reactive oxygen species (ROS) by activated monocytes. Increased development of proinflammatory cytokines and chemokines, as a hallmark of HIV immunopathogenesis, can also be synonymous with excessive oxidative stress. Among cell cultures originating from HIV-infected persons,
a significant increase of ROS levels has also been observed. The increased ROS production and intake of antioxidants are correlated with HIV infection. So many records indicate a decline in catalase (CAT), antioxidant enzymes, glutathione oxidase (GPx) and superoxide dismutase (SOD). Depletion of circulatory antioxidant and vitamins have also been observed in HIV patients with decreasing rates of antioxidants linked to lower CD4 counts. (Gwarzo and Muhammad, 2010)

Consequences of increased oxidative stress in HIV patients

Immune activation and immune response

Chronic immune stimulation with HIV increases ROS production. The immune system cells are also very susceptible to oxidative stress as they contain elevated amounts of polyunsaturated acyl lipid in their plasma membranes that are vulnerable to peroxidation. Higher levels of reactive oxygen species, which were recorded in HIV-infected individuals, lead to a higher level of 8-Oxoguanine (8-oxoG) and Malondialdehyde (MDA) due to promoting nucleic acid and lipid oxidation (Ivanov et al., 2016b). Peroxidation in the cell membrane of the polyunsaturated acyl chain induces the membrane integrity and changed membrane fluidity, thereby impairing the intracellular signalling and cell function. The resulting oxidative modifications of DNA are caused by gene mutations and deletions which have been involved in mitochondrial dysfunction, ageing and cellular senescence. High levels of stress-induced oxidative metabolites were also found to predict progression and death in HIV patients on an independent basis. (Masia et al., 2016)

Oxidative stress stimulates HIV replication

There is a vicious cycle of activation, oxidative stress and viral replication, through NFkB, leading to change in viral gene expression. (Gendron et al., 2011). Increased oxidative stress increases HIV replication, perhaps via nuclear factor Kappa B (NF-kB) activation. HIV expression can be triggered in vitro via oxidative stress. If NF-kB is complexed to a second regulatory molecule, called IkB, it is inactive in the cytosol. Dissociation of the complex between NF-kB and IkB with a number of the oxidative stimuli and subsequent translocation from NF-kB to the nucleus has been observed. In vitro oxidoreduction can be modulated for the NF-kB-binding function in the nucleus. Activated cells with a range of inflammatory factors, including oxidative stress, cytokines and mitogens, cause phosphorylation and degradation of the IkB-protein and releases active NF-kB heterodimers, that translocate to the nucleus to stimulate virus reactive transcript and to replicate. Some cytokines also promote replication of the virus, including tumour necrosis factor Alpha (TNF-α).

Oxidative Stress, HIV and DNA Damage

Lymphoid-borne malignancies in patients with AIDS are substantially elevated. In lymphocytes which are depleted with catalase and/or GSH, fail to repair. DNA strand breakages are more common in lymphocytes than in other cell types (macrophages) under similar circumstances (Ameisen and Capron, 1991). Since lymphocytes have a more active antioxidant function, they are more sensitive to DNA damage than macrophages. For HIV-affected people, chronic antigenic exposure is potentially valuable because it relates to levels of intracellular antioxidant involvement in the reparation of DNA damage. Another possible factor in the development of malignancies is due to DNA damage with mutagenic effects.

AIDS and Apoptosis

The apoptosis or programmed cell death of a tissue is a natural physiological process, but it is intensified in the course of oxidative stress. CD4+ T cell culture with HIV is related to the cytopathic action of the virus, which is demonstrated by cells ballooning and syncytia formation that contributes to cell death by both infected and non-infected cells. CD4 + T cell death can directly be mediated by HIV replication which disrupts the membrane of CD4 cell indirectly, by non-infected cells being primed into apoptosis, through gene expression (gp120-gp41). The initiation of apoptosis in both infected and non-infested cells is mediated by expression of the viral envelope gp120/gp41 complex in infected cells. Chronically HIV infected cells can serve as effector cells to bring apoptosis in the non-infected CD4+T cells. TNF-α is also a potent apoptosis inducer. (Sandstrom et al., 1994)

Oxidative stress in HIV patients on antiretroviral treatment

The induction of oxidative stress during antiretroviral therapy (ART) is a finding in HIV-1 redox biology. Several studies indicate that nucleoside and non-nucleoside inhibitors and inhibitors of the viral protease cause significant development of ROS in different cell types. In addition to the persistent redox imbalance of HIV-1 infection, several studies reported an increase in oxidant stress, due to a rise in oxidants and a decrease in serum antioxidant rates (Elias et al., 2013). The study carried out in 84 patients infected with HIV in 6 months of ART shown the rise in serum peroxidation, total hydroperoxide, MDA, advanced levels of protein oxidation, and glutathione levels decreased significantly in compari-
son with their rates before therapy and for healthier controls (Gil et al., 2011). This may be due to a rise in GSH utilisation or limited reduction of its oxidised form intracellularly. Experiments performed on cell lines exposed to ART and in laboratory animals showed that increased oxidised metabolite production is caused by mitochondrial dysfunction with ART results from altered mitochondrial DNA replication and inhibited antioxidative phosphorylation. (Day and Lewis, 2004)

CONCLUSION

In this analysis, we've summarised information on oxidants, antioxidants, pathology and the impact of HIV on the protection mechanism for antioxidative parameters like nitric oxide, total antioxidant level and correlate them with CD4 count and viral load which may be helpful during monitoring and giving efficient ART to the HIV patients.

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Conflict of Interest

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REFERENCES


