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Generation of reactive oxygen species and their impact on the health related parameters: A critical review

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Abstract

Reactive oxygen species (ROS) are highly reactive molecules produced during cellular metabolism and due to some environmental factors. These species have ability to induce damage in vital molecules. The body has several antioxidant systems to cope with but imbalance of oxidants and antioxidants creates a condition commonly known as oxidative stress. The excess production of ROS is the main cause of oxidative stress. In last forty years, oxidative stress was considered as major factors for pathological disorders and ageing. In body, ROS have dual role either beneficial or harmful in the biological systems. They either act as beneficial ($NO^{\bullet}$) in the form of signaling or harmful producing the health disorders. Mitochondrion is the major site for ROS production. The other sources include peroxisomes, endoplasmic reticulum, membrane and cytosol. ROS are studying for their pathogenesis of obesity, diabetes, cancer, inflammation, cardiovascular disease, neurodegenerative disorders and aging. In this review, sources, causes and consequences of superoxide radical, singlet oxygen, ozone, hydrogen peroxide, hydroxyl radical, peroxynitrite, peroxyl and alkoyl radicals and reactive nitrogen species are critically analyzed and discussed in details

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Introduction

The oxidative stress may be the disturbance between ROS and ability of antioxidant system to remove them in the biological systems. Jones (2006) defined it as disorder of redox signaling and control. According to Sies (1997) the term oxidative stress means serious imbalance between antioxidant and production of ROS (Datta *et al.*, 2015). ROS increase dramatically during the oxidative stress and causes lipid peroxidation, intact with nucleic acid, lipid and protein and causes loss of membrane integrity, functional changes and mutation. All these factors contribute to health disorder (Kataria *et al.*, 2010) which will be discussed latter in this review. In body it actually has two roles. It is either beneficial or harmful. In moderate concentration, superoxide anion, nitric oxide ($NO^{\cdot}$) and other reactive oxygen species play a critical role in signaling. In higher concentration due to over production of ROS causes the oxidative stress that leads to the pathological conditions including cardiovascular diseases, cancer (Sosa *et al.*, 2013), diabetes mellitus (Yang *et al.*, 2011), inflammation, neurodegenerative diseases and ageing (Oyinloye *et al.*, 2015).

Body has several enzymatic and non-enzymatic system including catalase (CAT), superoxide dismutase (SOD), GSH-Px and glutathione-S-transferase (GST), glutathione peroxidase, vitamin E components and glutathione (Memısoğullari *et al.*, 2003; Shafaq, 2012). Several synthetic and elements such as cerium also mimic the natural antioxidant scavenging the free radicals and ameliorated the oxidative stress (Khan *et al.*, 2015). Therefore, the aim of this review was to critically analyze the available literature for source types of reactive oxygen species and health disorder due to oxidative stress.

General concept about the free radicals and Oxidative stress

Oxygen is the most common source of free radicals in the biological systems. Over production of ROS inhibit the body normal function and cause damage to both cells and tissues (Wickens, 2001). It is basically produced from cellular substance of endogenous organelles include mitochondria, peroxisomes, cytochrome P_{450} and inflammatory cells (Inoue *et al.*, 2003). The exogenous sources are environmental sources including the ionization radiations, ultraviolet rays and pollutants (Klaunig *et al.*, 2010; Krumova and Cosa, 2016).

ROS primarily attacks on the cell membrane because it reacts with fatty acid of membrane and form the lipid peroxide. The accumulation of lipid peroxide leads to the production of maloiadehyde a potential carcinogen agent. This lipid peroxidation cause permanent loss of membrane elasticity and fluidity and leads to cell rupture(Klaunig *et al.*, 2010; Krumova and Cosa, 2016). The 2nd target of free radicals is body protein. The ROS oxidize the cross link between amino acid and permanent loss of enzymes and connective tissue function (Stadtman, 1995; Wang *et al.*, 2014). It also target the protein synthesis due to inhibition of photosystem II (Nishiyama *et al.*, 2011). The DNA is the 3rd major target of ROS in the cell. The ROS breaks the DNA after inaction and also causes cross linkage of the molecule (Chen *et al.*, 2014).

Sources and production of ROS

Endogenous sources

Mitochondria of the cell is the major site for ROS production and both complex I and Complex II found to be established for mitochondrial ROS generation (Dröse and Brandt, 2012). Many enzymes are also responsible for the generation of ROS including xanthine oxidase (Agarwal *et al.*, 2011), NADPH oxidase (Bylund *et al.*, 2010), α-ketoglutarate dehydrogenase complex (Ambrus *et al.*, 2011), dihydrolipoamide dehydrogenase (Zhang *et al.*, 2011; Kareyeva *et al.*, 2012) and d-amino acid oxidases (Fang *et al.*, 2002).

NADPHs oxidase peroxisomes present in the membrane are also the source of free radicals, which consume O_2 and generate H_2O_2 under normal condition. The H_2O_2 in the peroxisome is converted to water with the help of catalase enzyme. Oxidative stress occurs when the damaged peroxisomes unable

to convert it into water and release the H_2O_2 into cytoplasm directly. Under few conditions, H_2O_2 also reacts with $O_2^{\bullet-}$ and form highly oxidizing and toxic compound ($OH^{\bullet}$) in the Fenton and Haber-Weiss reaction (Fransen *et al.*, 2012).

Gastrointestinal tract is another site for free radical synthesis. $O_2^{\bullet-}$ is generated from xanthine oxidase and then it is converted to H_2O_2 in a reaction catalyze by gluthione peroxidase or catalase. H_2O_2 produced by neutrophils is utilized by meloperoxidase produce hypochlorite (OC^{1-}) ions which is very reactive with short reaction time and makes the membrane impermeable (te Velde *et al.*, 2008). The generated ROS in the gastrointestinal tract oxidize the protein, damage the DNA and protein creating diseased condition in colon (Sanders *et al.*, 2004).

Mitochondrial ROS production

The electron transport chain consisted of four complexes. The electrons move to complex I by NADPH and complex II by $FADH_2$. Then the electrons move to complex III and finally to IV depositing to the molecular oxygen and forming H_2O. however in some cases, electron leak prematurely to O_2 before reaching complex IV and formed superoxide instead of water in complex I to III (Muller *et al.*, 2007). It is estimated that about 1 to 2% of all consumed O_2 formed superoxide, and this production is increases independently in aging (Aung-Htut *et al.*, 2013; Breitenbach *et al.*, 2014).

Peroxisomal production

Investigation in the last decades revealed that endoplasmic reticulum and peroxisomes produce more ROS than mitochondria (Fransen *et al.*, 2012). Peroxisomes are filled with variety of enzymes like oxidoreductase/flavoenzymes that are considered involved in hydrogen peroxide. These enzymes are involved in oxidation of fatty acid or D-amino acid catabolism producing the hydrogen peroxide (Fransen *et al.*, 2012). Some studies also found peroxisomes not only involved in the hydrogen peroxide but also superoxide production. The production of superoxide is mainly due to xanthine oxidase during ischemia reperfusion injury (Cantu-Medellin and Kelley, 2013). Beside the production of ROS, peroxisome also generated the RNS by action of hemeprotein nitric oxide synthase that catalase the oxidation of L-arginine to nitric oxide (Stuehr *et al.*, 2001; Luis, 2011).

ROS production in Endoplasmic reticulum

The main contributors in the ROS production are the member of cytochrome P_{450} with the combination of disulfide isomers protein (PDI) and endoplasmic reticulum oxidoreducation EROI-1. In folding process, PDI protein induces disulfide formation in receptor protein. The isomers reduce this process and regenerated it by oxidio-reduction of ERO1. The ERO1 transferred the electrons to molecular oxygen through FAD. However, incomplete transfer led to superoxide production (Bhandary *et al.*, 2012; Benham *et al.*, 2013).

The family of P450 is found mainly in the ER and does the takes of xenobiotic detoxification by increasing solubility. In the process, electrons transferred from NADH to cytochrome P_{450} leading to xenobiotic hydroxylation. Sometimes the transfer of electrons results in the formation of superoxide radicals (Bae *et al.*, 2011).

Membranes and Cytosol production of ROS

Membrane also produces ROS due to activity of NADPH oxidases. Electrons move on form NADPH to FAD, two heme type to finally O_2 forming the superoxide (Rinnerthaler *et al.*, 2012).

In cytosol, ROS produced as byproducts of arachihonic acid metabolism. Cyclooxygenase and Lipoxygenase enzymes used the arachiodic acid as substrate and produce H_2 prostaglandin and leukotriene respectively. Both enzymes have ability to produce superoxide in the presence of NADPH and NADH (Kukreja *et al.*, 1986; Whicher and Evans, 2012). The level of arachiodic acid is generally low but increase in the skin due to skin inflammatory diseases including psoriasis apoptotic dermatitis leading to aging (Ziboh *et al.*, 2000; Whicher and Evans, 2012).

Additionally, iron of the cell and organelles react with oxygen forming ROS in the cytosol. In reaction which is Haber-Weiss reaction, the ferric iron reacts with superoxide forming the ferrous iron. In other reaction, which is Fenton reaction, ferrous reacts with hydrogen peroxide regenerating the ferric iron and very reactive hydroxyl radical ($OH^{\cdot}$) and hydroxide (OH^{-}). Both radicals are harmful (Chemizmu and Fentona, 2009). The skin acts as interface between environment and body the exogenous ROS production (Poljšak and Dahmane, 2012; Chen *et al.*, 2014).

Exogenous sources

Ionization radiations

Ionization radiations are believed to be involved in all the steps of carcinogenesis including initiations, promotion and progression (Little *et al.*, 2008). The damage done by these radiations include apoptosis, gene mutation and cancer (Riley, 1994; Kadhim *et al.*, 2013). The biological effects of ionization radiations are due to ROS which rapidly produced in radiolysis (Tulard *et al.*, 2003).

Nano-materials mediated ROS generation

Nanomaterial fullerenes and metals ions in nature induce oxidative stress (Bonner, 2007; Asghar *et al.*, 2016). The factors of nanoparticles induce oxidative stress are cell inaction, prooxodant functional group on nanoparticles and redox cycling on the surface of nanoparticles (Huang *et al.*, 2010). However, several studies revealed the active role of reactive particle surface in the generation of ROS (Schins, 2002; Khan *et al.*, 2015; Shakeel *et al.*, 2015; Khan *et al.*, 2015b; Asghar *et al.*, 2016). Nanoparticles produces the free radicals when oxidant and free radicals bound to the active surface of particles e.g. SiO and SiO_2 present in the quartz particles generated the OH- and $O_2^{\cdot-}$ species (Knaapen *et al.*, 2004). The other molecules such as nitrogen dioxide and ozone also generated the oxidative stress on surface of nanoparticles and induce oxidative stress (Buzea *et al.*, 2007).

The free radicals are generated on the nanoparticles due to one of following reasons.

a. Free radicals are produce when the nanomaterials are dissolved in the aqueous suspension (Fubini and Hubbard, 2003; Asghar *et al.*, 2015). Upon dissolving nanoparticles release the metals ions and induce the oxidative stress (Knaapen, *et al.*, 2004). For example, quartz particles produce the H_2O_2 and $O_2^{\cdot-}$ in the aqueous suspension.

b. Chemical and metal compounds on the surface of nanoparticles also enhanced the ROS production and oxidative stress (Wilson *et al.*, 2002).

c. Some transition metals like chromium and copper etc. found engaged in generation of ROS in Haber-Weiss and Fenton-type reaction.

d. Some metals nanoparticles such as Co, Ni etc. also activate the intracellular pathways of MARK and NF-αB for inducing the intracellular free radicals (Nel *et al.*, 2006).

e. Nanoparticles also activated the mitochondrial mediated ROS production. As major site, once nanoparticles get enter in to mitochondria, they impaired the electron transport chain, activate the NADPH like enzymes, depolarization of mitochondrial membrane and initiating the ROS production (Xia *et al.*, 2006).

f. Internalization of nanoparticles also imitated the immune response including neutrophils and macrophages contributing to the ROS production (Risom *et al.*, 2005).

g. Small particles having high surface to volume ratio reported to involved in the ROS production due to large number of active sites. The large surface area of surface particles are more exposed to reaction than interior molecules (Nel *et al.*, 2006). All these factors contribute to ROS generation and finally oxidative stress.

h.

Mostly xenobiotic are the exogenous sources of ROS productions and oxidative stress. These sources might

be barbiturates, chlorinated compounds, phorbol esters and metal ions (Hamid *et al.*, 2016). The exogenous sources produced the ROS by metabolism of primary radicals or by activating the endogenous sources. The resultant oxidative stress induces the DNA damage, lipid peroxidation and also modulating the antioxidant system (Klaunig and Kamendulis, 2004; Pu *et al.*, 2006). Some types of ROS discovered some of them discuss in table 1.

Table 1. Reactive species and their source and place of synthesis.

Reactive species	Place or source of synthesis
Hydrogen peroxide	Large number of reactions in the body
Superoxide anion	Mitochondria, cardiovascular system
Nitric oxide	Intestinal sub mucosa and some other cells by nitrogen oxide synthase
Nitrogen dioxide	During atmospheric pollution of dioxide
Peroxyl radicals	During oxidative damage of DNA, protein and sugar etc.
Ozone	In atmosphere pollutants
Peroxynitrite	Reaction of NO and superoxide in the body
Hydroxyl	In Fenton reaction
Alkoxyl	free radical chain reaction

Superoxide radical ($O_2^{\cdot-}$)

This reactive species is produced in the mithchondrial complex I, III and consider part of intracellular signaling (Murphy, 2009). The evidence supported that the superoxide involved in the intracellular signaling cascade by four ways.

Superoxide radical usually generated due to reduction of O_2. It is reactive than O_2 often associated with inflammatory pathway by the activation and regulation of inflamasome and inflammatory cytokines (Goetz and Luch, 2008; Zhou *et al.*, 2011). With long half-life involves in the inactivation of catalase, GPX and oxidation of glutathione in the absence of scavengers. Different studies revealed the role of superoxide in the development of disease state including cancer (Ambrosone *et al.*, 1999), cardiovascular (Collin *et al.*, 2007), inflammation (Afonso *et al.*, 2007) and neurodegenerative diseases (Waris and Ahsan, 2006).

The intracellular enzyme SOD is an antioxidant metaloenzyme and actively involved in scavenging the super oxide radicals. It has different isoforms with different metal ions (Copper, manganese and zinc) at active site (Zelko *et al.*, 2002). In animal models, the SOD protects the brain, heart and liver from ischemic and alcohol induced injury (Wheeler *et al.*, 2001). The mutation in SOD causes degradation of motor neuron and induced paralysis or death, susceptibility to type 2 diabetes, Alzheimer s disease and cancer (Tamai *et al.*, 2006; Wheatley-Price *et al.*, 2008). Over expression of SOD also causes the oxidative stress (Kowald *et al.*, 2006).

Singlet oxygen

It is not a free radical species with ample energy and no unpaired electrons but very reactive than O_2 with 10-5 s half-life. It is generated in the electronic excitation of molecular oxygen, termination of peroxyl radical in peroxidase- mediated reaction (Davies *et al.*, 1999). It was first observed in 1924 and found out the more reactive form of oxygen. The singlet oxygen targets the protein, nucleic acid, sterols and lipids consequence in the skin cancer. B-carotene and ascorbic acid actively involved in the scavenging of the singlet oxygen (Young and Woodside, 2001).

However, some studies also supported the beneficial role of singlet oxygen in the photodynamic therapy of carcinogenic cells. In the process of treatment carcinogenic cells accumulates the light sensitive agent during irradiation that produce the singlet oxygen and ROS in most of the cases. The singlet oxygen and ROS causes the cell death by cytotoxicity

and inducing the apoptosis (Juarranz *et al.*, 2008; Plaetzer *et al.*, 2009). Chemically, several compounds are used for scavenging the singlet oxygen including histidine, azide and 2-phenylisobenzofuran in laboratory (Foote *et al.*, 2012).

Ozone (O_3)

Ozone is acidic smelling, irritating, colorless gas and a form of elemental oxygen. It is also a powerful oxidizing agent than molecular oxygen. Its low concentration (0.5 ppm) causes the lung damage. Its few hours explore initiate inflammation, damage to macrophages of pulmonary tracts and this way decreasing the resistance to infection. It also causes the irritation of eyes and oxidation of lipid and protein. The inhaled O_3 reacts with ascorbates urate and GSH of the body. These compounds are scavengers of O_3 (Halliwell and Gutteridge, 2015).

Hydrogen peroxide (H_2O_2)

Like singlet oxygen, hydrogen peroxide (H_2O_2) is also not a free radical species and showed relatively stable state in most of the studies (Park and Imlay, 2003). However it gained much interest due to its ability of generating the ROS. Hydroxyl radical is its most important ROS. In biological systems, the H_2O_2 is produced in one of the following processes; (a) reduction of the superoxide by SOD generated the H_2O_2 (b) action of amino acid oxidase, glycolate oxidase and urate oxidase on their respective substrates also generates the H_2O_2 (Benov, 2001).

The H_2O_2 also showed the direct effect on intracellular signaling cascades by transduction of signals, genetic mutation of catalase enzyme and up regulation of SOD (mn) and SOD (Cu). Chen *et al.* (2014) suggested the proliferation of endothelial cells after the treatment with H_2O_2. Catalase and Gpx (cellular enzymes) actively involved in scavenging of H_2O_2 and conversion into the H_2O (Young and Woodside, 2001).

Hydroxyl radical (OH^-)

Hydroxyl radical is considered the most toxic species produced by reduction of H_2O_2 (Park *et al.*, 2004). Due to very short half-life (10-9 sec), the hydroxyl radical immediately reacts with biomolecules after the formation. Fenton- type reaction with Iron (II) and zinc (I) and Haber-Weiss reactions are the primary sources of cellular hydroxyl radicals.

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^-OH + HO^-$ (Fenton reaction)

$O_2^{\cdot -} + H_2O_2 \rightarrow {}^{\cdot}OH + O_2 + HO^-$ {Haber- Weiss reaction Haber and Weiss, 1932, (Haber and Weiss, 1934)}

It causes the oxidation of protein, lipid and nucleic acid. Base modification, DNA strand breaks and DNA cross linking was also observed after the treatment with hydroxyl radicals (Bar-Or *et al.*, 2001).

Peroxynitrite ($ONOO^-$)

The generated superoxide and nitric oxide in the cell reacts to form proxynitrite ($ONOO^-$) under the inflammatory condition. The peroxynitrate causes the lipid peroxidation and DNA damage. It is also involved in the ageing process because of damage to guanine in telomeres and decreases the production of collagen (Valko *et al.*, 2006; Afonso *et al.*, 2007). The other complications include the vasorestrication due to low availability of nitric oxide. Selenium in the form of selenomethionine and selenocystine found to have the protective role against single strand breaks in DNA due to $ONOO^-$ radical.

Peroxyl ($RO_2^{\cdot}$) and alkoyl ($RO^{\cdot}$) radicals

Both radicals are good oxidizing agent since they have ability of accepting electrons (Buettner and Jurkiwicz, 1996). Under the biological medium both reactive species undergo molecular rearrangement to form other radicals. $RO^{\cdot}$ found to initiate lipid peroxidation by two pathways including, fatty acid hydroperoxide (LOOH) independent pathway and LOOH dependent pathways (Aikens and Dix, 1991). The carbon centered radical of RO• has the ability to reacts directly with certain biological molecules like DNA and albumin. $RO_2^{\cdot}$ also induce peroxidation of lipids and damage the protein including lysosomes (Bailey *et al.*, 2003).

Metal based generated ROS

Metals ions such as Cu, Co, Mg, Ni, Zn are biological very important due to their contribution in normal

physiological functioning including, electron transport chain, oxygen transport, catalyst and part of various protein. However, metal ions are toxic too, if mis-regulated during homeostasis.

This condition leads to the oxidative stress due to generation of ROS and onset of many diseases like, anemia, hemochromatosis, Wilson s disease Monks diseases, cancer, diabetes, inflammation and neurodegenerative diseases (Beutler, 2007; Brewer, 2007; Jomova and Valko, 2011). Among the metal ions, copper and iron are more focused and their role in the generation of ROS is more extensively study. It is due to the fact both the metals ions are involved in the generation of $OH^{\cdot}$ (Beutler, 2007; Jomova and Valko, 2011). This hydroxyl radical induce the DNA damage and cell death in the fibroblast of mammalian.

Copper is the 3rd most found metal in the human body after zinc and iron (Brandolini *et al*., 2002). The copper also generated the hydroxyl radical during the Fenton-type reaction but 50 times faster than iron (Bar-Or *et al*., 2001; Shen and Anastasio, 2012). The copper concentration is closely maintained to avoid the toxic level (Que *et al*., 2008). However elevated level is associated with oxidative stress and related disease (Mishra *et al*., 2008; Que *et al*., 2008) including Alzheimer' s disease (Zappasodi *et al*., 2008), cancer (Gupte and Mumper, 2007) Wilson' s disease (Ala *et al*., 2007) renal diseases (Mishra *et al*., 2008) and cardiovascular disorders (Leone *et al*., 2006; Shen and Anastasio, 2012).

External environment polluted with some heavy metals are also the source of oxidative stress and toxicity. For example, Khan *et al*. (2015b) critically reviewed the toxicity and oxidative due to silver nanoparticles in fish model. Hamid *et al*. (2016) investigated the level of murrcury and its impact on the antioxidant system of fish.

Cell has inherent ability to cope with metal based oxidative damage. The cell has various metal binding sites in metaloprotein including transferrin, ceruloplasmin, metallothionein and ferritin (Letavayová *et al*., 2006; Kontoghiorghes *et al*., 2008). Some chelating drugs such as, deferiprone, ferrior amino B are used for iron and N. scetyl cysteine amide, tetrathiomolybdale and penicillamine for copper to minize the toxic effects (Zheng *et al*., 2008).

Reactive nitrogen species (NOS)

The byproduct of nitrogen oxide synthase form the 2nd group of free radicals mainly expressed in intestinal submucosa ad some selected cells (Ghafourifar and Cadenas, 2005). It has usually long half-life due to rapid diffusion into blood where it inactivated by hemoglobin. The nitric oxide has some vital role in the body including neurotransmission, immune-dilation and blood pressure regulation (Matheson *et al*., 2000). It also prevents the adhesion of leukocytes and toxicity of H_2O_2 to endothelial cells (Binion *et al*., 2000). However, the over production of RNS is responsible to impairment of antioxidative system and contributes the damage to large intestine mucous of membrane (Ya Sklyarov *et al*., 2011). The reaction between $O_2^{\cdot-}$ and $NO^{\cdot}$ also leads to the formation of aggressive oxidizing agent the peroxynitrte ($ONOO^-$) that causes the fragmentation of DNA and lipid peroxidation.

Impacts of ROS and RNS

Lipid peroxidation

Both RNS and ROS are responsible for lipid peroxidation particularly in the membrane. As the membrane is consisted of polyunsaturated lipids and lipoprotein, the membrane is primary target of lipid peroxidation; a hydroperoxy group is attached or introduced into unsaturated fatty acid with hydrophobic tail and causes the alterations. These structural alterations disturb the hydrophobic lipid-lipid interaction and create the hydroperoxy radicals and aldehydes derivatives. The ultimate end product of lipid peroxidation (Malondialdehyde) causes damage to protein by reacting with histidine imidazole group, lysine amino group and sulphydryl group of cysteine (Catalá, 2009). Severe lipid peroxidation was seen in the patients with inflammatory bowel disease depending upon the type

(Kruidenier *et al.*, 2003; Asghar *et al.*, 2016).

Aging

Aging research has targeted on a central finding that dates back to the year 1956. During year, Denham Harman projected that reactive oxygen species (ROS) accumulate over time and are a main contributor to the aging process (Harman, 1955). This idea was broadened sixteen years later by Harman himself identifying mitochondria because the main source of ROS, forming the basis for the mitochondrial radical theory of aging (Harman, 1972). In the last decade, serious doubts arose that ROS are indeed the foremost vital elements that are fueling aging (Hekimi *et al.*, 2011). Intrinsic aging is delineated as results of genetic factors and corporal changes that occur/appear throughout the conventional aging process, whereas extrinsic aging focuses on aging process accelerated by environmental influences (Farage *et al.*, 2008). It had been proposed that solely 3 % of all aging factors have a genetic background (Poljšak and Dahmane, 2012).The deterioration of metabolic processes and normal physiological functions cause aging. According to the free radical theory, the ROS is generated as byproduct of biological oxidation which induces damage to macromolecules ultimately dis-functioning and cell death(Harman, 1955).

Being the major site of intracellular superoxide production and major target of free radicals, mitochondria are closely associated with aging process. Mitochondrial ROS causes damage to mitochondrial constituents including mitochondrial DNA, protein and lipids (Park and Imlay, 2003; Belhadj Slimen *et al.*, 2014). The oxidant induces mutation in the mitochondrial DNA defer rationed mitochondrial normal bioenergetics function leading to the aging. The damages in mitochondrial DNA increase with age which leads to DNA break and somatic mutation. These mutation cause the impairment of respiratory chain complex and increasing the mutation and oxidative damage, energy supply and normal cellular function alters leading to the apoptosis (Judge and Leeuwenburgh, 2007).

Effect on DNA

Several chemical reactions that involved the oxygen generated the reactive intermediate that damages the DNA. This damage causes mutations leading to the cancer. The researchers are trying to explore the role of reactive intermediate in the carcinogenesis (Gupta *et al.*, 2013).

Hydroxyl radical produced during Fenton reaction causes oxidation of nuclear DNA. The reaction of ROS with free radicals also leads to deleterious effect on DNA and produced mutagenesis. Most familiar DNA alteration induced by oxidative stress is 8-oxo-2´ deoxyguanosine which pair both adenine and cytosine forming the GC and TA transition (Kaiser *et al.*, 2004). This mutation was seen in the skin especially in aging (Sauvaigo *et al.*, 2010). Along with modification in DNA base, ROS also produce double and single DNA breaks (Caldecott, 2008).

Effect on protein

Oxidative stress induces reversible and irreversible oxidative modifications in the protein. Irreversible modification including tyrosine nitration and carbonylation are associated with oxidative stress and used as biomarkers in diseases and aging (Prokai *et al.*, 2007; Rao and Møller, 2011). Reversible modification includes cysteine modification (Cai and Yan, 2003). It reflects the change in cellular redox state and involved in the singling cascades (Finkel, 2011; Chung *et al.*, 2013).

Cancer

Hydroxyl radical has ability to react with guanosine of nucleotide chain and form 8-oxo-2´ deoxyguanosine and high frequencies of this dimer were observed in tumor (Kuppusamy and Zweier, 1989; Ishikawa *et al.*, 2008; Kumar *et al.*, 2008). Basal cell cancer, melanoma and squamous cell cancer were the most frequent cancer types in skin and due to mutation in tumor suppressor gene P_{53} (Brash *et al.*, 1996). Transversion of G to T is the most prominent mutation in P_{53} gene and due to oxidation of guanine

(Waris and Ahsan, 2006).

Mutation in P_{53} increases the risk of cancer due to cell inability of apoptosis. Various studies revealed ROS not only involve in the inducing of tumor but also in its progression. The tumor produces significant amount of H_2O_2 that promote the tumor progression (Waris and Ahsan, 2006). ROS also promotes the release of calcium from locally endoplasmic reticulum store which activates the protein kinase C (PKC). The PKC was found to involve in the cell apoptosis, cell migration, proliferation and reorganization of cytoskeleton (Klaunig *et al*., 2010). Alteration in the signaling pathway is the most common reason for cancer genesis (de Gruijl *et al*., 2001). Most of studies revealed the generation of ROS in the melanomas. As melanocyte transformations the melanomas tend to disorganized and promote the ROS. This ROS activate the proto-oncogene pathways (Fruehauf and Trapp, 2008).

Neurodegenerative diseases

Oxidative damage to neurons is the most common reason for most of neurodegenerative diseases. The oxidative stress is responsible for death or dysfunction of neural cell leading to pathogenesis.

Acrolein, 4-hydroxy-2,3-nonenal (HNE), F2-isoprostanes and malondialdehyde (MDA) are the most important products of lipid peroxidation. Elevated level of HNE is the reason for Alzheimer s disease (Selley *et al*., 2002). The oxidative stress modifies the DNA base pairing by hydroxylation which increases the level of 8-hydroxyguanine and 8 hydroxy 2 deoxyguanine initiating the AD (Gabbita *et al*., 1998; Nakabeppu, 2014). In AD the activity of catalase, glutathione peroxide, superoxide dismutase and glutathione reductase increase and use as biomarker of oxidative stress related diseases (Pappolla *et al*., 1992; Zhao and Zhao, 2013).

Heart Disease

Several lines of evidence demonstrate that oxidative stress plays a very important role in the pathological process and development of cardiovascular diseases, as well as hypertension, atherosclerosis, dyslipidemia, angina pectoris, myocardial infraction, and heart failure (Linke *et al*., 2005; Little *et al*., 2008; Csányi and Miller, 2014). Many studies conjointly support the role of OS in disease pathological process of coronary heart disease**.** Paradoxically, though moderate exercise poses an acute oxidant stress, regular endurance exercise is related to improved cardiovascular operate and a reduction in traditional CHD risk factors. These new findings are consistent with the hypothesis that adaptations elicited by acute exposures to exercise-induced oxidative stress result in long-term vascular protection. This happens through activation of signaling pathways that result in accumulated synthesis of intracellular antioxidants and antioxidant enzymes and shrunken ROS production during exercise (Pandey and Rizvi, 2009).

In case of atherosclerosis, several evidences supported the free radicals role in the development of pathogenesis (Steinberg, 1997). In this hypothesis, low density lipoprotein (LDL) that are the main circulating part leave the antioxidant replete plasma, enter to sub-endothelial space of arteries and got oxidized there. The oxidized LDL initiates the process of formation of atherosclerotic lesions. The microphages take these oxidized LDL and release the other factors that stimulate the proliferation of smooth muscles. These oxidized LDL also facilitates cellular adhesion and binding of leukocytes, speed up the plaque formation and causes the stroke and heart attacks (Steinberg, 1997; Singh *et al*., 2015). Moreover the LDL also showed involvement in the blockage of nutritional antioxidant system (Tribble and Committee, 1999).

Stroke is the pathological condition and occurs due to the cell death because of oxidative stress in the condition of ischemia (Alexandrova *et al*., 2004; Bretón and Rodríguez, 2012). There are two types of strokes including ischemic stroke and hemorrhagic stroke. Several evidences support the role and generation of ROS in both conditions leading to oxidative stress. The oxidative stress interrupts the normal flow of blood flow and metabolic pathways which is the leading cause of ischemic strokes

(Piantadosi and Zhang, 1996). In the hemorrhagic strokes, the blood borne cells (neutrophils, macrophages/monocytes) accumulates and increases the oxidative stress and eventually causes the disease.

Effect on visceral obesity

Obesity showed close relationship with metabolic syndrome. It is now recognized that adipose tissue being metabolically active play a critical role in the regulation of homeostasis of energy and pathological effects in obesity related diseases. The role of white adipose tissue medaling inflammation in cardiovascular and diabetes disease is the hot topic of current investigation. Infiltrated adipose tissue by bone morrow produced the macrophage that secrete adipose and cytokinase in the systematic circulation result chronic inflammation (Wellen and Hotamisligil, 2005; Lugogo *et al.*, 2011). Obese persons have higher level of oxidative stress marker and these markers increase with gain of weight (Vincent *et al.*, 2010; Savini *et al.*, 2013). Multiple sources are found associated to obesity including inherent source that increase fat distribution and adiposity. Some other sources are behavioral changes that make a person obese. Increased adipose tissue significantly correlated with increased level of oxidative stress biomarkers (Fujita *et al.*, 2006; Steffes *et al.*, 2006; Bonomini *et al.*, 2015).

Obesity is also associated with several conditions including, insulin resistance, hyper tension, hyperlipidemia and diabetes. Each of these increases the oxidative stress in the obese persons (Shaheen *et al.*, 2007).

Eating balance and rich in antioxidant is necessary to maintained the healthy life and reduce the oxidative stress (Khan *et al.*, 2016). Unfortunately this protection is less effective in obese person having sedentary life style with diet of lower dietary antioxidant and lover vitamin level (Nayak *et al.*, 2000). Obesity increases the chronic oxidative stress that causes the endogen damage found mostly in the cardiovascular system and nonalcoholic hepatic steatosis (Roberts and Sindhu, 2009; Kizhakekuttu and Widlansky, 2010).

Conclusion

Reactive oxygen species and reactive nitrogen species are very reactive species produce during the oxidative metabolisms and due to environmental pollutant sources. These ROS causes oxidative stress in the organisms including the human beings. The oxidative stress causes damage to genetic materials inducing the health disorder and aging. Therefore, it is recommended both natural and synthesis antioxidant should be used to cope with imbalance of oxidatant and antioxidant level.

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