T. R.
VAN YUZUNCU YIL UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCE
DEPARTMENT OF CHEMISTRY

DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN RHEUMATOID ARTHRITIS

M. Sc. Thesis

PREPARED BY: Darya Assi YOUNUS
SUPERVISOR: Prof. Dr. Halit DEMİR

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled "**DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN REUMATOID ARTHRITIS**" and prepared by Darya Assi YOUNUS under consultation of Prof. Dr. Halit Demir in Department of Chemistry, on date of 1/7/2019 it has been successful with a unanimous vote by the following jury and it has been recognized as a Master's Thesis, in accordance with Postgraduate Education and training regulation with the relevant provisions.

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This thesis has been approved by the committee of The Institute of Natural and Applied Science on 02../07../2019 with decision number 2019.1.36-I

Signature
Prof. Dr. Suat ŞENSOY
Director of the Institute

THESIS STATEMENT

All information presented in the thesis that ethical behavior and academic rules were obtained in the frame, as well as all kinds of work that does not belong to me in this statement prepared in accordance with the rules of writing theses and reports that I referred to the complete information source.

Signature

Darya Assi YOUNUS

ABSTRACT

DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN RHEUMATOID ARTHRITIS

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It is well known that the oxidative stress and antioxidant status play a crucial role in the Rheumatoid arthritis. To further investigation about the levels of the oxidative stress and the antioxidant parameters, 60 subjects were conducted in this study, 30 patients who are suffering from Rheumatoid arthritis and 30 of them are healthy subjects that they have not any diseases as a control group. The study is carried out in Rheumatology Department of Rizgary Teaching Hospital and CMC private hospital and general hospital Taq Taq in Erbil north of Iraq. Blood samples from the subjects were collected and the sera of both groups were used to determine the malondialdehyde (MDA) level which is the end product of lipid peroxidation and the antioxidant enzyme activities superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). The data show that the mean level of serum (MDA) level of the patients group is significantly higher compared to control group. Whereas the mean level of superoxide dismutase (SOD) activity of patients group is significantly lower than that of healthy control group in rheumatoid ($p<0.05$) (Table 3.1) There are significant differences in the mean levels of serum GSH and CAT of patients group compared to the control groups. This study show that the oxidative status affects very well the cellular damage of the tissue in RA patients.

Keywords: CAT, MDA, Rheumatoid arthritis, SOD.

ÖZET

ROMATOİD ARTRİTLERDE OKSİDATİF STRES SEVİYELERİNİN VE BAZI ANTİOKSİDAN ENZİM FAALİYETLERİNİN BELİRLENMESİ

YOUNUS, Darya Assi
Yüksek Lisans Tezi, Kimya Bölümü
Tez Danışmanı: Prof Dr Halit DEMİR
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Oksidatif stresin ve antioksidan durumunun Romatoid artritte çok önemli bir rol oynadığı iyi bilinmektedir. Oksidatif stres düzeyleri ve antioksidan parametreler hakkında daha fazla araştırma yapmak için, bu çalışmada 60 denek çalışılmış, Romatoid artrit şikayeti olan 30 hasta ve bunlardan 30'u kontrol grubu olarak herhangi bir hastalığı bulunmayan sağlıklı deneklerdir. Bu çalışmada, Kuzey Irak Bölgesi Rizgary Eğitim Hastanesi Romatoloji Bölümü ve CMC özel hastanesinde kan örnekleri toplandı. Hasta ve sağlıklı bireylerden kan örnekleri alındı. Her iki grubun serumları, lipit peroksidasyonunun son ürünü olan malondialdehit (MDA) seviyesi ve antioksidan enzim aktivitelerinden süperoksit dismutaz (SOD), glutatyon (GSH) ve katalaz enzim aktivitesi belirlendi. Veriler, hasta grubundaki ortalama serum (MDA) düzeyinin kontrol grubuna göre anlamlı derecede yüksek olduğunu göstermektedir ($p<0.05$). Hasta grubunun ortalama süperoksit dismutaz (SOD) aktivitesi romatoid artrit grubu sağlıklı kontrol grubundan anlamlı derecede düşük bulundu ($p<0.05$) (Tablo 3.1). Hasta grubunun ortalama serum GSH ve CAT düzeylerinde kontrol grubuna göre anlamlı bir fark bulundu ($p<0.05$). Bu çalışmada, oksidatif stresin, Romatoid artrit (RA) hastalarında dokunun hücresel hasarını çok iyi etkilediğini göstermektedir.

Anahtar kelimeler: CAT, MDA, Rheumatoid arthritis, SOD.

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2019

Darya Assi YOUNUS

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SYMBOLS AND ABBREVIATIONS INDEX

Some symbols and abbreviations used in this study are presented below, along with descriptions.

Symbols	Description
Cu	Cupper
Fe	Iron
Zn	Zinc
O_2	Oxygen
O_3	Ozone
O_2^-	Superoxide
OH^-	Hydroxyl
ºC	Centigrade temperature
min	Minute
g	Gram
h	Hour
L	Liter
Ml	Milliliter
µl	Microliter
mg	Milligram
mM	Mill molar
M	Molar
N	Normal
rpm/min	Round/Minute
µg	Microgram
µm	Micrometer
β	Beta
α	Alfa
kDa	Kilo Dalton
U/L	Unit/liter

Abbreviations	Explanation
RA	Rheumatoid arthritis
Apo	Apolipoprotein
ATP	Adenine triphosphate
CAT	Catalase
DNA	Deoxyribo nucleic acid
RNA	Ribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
HDL	High density lipoprotein
H_2O_2	Hydrogen peroxide
Kcal	kilocalorie
LDL	Low density lipoprotein
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide Phosphate
NO_2	Nitrogen dioxide
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RSS	Reactive sulphur species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
UV	Ultraviolet
GSH	Reduced glutathione
Mn	Manganese
Ni	Nickel
GSSG	Oxidized glutathione
KOH	potassium hydroxide

Abbreviations	Explanation
Na-azide	Sodium azide
Na_2CO_3	Sodium carbonate
BSA	Bovine Serum Albumin
$NH_4 (SO_4)$	Ammonium sulphate
$CuCI_2$	Copper chloride
NaOH	Sodium hydroxide
DTNB	5,5'dithiobis-(2-nitrobenzoic acid)
BHT	Butylhydroxytoluene solution
TBA	Thiobarbituric acid solution
TCA	Trichloro acetic acid solution
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-Sulphonic acid)
TNF	Tumor necrosis factor
TH	T-helper
IL	Interleukin

1. INTRODUCTION

Oxygen is an essential component for any living organism that has evolved to live on oxygen-based physiological systems. The most significant role of oxygen for living organisms is arguably its capability to oxidize other molecules. While oxidation is necessary for various physiological phenomena, it is also a double edged sword as it can also cause cellular damage due to oxidative stress it induces on them (Shinde et al., 2006).

This is closely related to the presence of free radicals in biological systems. A free radical is basically an element with an atom or molecule that has an unpaired electron in its outer orbits. This free electron is harmful to various biological systems as it is capable of "stealing" an electron off of a stable molecule, and causes it to become a free radical as well. This can go on like a chain reaction and when this occurs on healthy tissues the tissue may lose some of its functionality (Valko et al., 2006).

The presence and negative effects of the free radicals were first noticed in the last couple of decades. Together with other toxins present in the body that is formed as a result of normal physiological reactions, these can create stress on the metabolic functionality of the body. This is most important in terms of the energy regulation systems of the body, where the carbohydrates, lipids, and proteins are oxidized through aerobic and anaerobic reactions, both of which can produce free radicals. When the free radicals are produced in the body faster that they can be "neutralized", this creates a loss of radical balance and various tissues of the body can be harmed due to the excessive presence of free radicals. The most susceptible elements are the lipid molecules of cell membranes, where the oxidative damage can break them apart or cause them to lose their flexibility, which may lead to complete cell wall destruction. Such a cell either dies or becomes dysfunctional. Besides the lipid structures in the cell wall, RNA and DNA protein regulating enzymes are also susceptible to the oxidative stress.

Certain agents taken into the body from the environment with various paths are known to lead to free radical production in the body. Some of these are listed below (Langseth, 1996). Lead toxicity, Pesticides, Cadmium toxicity, Alcohol consumption, Ionizing radiation and smoking.

Due to the unpaired electron it contains, the atom, ion, or molecule of the free radical is very reactive. This is due to the fact that the distribution of electrons in the orbits of an atom decides its reaction potential. The configuration of free radicals makes them quite efficient in pairing up with other molecules. When this pairing occurs with a free electron in the body it may have no significant effect on the body, but when it occurs with a hydrogen atom in a molecule, for example, it may prevent a critical step of a physiological reaction. The element with the free radical may bind another molecule or may form harmful compounds with other free radicals (Tiwari, 2004).

There are three primary sources of free radicals that make the majority of all free radicals. These are oxygen, sulfur, and reactive nitrogen species. Reactive oxygen and nitrogen species ROS and RNS are naturally produced in the body as a result of normal physiological reactions. ROS and RNS partake in both beneficial and harmful reactions, and a healthy human is believed to be subject to 10 to 20 thousand free radicals of these types every day (Valko et al., 2006)

While the reactive free radical species are more reactive compared to non-radicals, they are also less stable (Pham-Huy, 2008). In the body, free radicals form when a chemical bond gets broken by redox reactions. A free radical is quite capable of stealing electrons from other non-radicals and turning them into free radicals, initiating a chain reaction (Bahorun et al., 2006; Valko et al., 2006). Below are the examples to ROS and RNS species (Pham-Huy., 2008). Superoxide radical (O_2•), peroxy (RO_2•), hydroperoxyl (HO_2•), alkoxyl (RO•), hydroxyl (OH•), peroxyl (ROO•), nitrogen dioxide (NO_2•), nitric oxide (NO•), lipid peroxyl (LOO•)

There are also non-radical ROS and RNS species, and some of them are (Pham-Huy., 2008). hydrogen peroxide (H_2O_2), singlet oxygen peroxynitrite (ONOO), ozone (O_3), hypochlorous acid (HOCl), nitrous acid (HNO_2), lipid peroxide (LOOH), dinitrogen trioxide (N_2O_3)

There are also reactive sulfur species which can enter into reaction with thiols to form reactive oxygen species (Lu et al., 2010). These are molecules like sulfite and sulfonic acid, and sulfenyl radicals, which contain oxygen in their centers. Various oxidants as such can lead to free radical production even though they are not radicals themselves. Oxygen-derived radicals are considered to be the most important radical species (Miller et al., 1990; Valko et al., 2006).

As mentioned before, certain reactive species can have both beneficial and harmful effects.

Nitric oxide (NO•) at low levels, for example, is essential in preserving the homeostasis, while it becomes toxic at higher concentrations and harms various organic molecules, leading to pathological conditions or mutations. The effects of various oxidants are fought back by antioxidant defense mechanisms, which consist of various molecules that are capable of neutralizing free radicals, reducing the oxidative stress. Superoxide dismutase, for example, reacts with superoxide (O_2•) and transforms it into hydrogen peroxide, while glutathione peroxidase transforms hydrogen peroxide (H_2O_2) into water. Vitamins A, E, and C also are low-molecular-weight defenses that are capable of acting as antioxidants (McCord, 2000).

ROS can be organized in three main categories, namely the superoxide, hydroxyl, and hypochlorite groups (Pevcival, 1998). These are created as part of inter and intracellular enzymatic cycles, namely the endogenous reactions (Inoue et al., 2003). These can cause auto-catalytic reactions as they are byproducts of aerobic cellular reactions. As mentioned before, they can advance their effects through chain reactions, which can occur between all kinds of substances like proteins, enzymes, and membrane lipids. The damage they cause is therefore related to the agent they are interacting with (Pevcival, 1998). Most of the endogenous reactions take part in microsomal peroxisomes, mitochondria electron transportation reactions, and inflammations with cell activation neutrophils and cytochrome p_{450} present (Cadenas, 1989; Inoue et al., 2003). ROS can also be taken in through exogenous paths, however, like various ions, metals, chlorine, radiation (x-rays, UV rays), and xenobiotic (Valko et al., 2006).

A connection between RNS and septic shocks, asthma, and atherosclerosis was drawn. Nitric oxide and nitrogen dioxide are the primary examples of RNS. Nitric oxide is formed by the nitric oxide synthase (NOS) enzyme and is a very reactive free radical species that can alter carbohydrates, fats, proteins, and the cell nucleus. The damage can be to such an extent that the body starts an inflammatory reaction, which may lead to tissue damage and various adhesions. Nitric oxide has other effects like suppressing platelet aggregation and relaxing the arterial and venous muscles (Agavwal et al., 2005).

When thiols and disulfides are oxidized, they create the reactive sulfur species which are essential sulfur in increased oxidation state. RSS like disulfide, thiyl radicals, and sulfonic acid inhibit the thiol proteins and enzymes by rapidly oxidizing them. Various studies have revealed that sulfur may be in oxidation states varying from two to six, which also indicates that the amount of thiols it can potentially reduce (Giles et al., 2002). Sulfite radicals and disulfide-S-oxide are particularly capable of producing higher amounts of secondary oxidation products. Various experiments have shown that sulfite slowly but steadily oxidize fats and sulfhydryl's and that RSS can also be an important factor in lipid oxidation (Robert et al., 2010).

While every cell and tissue in the body has its own antioxidant presence, these are consumed during the neutralization of oxidizing agents, and if they can't be replaced quickly enough under the presence of high amounts of reactive species from exogenous sources combined with the endogenous agents, they can be depleted. In such a case the cells or the tissue resistance to oxidizing agent's drops, and the aforementioned cellular destruction may occur (Çekiç et al., 2013).

The relationship between oxidative stress and aged cells in the body with various diseases were evaluated and proved in numerous studies. Modern lifestyle choices were shown to have reduced the antioxidant production and increased the oxidative stress the society experiences (Jimenez et al., 2016). Oxidative stress, in turn, was shown to have a strong relationship with all types of cancers, various cardiovascular diseases, diabetes mellitus, Alzheimer's disease, autism, kidney failures, liver damage, and aging (Lu et al., 2010).

Rheumatoid arthritis is a chronic autoimmune disease and is present all around the world (Doherty et al., 2006). While T-cells and cytokines are influential in the development of RA, the macrophages that are activated as part of the reaction also take part by creating oxygen radicals as explained above. These inflammatory cells move into the synovium and show their effects primarily inside by releasing certain pro-inflammatory mediators (Edwards et al., 1997; Bellucci et al., 2016). These changes cause synovial hyperplasia, and over extended periods of time, they damage the bones and cartilages under influence permanently.

RA is believed to be prevalent in 0.5 to 1% of the world's population (Senna et al., 2004). Women are shown to be three times more susceptible to RA compared to

men. Similarly, while RA can be seen in all age groups, the ages between 40 and 50 are where the disease mostly occurs. Despite having some insight on how it may be developing, the exact cause of rheumatoid arthritis is still unknown (Haris et al., 1989; Krane et al., 1989). Still numerous studies have proposed that genetic factors triggered by environmental conditions like infections and smoking could be the underlying cause of immune regulation problems and inflammatory systems that partake in joint tissue damage (Ozkan et al., 2007).

The agents that initiate the inflammation in rheumatoid arthritis were clearly defined, and consist of, cytokines, growth factors, chemokine, adhesion molecules and matrix metalloproteinase. Such agents attract and activate the above mentioned cells from the circulation and cause synoviocytes to proliferate and activate. The overall change cause, particularly by proteases, is quite similar to a localized tumor, which can invade and damage articular cartilages, bones, tendons, and ligaments (Sommer et al., 2005). Various studies support this claim and report that ROS are influential in the pathophysiology RA (Ozturk et al., 1999). RA animals were shown to have increased ROS formation and lipid and protein oxidation indicators. Rheumatoid arthritis patients were also shown to have altered oxidative status in their serums, brains, livers, and vascular tissues.

The primary objective of this study was to measure the GSH, CAT, and SOD enzyme levels in RA patients in order to reveal the presence and influence of lipid peroxidation indicators like malondialdehyde MDA, along with various antioxidant enzymes.

2. LITERATURE REVIEW

2.1. Reactive Oxygen Species and Free Radicals

Free oxygen radicals numerous a long times back, an expanding body of the test proven on the inclusion of free oxygen radicals and reactive (O_2) species in different pathophysiological states stimulated assumptions on the function of reactive oxygen species within the etiopathogenesis of different diseases. The substance of this see is based on discovered that ROS easily react with most biological macromolecules causing their degradation and destruction (Dargel et al., 1992). Consequently the term of Free Radical Diseases was introduced for those disorders, where (ROS) were considered to play a causal role in tissue injury resulting in organ dysfunction. This has been suggested for adult respiratory distress syndrome, atherosclerosis, inflammation, rheumatoid arthritis and other autoimmune diseases, degenerative disorders associated with aging, diabetes millets complication, stress induced injuries, processes of mutagenesis and cancerogenesis, post ischemic and post hypoxic damages organ transplantation complications (Halliwell et al., 1992).

Reactive oxygen species are little particles derived from oxygen molecules consist of free oxygen radicals like superoxide anions ($O_2\cdot$), hydroxyl radical ($\cdot OH$), peroxyl ($RO_2\cdot$), and alkoxyl (RO), they are many kind of reactive oxygen species can most impact irreversible oxidative effect to biology molecules, such as proteins, lipids, and DNA inside many cell and tissue because enzymes activity and destroy membrane function (Aliahmat et al., 2012).

Reactive oxygen species could be a collection containing oxygen radical's superoxide, hydroxyl radical, peroxyl and alkoxyl can appear the (Figure 2.1) non-radicals are either oxidizing agents or easily changed inside radicals such as hypochlorous acid (HOCl), ozone (O_3), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), which are called non-radicals. Nitrogen containing oxidants, such as nitric oxide (NO.) peroxynitrite (ONOO.), nitrogen dioxide (NO_2) is called reactive nitrogen species (Bedard et al., 2007).

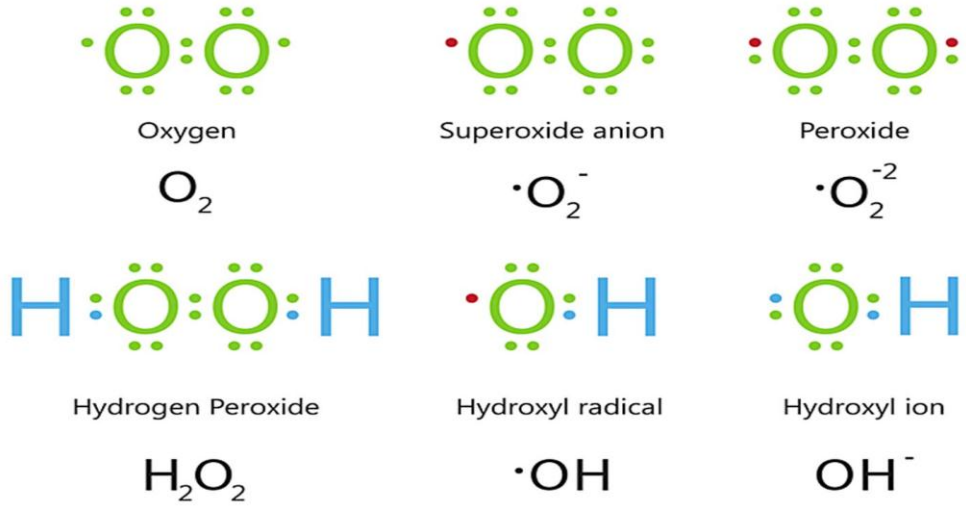

Figure 2.1. Reactive Oxygen Species (· unpaired electron) (Bowen, 2003).

Reactive oxygen species are continuously made inside the cells via a number of metabolic processes. Macromolecules and the function activities, they have numerous functions inside gene expression, cell signaling and transportation of iron (Halliwell, 2001). Organisms have created a sophisticated antioxidant system that keeping them adequately from the hazardous effects of reactive oxygen species.

Many group of reactive oxygen species is observing controlled in vivo by a wide range of spectrum antioxidants consist of both enzymatic molecules .Antioxidant non-enzymatic systems, consist of glutathione systems, bilirubin and numerous vitamins A, C and E also that is refer antioxidant enzymatic systems such as catalase (CAT), malondialdehyde (MDA), superoxide dismutase (SOD) and reducing glutathione (GSH) (Artur et al., 1991).

The high amounts of reactive oxygen species can influence harming of molecules presenting lipid, protein, DNA and RNA, due to they have high chemical reactivity and tiny. Bases pair inside nucleic acids, on amino acid side chain in protein and double bonds in unsaturated fatty acids may be attacked via (ROS) during (.OH). It has a high oxidant of the free radical (Lu et al., 2010). Reactive oxygen species and reactive nitrogen species are involved in a wide range of biological important function from physiological events consist of neurotransmission to pathological situations such as inflammation cancer rheumatoid arthritis, neurodegeneration and cardiovascular diseases. Particularly both kinds of substances play a critical role in the continuously of vascular homeostasis and injury (Förstermann et al., 2008).

In addition concentration of reactive oxygen species may be require for normal cell functioning and ROS takes part within the growth of plants and animals. Enivarmental or behavioral stressors sunlight, exposure, pollution, smoking and increases alcohol most of consumption. Also a tiny many function inside the formation of antioxidant can due to free radicals increases that are known oxidative stress. That the moves redox equilibrates between oxidant and antioxidant is converted to strong oxidative (Çekiç et al., 2013). (Figure2.2) as a rule cell is able to keep the once more ROS impact by the aid of intracellular enzymes. Whereas in the environmental stress and cell abnormality period ROS levels increases and cellular damages occur significantly in the human body (Lu et al., 2010).

RNS/ROS are found inside the atmosphere as pollutant and may be generated, during UV light irradiation, by X-rays and gamma rays, during metal catalyzed reactions, through neutrophils, esinophils and macrophages during inflammatory cell activation, as by-products of mitochondrial catalyzed electron transport reactions, via cytochrome P-$_{450}$ metabolism and the enzyme xanthine oxidase, which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid (Valko, 2007).

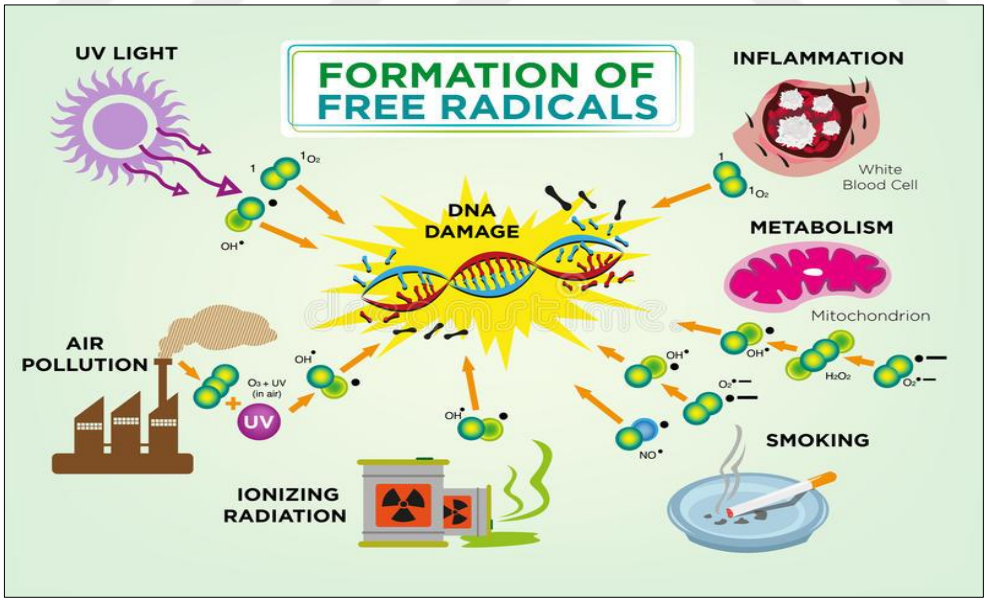

Figure 2.2. Formation of free radicals (Linda Peters et al., 2018).

The maximum vital endogenous sources of oxidizing agents take part in aging are mitochondrial electron transport chain and nitric oxide synthase reaction. Without mitochondrial sources of free radicals are Fenton's reaction, microsomal cytochrome

P-$_{450}$ enzymes, peroxisomal beta-oxidation, and respiratory burst of phagocytic cells (Gilca et al., 2007). In order to protect or reduce the oxidative damage, generated by the ROS, the human body and other organisms have improved an antioxidant protecting system that found enzymatic, metal chelating and free radical scavenging activities to neutralize these radicals after they have formed.

Additionally, consumption of dietary antioxidants can assist to maintain a sufficient antioxidant status in the body, which antioxidant molecules can react at once with the reactive radicals to demolish them. In any other case, they can turn into less active new free radicals, that live longer, less risky than the radicals that had been neutralized by them (Lü et al., 2010).

When ROS increased the reaction occurs with fatty acids to generate the liberation of toxic and reactive aldehyde metabolites, consist of polyunsaturated malondialdehyde that is the end product of lipid peroxidation process. Lipid peroxidation is associated with aging and some kinds of chronic health illnesses, like cancer and atherosclerosis. Most of studies have recommended that antioxidants may prohibit the oxidation of different macromolecules consist of DNA, proteins, and lipids, hence preventing the aging process and extending the lifespan of the organisms (Aliahmat et al., 2012). Reactive oxygen species production and the antioxidant defense activity seem to be more or less balanced in vivo. As already mentioned, actually the balance may be slightly tipped in favor of the reactive oxygen species therefore; there is maintain ROS formation and low-level oxidative damage inside the human body. (Figure 2.3).

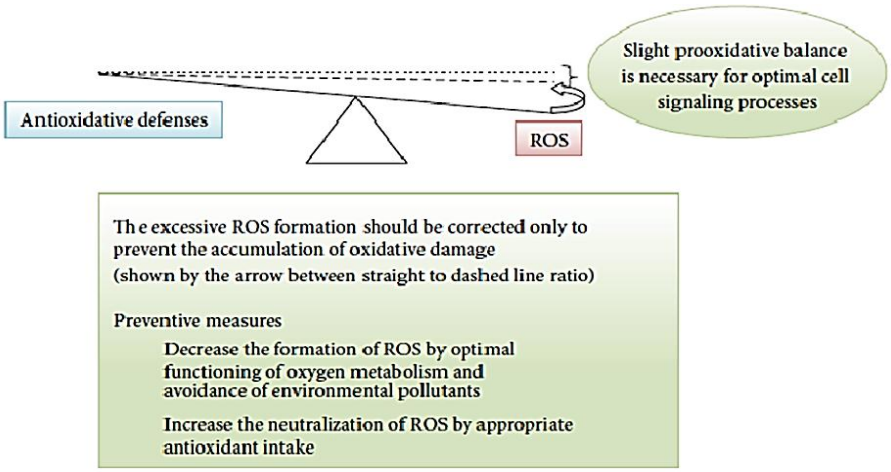

Figure 2.3. Model antioxidative and oxidative balance of an adult person (Poljsak et al., 2003).

The balance is slightly moved closer to the increased ROS production (dashed line). The physiological balance is represented by mean of the dashed line and not the dotted line geometrical balance since slight prooxidative balance is necessary for the optimal immune system and cell signaling processes.

Cellular redox homeostasis is carefully preserved through a detailed endogenous antioxidant protection system, those present endogenous antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione, proteins, and low molecular weight scavengers, like uric acid, coenzyme Q10, and lipoic acid (Halliwell, 2011). It appears that defeats of some or all of the repair systems help more too aging and age-concerned diseases than moderate oscillations in antioxidants and ROS formation (Poljsak et al., 2013). This forms a demand for a second category of endogenous antioxidant defense system in order to eliminate or reform damaged biomolecules before they gather and result in changed cell metabolism and permanent damage (KH et al., 1993)

Nucleic acids damaged oxidative are repaired via many specific enzymes, oxidized proteins are removed by proteolytic systems, and oxidized lipids are repairing via phospholipases, peroxidases, and acyl-transferases (Poljsak et al., 2013).

2.1.1 Types of free radicals

Numerous sorts of free radicals had been identified in human and different mammals consist superoxide molecules, hydroxyl groups, nitric oxide and hydrogen peroxide. Superoxide, the excellent known free radical all of the oxygen derived species is a fundament a part of the method of phagocytosis via leucocytes (Halliwell, 1994). Hydroxyl is the most poisonous of the oxygen based radicals and it reeks inside the cells, particularly macromolecules (Reiter et al., 1995) Hydroxyl radical is brief lived however maximum destructive radical inside the body. Hydrogen peroxide isn't a free radical however fall in the category of creative oxygen agent. Nitric oxide is some other physiological free radical that is made through vascular endothelium as a relaxing factor, and also via phagocytes and inside the brain. It has many essential physiological functions but extra may be toxic (Moncada et al., 1993).

It is known to be concerned in various age associated diseases like hypertension, atherosclerosis and in many different biological effects such as neurotransmission, blood vessel dilatation, signaling, regulation of hair follicle activity and immune response. High nitric oxide may also contribution to the development of oxidative stress at some point of the aging and rheumatoid arthritis (Maurya Pawan et al., 2009).

2.1.2. Superoxide anion

Superoxide anion ($O_2^{\cdot}$) the most common ROS is generated in mitochondria, in cardiovascular system and other parts of the body (Drew, 2002; Salman et al., 2012). The electron transport chain (ETC) is responsible for most of the superoxide generation through partial reduction of oxygen (Bolisetty et al., 2013).

In aerobic organism most of the oxygen is reduced to water in mitochondrial respiratory chain. However, a small proportion of the oxygen molecules 1 to 2 % are converted to superoxide anion radical. These reactions occur in respiratory chain by (NADH: ubiquinone oxidoreductase) and (ubiquinol: cytochrome coxidoreductase) ((Bolisetty et al., 2013). Another important pathway to form superoxide is represented by heme oxidation. The iron of heme group is reduced to ferrous Fe^{2+} in the

deoxyhemoglobin and when it attaches to oxygen an intermediate structure is formed (Buonocore et al., 2010).

Heme Fe + O_2 → O2▪ + Heme Fe

In addition to this, dihydrorotate dehydrogenase, aldehyde oxidase, and xanthine oxidase areoxidative enzymes that can also produce superoxide anion.

2.1.3. Hydrogen peroxide

Hydrogen peroxide also can be generated through a dismutation reaction from superoxide anion by superoxide dismutase. Enzymes such as amino acid oxidase and xanthine oxidase also produce hydrogen peroxide from superoxide anion. Hydrogen peroxide is highly diffusible and crosses the plasma membrane easily. Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. Hydrogen peroxide is a weak oxidizing and reducing agent and is thus regarded as being poorly reactive. Hydrogen peroxide can generate the hydroxyl radical in the presence of metal ions and superoxide anion (Halliwell, 1997).

$\bullet O_2 - + H_2 \rightarrow \bullet OH + OH + O_2$

Hydrogen peroxide can produce singlet oxygen through reaction with superoxide anion or with HOCl or chloramines in living systems. Hydrogen peroxide can degrade certain heme proteins, such as hemoglobin, to release iron ions.

2.1.4. Singlet oxygen

Singlet oxygen ($^1O_2$), it is a non-radical, may be generated with the aid of an input of energy that rearranges the electrons, it is miles alternatively mild and nontoxic for mammalian tissue. It is shaped all through chemical reactions and photosensitization (Salman et al., 2013). Inside the human ($^1O_2$) they are a signal and a weapon, with

therapeutic potency against numerous pathogens which include viruses, microbes and cancer of the cells.

Two one of a kind pathways in biology can produce singlet oxygen. ($^1O_2$) can at once oxidize proteins, DNA and lipids also it had been known to be involved in cholesterol oxidation that may be take part in Dielse-Alder reactions. It is able to be generated by chemical processes, consisting of spontaneous decomposition of hydrogen trioxide in water or the reaction of hydrogen peroxide with hypochlorite.

2.1.5. Hydroxyl radical

The hydroxyl radicals (•OH) it is the impartial shaped of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a totally dangerous radical with a very brief in vivo of the half-life (Pastor et al., 2014). Hence while produced in vivo (•OH) reacts near its site of formation. The redox state of the cell is basically related to an iron like copper redox couple and is maintained within strict physiological limits.

It's been counseled that iron regulation guarantees that there may be no free radicals intracellular iron however, in vivo below stress condition an excess of superoxide releases free iron from iron containing molecules. The release of iron with the aid of superoxide has been confirmed cluster containing enzymes of the dehydratase-lyase family (Liochev et al., 1994). The released Fe^{2+} can take part inside Fenton reaction, generating highly reactive hydroxyl radical.

$$Fe^{3+} + O_2\bullet^- \rightarrow Fe^{2+} + O_2$$

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

2.1.6. Peroxyl and alkoxyl radicals

They are two radicals Peroxyl and alkoxyl are good oxidizing agents, having more than a thousand mV of standard reduction potential. Irradiation of UV light with the found of transition metal ions may cause hemolysis of peroxides to provide peroxyl and alkoxyl radicals.

Aromatic alkoxyl or peroxyl radicals are less than respective open chain radicals due to the delocalization of electrons in the ring. ROO is reactive and formed from lipids, proteins, DNA and carbohydrates all through oxidative damage (Salman et al., 2013). Peroxyl radicals are generated through a direct reaction of oxygen with alkyl radicals (Lee et al., 2004).

2.2. Scavenging of free radicals

Evaluation of the antioxidant activities of natural substances has been of interest in recent years. Antioxidants scavenge free radicals and reactive oxygen species may be extremely significant in inhibiting oxidative stress that due to degenerative most diseases such as Rheumatoid arthritis, heart diseases, cancers, lung diseases (Cardador et al., 2002). Free radicals were implicated as playing a function within etiology of cardiovascular, cancer, Alzheimer's and Parkinson's disease. Antioxidant capability of maximum plant food sources is normally related to their phenolic content. Even through plant polyphenols such as tannins and flavonoids have problems of astringency and protein binding which have grouped them under the category of anti-nutrients (Enujiugha et al., 2003; Enujiugha, 2005).

They have been found useful as natural antioxidants in scavenging deleterious free radicals released in the body by fat metabolism (Enujiugha, 2010) since the well-known synthetic antioxidants; butylated hydroxyanisole and butylated hydroxytoluene are reported to confer some degree of carcinogenicity (Ito et al., 1982). Current research efforts are channeled to-wards exploiting the antioxidant potentials of natural phenolic. Such compounds are found to be abundant in fruits, vegetables, The DPPH method is rapid simple accurate and inexpensive assay for measuring the ability of different compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidant activity of foods and beverages (Prakesh, 2001).

The DPPH method is described as a simple, rapid and convenient method independent of sample polarity for screening of many samples for radical scavenging activity (Marxen et al., 2007). The method DPPH is widely used for measurement of free radical scavenging ability of antioxidants (Perez et al., 2008). For determination of radical scavenging activity of different foods, beverages and substrates were elaborated a great

variety of methods with utilization of DPPH (1,1-Diphenyl-2-picrylhydrazyl). They are based on the original methods of (Brand et al., 1995). The great diversity of methods modifications is evident from its different names. It is known many methods using DPPH for determination of the radical scavenging activity. The most of popular working on the spectrophotometer for determination of every antioxidants.

2.2.1. Superoxide scavenging and ROS

Superoxide anion radical (O_2•-) is generated by four-electron reduction of molecular oxygen into water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain. Superoxide radicals (O_2•-) are also formed by activated phagocytes such as monocytes, macrophages, esinophils and neutrophils and the production of (O_2•-) is an important factor in the killing of bacteria by phagocytes. In living organisms (O_2•-) is removed by the enzymes called superoxide dismutases also can remove superoxide anion radical (O_2•-).

It is the most common free radicals that number of a harmful of many diseases and increases in per-oxidative process and connected of low antioxidant (Packer et al., 1990). Superoxide radicals this type of free radicals is the most powerful than other type of free radicals superoxide anion is product of mitochondrial respiration as well as many other enzymes such as NADPH oxidase exogenous chemical and endogenous metabolic processes in the human body or in food system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage. Oxidative damages play a significantly pathological role in human diseases. Cancer emphysema, cirrhosis, arteriosclerosis, and arthritis have all been correlated with oxidative damage (Halliwell et al., 1985).

Also, excessive generation of reactive oxygen species induced by various stimulating and which exceed the antioxidant capacity of the organism leads to a variety of pathophysiological processes such as inflammation, rheumatoid arthritis, diabetes, genotoxicity and cancer. However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage (Gülçin et al., 2002).

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods. Therefore, antioxidants play a very important role in the food industry. Spices used in different types of food to improve flavors, since ancient times, are well known for their antioxidant properties (Madsen et al., 1995; Gülçin et al., 2002). Synthetic antioxidants, such as butylated hydroxyanisole, butylated hydroxytoluene (BHT), and tertbutylhydroquinone (TBHQ) are widely used in the food industry, but (BHA and BHT) have suspected of being responsible for rheumatoid arthritis, liver damage and carcinogenesis. Therefore, the development and utilization of more effective antioxidants of natural origin are desired (Liu et al., 1997).

2.2.2. Hydroxyl radicals scavenging and ROS

Hydroxyl radical (▪OH) is very reactive, additional toxic than different radical species and might attack biologic molecules consist of proteins, DNA and lipids. (▪OH) is wide believed to be generated from the Fe^{2+} or Cu metal. (H_2O_2) Fenton reaction system, by merely incubating ($FeSO_4$ and H_2O_2) in solution. Hence, (▪OH) scavenging activity of antioxidant may be performed via direct scavenge or prevent of ▪OH formation via the chelation of free metallic ions or changing (H_2O_2) to different harmless compound.

The scavenging ability of antioxidants can be determined via Gutteridge technique that is monitored within the Fe^{3+} EDTA and H_2O_2 deoxyribose system the extent of deoxyribose degradation via the hydroxyl radicals shaped can be measured without delay with the in the aqueous phase via thiobarbituric acid reactive species (TBARS) assay at 532 nm (Figure 2.4). This method is basic on the fact that the degradation of deoxyribose via hydroxyl radical shaped reactive oxygen species within malondialdihyde, which forms adduct with thiobarbituric acid. Adduct, MDA-TBA, has an absorption at 532 nm that can be assayed spectrophotometric (Lü et al., 2010).

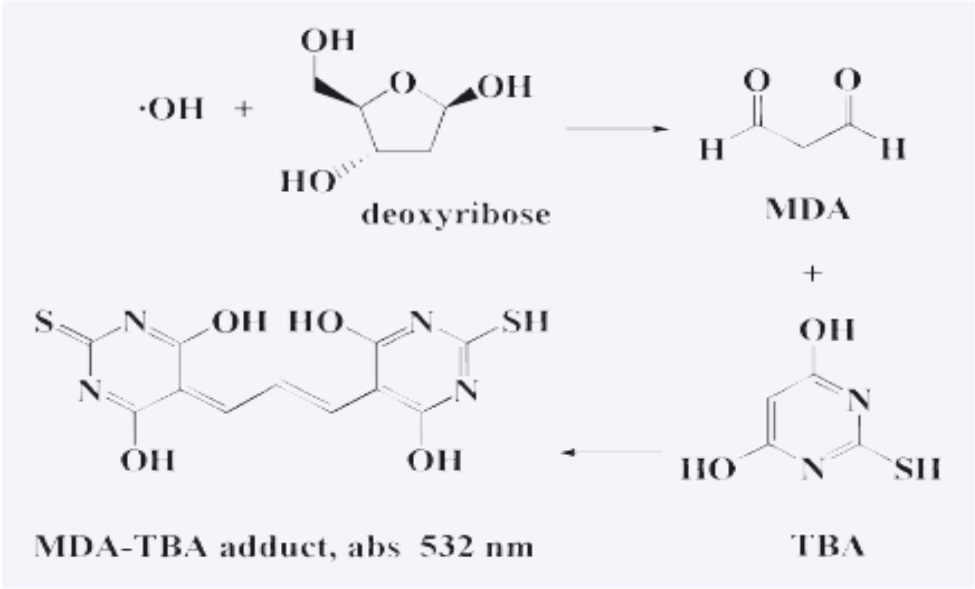

Figure 2.4. Reaction pathway of TBARS assay of ·OH (Lü et al., 2010).

2.2.3. Sources of ROS generation

The amount of free radical production is determined by the balance of many factors, and ROS are produced both endogenously and exogenously. The endogenous sources of ROS include mitochondria, cytochrome P-$_{450}$ metabolism, peroxisomes, and inflammatory cell activation (Chance et al., 1997). In general, ROS can be generated during UV light irradiation and by X-rays and gamma rays. Produced during metal catalyzed reactions, present in the atmosphere as pollutants, produced by neutrophils, esinophils, and macrophages during inflammation, by-products of mitochondrial catalyzed electron transport reactions and various other mechanisms (Kunwar, 2011; Shinde et al., 2012).

There are numerous cellular systems that can produce ROS. The major source of ROS production in the cell is the mitochondrial respiratory chain that utilizes produced in the body.

Another major source of ROS, especially in the liver, is a group of enzymes called the cytochrome P-$_{450}$ mixed function oxidases. There are many variants of these iron-containing enzymes, some of which are responsible for removing or detoxifying a variety of compounds present in our environment and ingested foods or drugs including alcohol (Proulx et al., 1995).

Some cytochrome P-$_{450}$ enzymes also are important for metabolizing substances that naturally occur in the body, such as fatty acids, cholesterol, steroids, or bile acids (Cederbaum, 2001). The cytochrome P-$_{450}$ molecules in their biochemical reactions catalyzed use O_2 and during these reactions small amounts of ROS are generated. The extent of reactive oxygen species generated varies considerably depending on the compound to be degraded and on the cytochrome P-$_{450}$ molecule involved. One type of cytochrome molecule that is especially active in producing ROS is known as CYP2E1, whose activity increases after heavy alcohol exposure (Lieber, 1997).

ROS also are produced by a variety of oxidative enzymes present in cells, such as xanthine oxidase. Xanthine oxidase under normal physiological conditions acts as a dehydrogenase, wherein it removes hydrogen from xanthine or hypoxanthine and attaches it to NAD, thereby generating NADH.

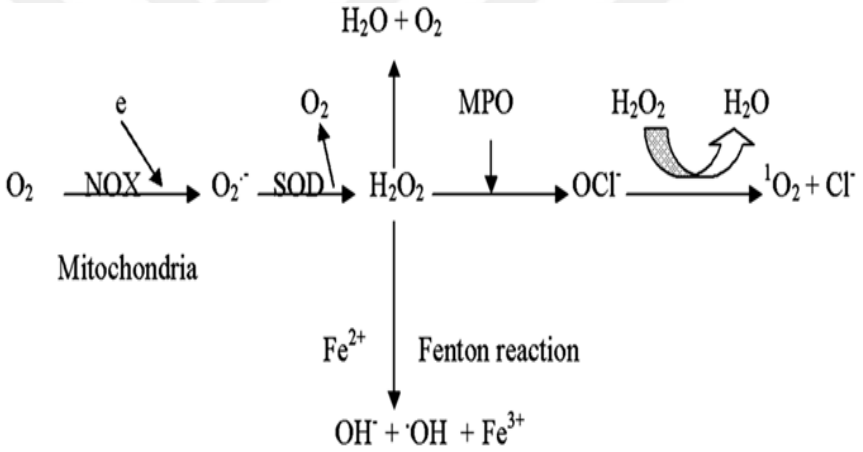

Figure 2.5. Generation of ROS in neutrophils (Rani et al., 2015).

The generation of superoxide ($O_2^{\cdot}$) from oxygen (O_2) is mediated either by the NADPH oxidase complex (NOX) or in mitochondria by cytochrome c peroxidase or xanthine oxidase. Superoxide will be converted to hydrogen peroxide (H_2O_2) either spontaneously or mediated by superoxide dismutase. Hydrogen peroxide can be converted to $H_2O + O_2$ in the presence of catalase and glutathione (GSH) peroxidase, or hydrogen peroxide can act as a source of hydroxyl radical (•OH) via the Fenton reaction. Myeloperoxidase uses hydrogen peroxide as substrate for the formation of halogenated ROS such as hypochlorite (OCl-). Reaction of hypochlorite with hydrogen peroxide results in the formation of singlet oxygen ($^1O_2$).

However, under certain conditions, such as the disruption of blood flow to a tissue, xanthine dehydrogenase is converted to a ROS producing oxidase form (Sultatos, 1988). Other sources of ROS in the body are two types of immune cells called macrophages and neutrophils, which defend the body against invading microorganisms (Figure 2.5).

Reactive oxygen species production here is beneficial and even essential to the organism as it functions to destroy foreign pathogens (Rosen et al., 1995). Macrophages and neutrophils contain a group of enzymes called the NADPH oxidase complex, which, upon activation generates superoxide radicals and hydrogen peroxide. Hydrogen peroxide then interacts with chloride ions present in the cells to produce hypochlorite, which in turn destroys the pathogen. The NADPH oxidase complex and the resulting ROS production are critical to the body's defense against all kinds of diseases, as is evident in patients with a condition called chronic granulomatous disease, in which ROS production by the NADPH oxidase complex is drastically reduced. Patients with this condition are highly sensitive to infections and usually die at an early age (Kohchi et al., 2009). Another peroxidase enzyme that is abundantly expressed in neutrophils is myeloperoxidase (Figure 2.5). MPO in the presence of heme as a cofactor produces hypochlorous acid (HOCl) from hydrogen peroxide and chloride anion with the equivalent from a non-chlorine halide (Klebanoff, 2005).

It also oxidizes tyrosine to tyrosine radical in the presence of hydrogen peroxide. Both HOCl and tyrosine radical are cytotoxic and are used by the neutrophil to kill pathogenic organisms (Heinecke et al., 1993). Humans are constantly exposed to environmental free radicals, including ROS, in the form of radiation, UV light, smog, tobacco smoke, and certain compounds called as redox-cycling agents, which include some pesticides as well as certain medications used for cancer treatment. The toxicity of these medications against tumor cells as well as normal body cells results because of their modification by cellular enzymes to an unstable intermediate that then reacts with molecular oxygen to produce the original product plus a superoxide radical. Thus, vicious cycle of chemical reactions involving these compounds continually produces ROS (Cederbaum, 2001).

1.2.4. Physiological functions of reactive oxygen species

Reactive oxygen species are recognized to play a twin function in biological systems, since they may be either dangerous or useful to living systems (Valko et al., 2006). These species are maintained at low, but measurable, concentrations inside the cells, via a balance between their production of rate and their rates of removal with the aid of antioxidants. As a consequence, every cell is characterized by a particular concentration of electrons redox state stored in many cellular constituents, and the redox state of a cell and its oscillation determines cellular function temporary shift of the intracellular redox state towards more oxidizing conditions results in a temporary imbalance that represents the physiological basis for redox regulation (Schafer et al., 2001). large number of physiological functions are controlled through redox-responsive signaling pathways (Droge, 2002).

Numerous evidences suggest that ROS take part in the defense against intrusion of foreign bodies (Babior et al., 2003) Activated neutrophils and macrophages producing big quantities of ROS through the phagocytic isoform of NADPH oxidase, with the purpose to kill the pathogens. This massive production of ROS at the same point an inflammatory occur is called "oxidative burst" also plays a significant role as the first line of defense in opposition to environmental pathogens (Nordberg et al., 2001; Valko et al., 2006). At a smaller scale, some form of non-phagocytic cells, like fibroblasts, vascular smooth muscle cells, cardiac myocytes and endothelial cells are also are recognized to provide ROS through NADPH oxides to regulate intracellular signaling cascades. For that reason, ROS play an important function in the regulation of cardiac and vascular cell functioning (Griendling et al., 2000).

Nitric oxide plays several regulatory functions. In fact, its own production by NOS, the only isoform of NOS that is not constitutively expressed, is regulated at the transcriptional and post-transcriptional levels by signaling pathway involving redox-dependent transcription factor NF-κ B or mitogen-activated protein kinases. In addition, (•NO), in combination with hydrogen peroxide leads to the activation of the enzyme soluble guanylate cyclase, which catalyses the formation of cyclic guanosine monophosphate that, by its turn, is used as an intracellular amplifier and second messenger in a variety of physiological responses, such as modulation of protein

kinases, ion channels, smooth muscle tone and inhibition of platelet adhesion (Droge., 2002; Martinez-Ruiz et al., 2004).

In higher organisms, oxygen homeostasis is maintained by a tight regulation of the red blood cell mass and respiratory ventilation. It has been proposed that changes in oxygen concentration are sensed independently by several different ROS-producing proteins, including a type cytochrome. Other studies also suggested that a change in the rate of mitochondrial ROS may play a role in this oxygen sensing by the carotid bodies, which are sensory organs that detect alterations in arterial blood oxygen. Other responses to changes in oxygen pressure include the regulated production of certain hormones like erythropoietin controlled by the transcription hypoxia inducible factor-1(HIF-1) (Semenza et al., 2000).

ROS also seem to be involved in cell adhesion, a mechanism that plays an important role in embryogenesis, cell growth, differentiation, wound repair, among other processes. The expression of cell adhesion molecules is stimulated by bacteria lipopolysaccharides and by various cytokines such as TNF, interleukin-1a, and interleukin-1b. The adherence of leukocytes to endothelial cells is also induced by ROS. Moreover, the oxidant-induced adherence of neutrophils is inhibited by hydroxyl radical scavengers or iron chelators, suggesting that the induction of adherence may be mediated by hydroxyl radicals generated from hydrogen peroxide within the cell (Droge, 2002).

Reactive oxygen and nitrogen species can directly affect the conformation and/or activities of all sulfhydryl-containing molecules, such as proteins or glutathione (GSH), by oxidation of their thiol moiety. This type of redox regulation affects many proteins important for signal transduction and carcinogenesis, such as protein kinase C, Ca^{2+}ATPase, collagenase and tyrosine kinases, among many other enzymes and membrane receptors (Dalton et al., 1999). In addition, ROS and RNS are known to trigger apoptotic cell death, by causing (Bcl-$_2$) a protein located in the outer membranes of mitochondria to activate a related protein by its turn leads to the release of cytochrome C from mitochondria (Nunes et al., 2008). This release then results in the activation of several other proteins.

2.2.5. Inhibition of free radical generating enzyme

NADPH oxidases are a place of the plasma membrane common enzymes that moved one electron from the cytosolic donor NADPH to a molecule of extracellular oxygen, this is can produce (O_2•). Xanthine oxidase is an enzyme participate inside the formation of uric acid within the human body, that catalyses the oxidation of hypoxanthine to xanthine and to uric acid yielding O_2• and H_2O_2 also high amount the oxidative stress stage in an organism (Figure.10). Those enzymes are the main resources of the free radicals and in diverse physiological with pathological situation (O_2•) is likewise via product of mitochondrial respiration, additionally several different enzymes such as NADH oxidase, mono oxygenases, and cyclooxygenases. That is biologically pretty toxic and is deployed by means of the immune system to kill invading microorganisms. Inside phagocytes (O_2•) is produced in large quantities by the enzyme NADPH oxidase to be use in oxygen dependent killing mechanisms for invading pathogens. The controlled manufacturing of reactive oxygen derivatives at some of the respiratory burst is need for defense of an organism in opposition to invading microorganisms without causing a great loss of tissue functions. Even though excessive ROS promote oxidative stress which includes low density lipoprotein (LDL) oxidation. Additionally that is enzymes can NADPH oxidase to avoid overproduction of ROS. (Lü et al., 2010).

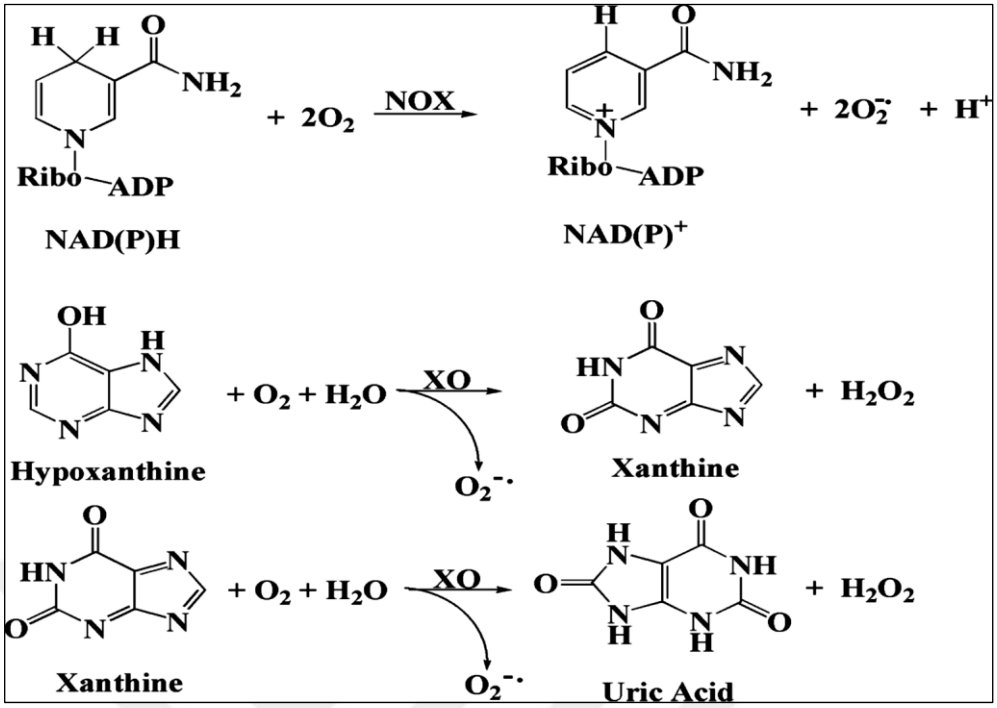

Figure 2.6. Superoxide and hydro peroxide generation which counterproductive from NADPH oxidase (NOX) and XO (Lü et al., 2010).

2.2.6. Determination of ROS

Free radicals which one of the most risky of the cell and tissue in human body many be causes the sicknesses, that is because the short life, free radicals it's far very hard to detection inside the laboratory. Now days we have may additionally to technique to realize for measurement of oxidative stress. Ever manner with their advantages and disadvantages. Most of them approaches are possible. Identification of free radicals either without delay via paramagnetic electron resonance like electron spin resonance. And additionally indirectly through identifying some more stable intermediates assessment of the traces of radical assault on biological molecules by means of high overall performance such as liquid or gas chromatography and colorimetric investigation .The measurement of antioxidant status may be evaluated via like colorimetric, immune, and enzymatic ways. The direct ways of ROS detection methods measure superoxide and H_2O_2 and ·OH. These are very reactive species and their quantitation is difficult. In vivo ESR is relatively mindless and necessary steady-state concentrations of free radicals in the micro molar variety, which limits its use for measuring ROS in patients. ESR may be implemented only via the technique of spin

trapping for in vivo cases. Although it appears that toxicity isn’t a several problem for maximum traps, there are not any effective spin traps to be administered to humans. Indirect methods are used so as to overcome these problems. Indirect strategy usually measure the converted in endogenous antioxidant defense systems or determination of the ROS-induced impact of cellular components. Measuring the harmful due to by ROS in place of direct measuring of ROS seems logical, since it's the dangerous caused by ROS that is significant more than the general amount of generated ROS. Methods had been advanced to detect and quantify oxidative damage to lipid, protein, and DNA. The principle at the back fingerprinting ways is to measure products of damage by ROS, and additionally we can detection on the oxidative stress that due to high amount concentration of the ROS or that is lowering antioxidant (Poljsak et al., 2013).

2.2.7. Function of ROS in cells

ROS plays useful capabilities in our body redox stage must be maintained. This is the mediator of apoptosis, phagocytosis, detoxification equation executioner of precancerous cells and infections. It is beneficially involved in signaling pathways to maintain cellular homeostasis in body. The ROS regulates many metabolic and cellular processes including proliferation, migration, gene expression, immunity and wound healing (Salganik, 2001). Biochemical reactions are involved in the synthesis of prostaglandins, hydroxylation of proline and lysine with oxidation of xanthine and different oxidative processes (Spooner, 2011).

2.3. Antioxidant

Antioxidants are believed to play a totally important role within the body defense system in opposition ROS (Vivek et al., 2006; Boxin et al., 2002). In some other term antioxidant is any substance that when present at low concentrations compared with that of an oxidizable substrate, substantially delays or inhibits oxidation of that substrate (Halliwell et al., 2007). That an antioxidant is any substance that delays prevents or removes oxidative harm to a goal molecule. The damage to DNA, lipid, or protein is usually a outcome of the action free radicals antioxidants are an inhibitor of

the method of oxidation, even at relatively small concentration and hence have various physiological function within the body.

Antioxidant constituents of the plant material act as radical scavenger and helps in converting the radicals to much less reactive species. An expansion of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea. This evaluation presents some statistics about the antioxidant their role in our body and additionally their presence in herbs and spices (Nema et al., 2009).

Antioxidants are our first line of defense in opposition free radical damage, and are vital for continually the best health and well-being. Regular consumption of anti-oxidant vegetables and fruits has been recognized as lowering the risk of chronic diseases (Dembinsk et al., 2008). Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have numerous physiological roles inside the body. Antioxidant constituents of the plant material act as radical scavengers and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is discovered in dietary sources like vegetables, fruits and tea.

This assessment gives some information about the antiradicals with antioxidant and their function in our body and additionally their presence in spices and herbs (Hall, 2001). Oxygen is surely essential for the life of aerobic organism but it may causes toxic if supplied at higher concentrations. Dioxygen in its ground state is pretty unreactive its partial reduction gives rise to active oxygen species such as superoxide radical anion, hydrogen peroxide and singlet oxygen. This is partly due to the oxidative stress that is basically the adverse effect of oxidant on physiological function. Free oxygen radicals performs cardinal role within the etiology of numerous diseases like atherosclerosis, cancer with rheumatoid arthritis.

The oxidative damage to DNA may additionally play critical function in aging and the presence of intracellular oxygen also can be responsible to initiate a chain of inadvertent reaction at the cellular level and these reaction cause damage to critical cell bio-molecules. Those radicals are incredibly poisonous and for that reason generates oxidative stress inside human body these the free radicals are deactivated by antioxidant system consist of each non-enzymatic and enzymatic antioxidant. The non-enzymatic system such as vitamin C tocopherol, carotenes and ascorbic acid. And enzymatic

system includes superoxide dismutase, catalase, glutathione reductase, and malondialdehyde. The function of this antioxidant system is to scavenge the toxic radicals throughout oxidative stress the intake of antioxidant compounds present in food is an essential health protecting factor. It is regarded that compounds belonging to several classes of phytochemical components which include flavonoids, phenols, and carotenoids are capable to scavenge free radical such as O_2 and OH or lipid peroxyl radical LOO inside plasma. The effective consumption of single food antioxidants and their destiny within the human body have been described best for some compounds (Devi et al., 1999; Ruberto et al., 2000). It is reasonable that the better the antioxidant content material in meals. Herbal antioxidants occur in all elements of the plants.

These antioxidants encompass carotenoids, vitamins, phenols, flavonoids, dietary glutathione, and endogenous metabolites. Plant-derived antioxidants were shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists. The maximum contemporary research on antioxidant action specializes in phenolic compounds inclusive of flavonoids. Fruits and vegetables contain different antioxidant compounds, such as vitamin-C, vitamin E and carotenoids, whose activities had been established in current years. Flavonoids, tannins and different phenolic ingredient found in food of plant beginning are also capability antioxidants (Smith et al., 2000; Sas et al., 2007).

Antioxidant several the enzymes inclusive tocopherol, buthylhydroxyaniso, butyl hydroxyl toluene to the superoxide dismutase, catalase, glutathione reductase and malondialdehyde deoxyribonucleic acid reducing ROS. Radical oxygen species antioxidants slow down the process of degradation so that the energetic action of the environment can lead to higher sustainability. They interact with free radicals, making variable their reaction with oxygen. Antioxidants can be grouped into two classes– synthesis antioxidants and natural antioxidants. The difference between the two categories is that maximum synthesis antioxidants generate material that increases most cancer or different illness (Giacalone et al., 2011).

Classification of antioxidants may be accomplished relying on their role or nature. Depending on their function, there are. Primary antioxidants antioxidant proper ascorbic acid and its derivatives, tocopherols, the esters of gallic acid, erythorbic acid and its sodium salt, BHA, BHT and other substances THBP and TBHQ. Secondary

antioxidants substances with antioxidant action but that have other capabilities as good Sulphur dioxide and sulphites in addition lecithin are secondary types of antioxidants.

Antioxidants slow down the process of degradation so that the energy action of the environment can lead to better sustainability they interact with FR, making possible their reaction with oxygen.

After the antioxidant is exhausted in those reactions, the manner of self-oxidation starts again, at excessive velocity. To prolong the period of action of the antioxidants, we will use reducing agents R-SH or C-SH, able to regenerating the antioxidant by releasing a hydrogen The using two AA will have a good effect due to their synergism, their optimal ratio being established for each case apart. AA is efficient in a single domain of concentration at higher concentrations; it may have even a pro-oxidative impact. Any other possible mechanism through which antioxidants act is oxygen capture, which now not gets to oxidase the lipid substrate (Takabe et al., 2010). A third possible mechanism of action of antioxidants is the inhibition of some oxidative stress like polyphenol oxidase or lipoxygenase, which avoid oxidation reactions catalyzed via those enzymes.

2.3.1. Antioxidant defense system

The oxidation technique is vital for survival and aerobic cell of the life, however the increases produced level of reactive oxygen species is harmful, and therefore this must should be balanced. Therefore, the human body has the protection in opposition to risky ROS impact. That is known as the antioxidant defense system for the rheumatoid arthritis, which may be divided into two enzymatic and non-enzymatic (Table 1.1). Even though the enzymatic system such as SOD, CAT, GP_X, and GR_X that at once and indirect helps to protect against the ROS. In fact, the non-enzymatic antioxidants are the scavengers of (ROS, RNS), including vitamin (E and C) and glutathione that is avoid oxidation of membrane lipid, and also uric acid is the scavenger of proxy-nitrite (ONOO-) in serum, plasma, albumin, and melatonin which straight reacts with ROS and form disulfides (Buettner, 1993).

Antioxidants protection system work by numerous mechanisms characterizes that consisting. Preventive free radicals, scavengers, sequestration of elements by means

of chelation, and quenching, which antioxidants can alter active oxygen species to more stable forms, which include carotenoids and α-tocopherols steady singlet oxygen ($^1O_2$) radical, forming less reactive hydrogen peroxide (McDowell et al., 2007).In addition antioxidants system performs roles in the prevention and counterbalances the impact of oxidants are the primary leads to cellular damage oxidant are the main mechanism for plenty diseases including rheumatoid arthritis.

Table 1.1. Some of enzymatic and non-enzymatic

Enzymatic antioxidants	Non-enzymatic antioxidants
A. Superoxide dismutase (SOD) B. Catalase (CAT) C. Glutathione peroxidase (GP_X) D. Glutathione-S-transferase (GST) E. Glutathione reductase (GSH)	A. vitamins - α- tocopherol - ascorbic acid - carotenoids B. Glutathione C. Trace elements . selenium, . copper, . manganese, and zinc, D. coenzyme Q, dietary polyphenols E. other, uric acid, flavonoid, melatonin

2.3.2. Synthetic antioxidant

Antioxidant synthesis are chemically synthesized given that they do not now arise in nature and are brought to food as preservatives to assist avoid lipid oxidation (Shahidi et al., 1992). Those antioxidants fall into many classes categories depending on their mode of action number one antioxidants and Second antioxidants. The number one antioxidants which avoid the formation of free radicals for the duration of oxidation.

2.3.3. Endogenous antioxidants

Similarly to dietary antioxidants, the body improves on numerous endogenous defense mechanisms to help hold closer to free radical-triggered cell impact. The antioxidant enzymes inclusive catalase, glutathione peroxidase and superoxide

dismutase metabolize oxidative poisonous intermediates and require micronutrient cofactor which includes iron, zinc, copper, selenium with manganese for optimum catalytic activity. It's been cautioned that an inadequate nutritional intake of these trace minerals can also compromise the effectiveness of these antioxidant protection mechanisms (Duthie et al., 1994; Brown et al., 2007).

Glutathione this is a significant water-soluble antioxidant is synthesized from the amino acids g glutamate, lysine, and cysteine. Glutathione directly quenches ROS like lipid peroxides, and also performs a major function in xenobiotic metabolism. Exposure of the liver to xenobiotic materials induces oxidative reactions through the up regulation of detoxifying enzymes together with cytochrome $P\text{-}_{450}$ blend function oxidase. When a character is exposed to excessive degree of xenobiotic, more glutathione is carried out for conjugation a key step inside the body's detoxing method making it much less to be had to function an antioxidant. Research suggests that glutathione and vitamin C work interactively to quench free radicals and that they have got a sparing impact upon every different (Jocab, 1995). Lipoic acid however every other important antioxidant, classified as a biothiol or thiol is a sulfur containing molecule this is regarded for its involvement within the response that catalyzes the oxidative decarboxylation of alpha-keto acids, inclusive pyruvate and alpha ketoglutarate, within the Krebs cycle. Lipoic acid may also exert its antioxidant impact with be aid of manner of chelating with prooxidant metals. Research similarly suggests that lipoic acid has a frugality effect on one of kind antioxidants (Kagen, 1992).

2.3.4. Exogenous Antioxidants

Exogenous antioxidants can derive from natural assets such as flavonoids, vitamin a few mineral compound also can be synthesis compound like butylhydroxyanisole, butylhydroxytoluene with gallates (Litescu et al., 2011). There is a growth interest in antioxidants, especially with the ones supposed to avoid the presumed deleterious results of free radicals inside the human body, in addition to deterioration of fat and different materials of foodstuffs (Molyneux, 2004). Apart from enhancing the performance of antioxidant gene regulation, exogenous antioxidants also exert their results via extra mechanisms of action. In cases which consists tocopherols

and resveratrol, two or three special move may be simultaneously finished to counter the impact of negative redox modulations (Kamal et al., 1996; Alarc´on et al., 2007). Exogenous antioxidant carries out their impact through extra mechanism of action. That Show the mechanism (figure 2.7)

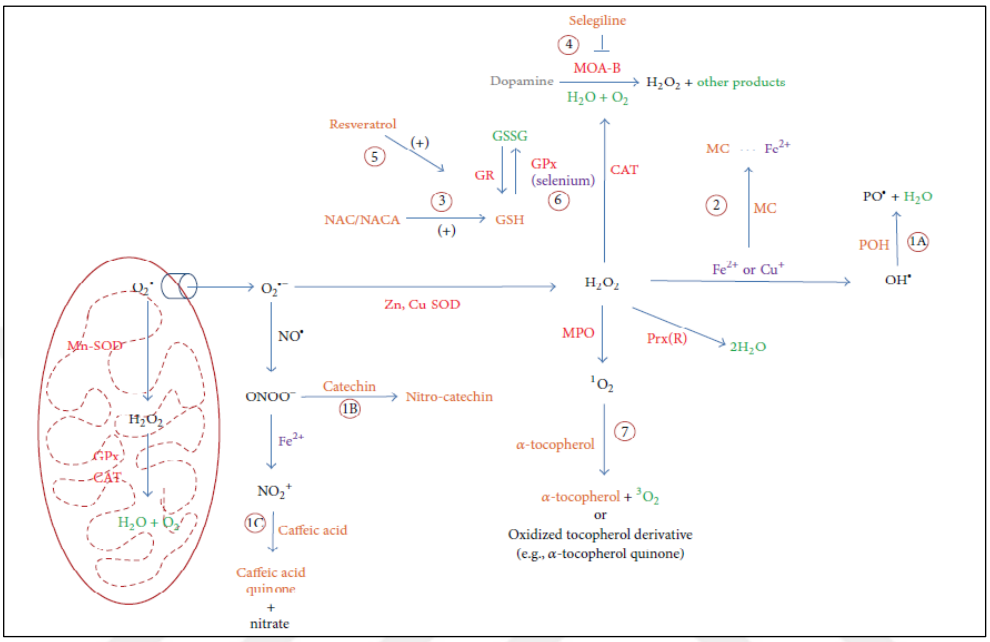

Figure 2.7. Mechanisms of action exogenous antioxidant (Fraunberger et al., 2016).

Red: enzymes; green: other products; purple: cofactor or substrate; black reactive species; CAT, catalase; MC, metal chelators; POH, polyphenol; GSSG, oxidized glutathione; MPO, myeloperoxidase. Reaction legend 1A: H-atom transfer, 1B: electron donation, 1C: direct scavenging, 2: metal chelation, 3: restoration of endogenous antioxidants, 4: inhibition of RS generating species and reactions, 5: support of endogenous antioxidant enzymes, 6: cofactor in antioxidant enzymes, 7: singlet oxygen quenching (Fraunberger et al., 2016).

2.3.5. Enzymatic antioxidant defense system

2.3.5.1. Superoxide dismutase

These enzymes become discovery in 1969 years both the scientist (McCord and Fridowich).Superoxide dismutase (SOD, E. C.1.15.1.1) that is metalloenzymes catalyzes dismutation of the superoxide radical (O_2-) into hydrogen peroxide (H_2O_2) and elemental oxygen (O_2) consequently form a crucial part of the cellular antioxidant protection mechanism (Maier, 2002) and this is one of the most important antioxidant enzymes. Superoxide dismutase is necessary to continue biological homeostasis via numerous functions involving numerous cellular signal transduction pathways.

$$2O_2^{\bullet-} + 2H^+ + SOD \rightarrow H_2O_2 + O_2$$

The stability between oxidants and antioxidants is enough to prevent the disruption of normal physiologic functions. But either will increases in oxidants or decreases in antioxidants can disrupt this stability giving rise to elevated stage of reactive oxygen species .The primary antioxidants systems are characterized by a group of enzymes working both sequential to reducing or elimination (Halliwell, 2007).

The amount of SOD present intracellular and extracellular environments is very significant to avoid of the disease related to oxidative stress (Longfeng et al., 2017) SOD also seems to be important in prevention of other diseases along with rheumatoid arthritis, Alzheimer's, Parkinson's disease. The response catalyzed via SOD is extremely speed and the found of sufficient amount of the SOD in cells and tissues normal keep the concentration of superoxide anion and it is very low (Cases et al., 2017).

Is one of the maximum important antioxidant enzymes superoxide dismutase enzyme are categories inside three groups the most important antioxidant enzyme superoxide dismutase. Include copper-zinc superoxide dismutase one (Cu/ZnSOD, SOD1). Is positioned within the nucleus, cytoplasm and extracellular SOD and the second one of sort of SOD manganese superoxide dismutase (MnSOD, SOD2) is localized inside the mitochondria. That is synthesized within the cytoplasm and translocate to the inner matrix of mitochondria. Also the third sort of SOD extracellular superoxide dismutase (EC-SOD, SOD3) is the primary extracellular enzyme SOD.

Different compartmentalized antioxidant enzymes such as catalase, which is present in cytoplasm, peroxisomes, with GPx, which may be found in lots of sub-cellular compartments which include the mitochondria and nucleus depending at the family member. As a result the many forms of each of these enzymes reducing oxidative stress in the various parts of the cell. Additionally antioxidant proteins with similar enzymatic activity may have different effects after modulation due to different localizations within cells. In the (figure 2.8) shown all form of superoxide dismutase.

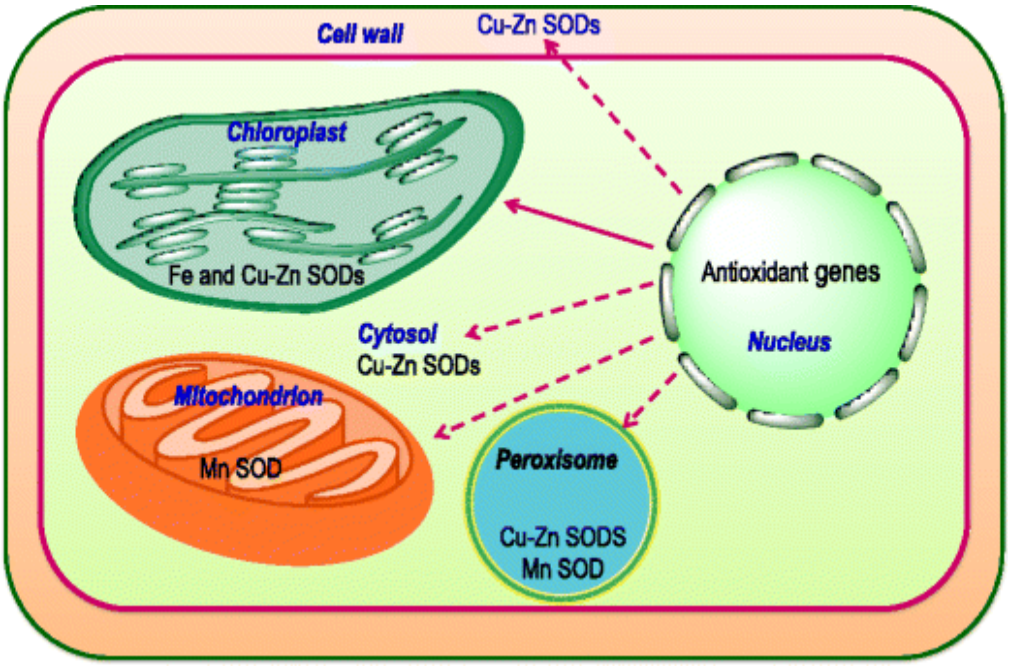

Figure 2.8. Superoxide dismutase three isoenzymes and location (Gill et al., 2015).

Superoxide dismutase (SOD) are the principle scavengers of reactive oxygen species and superoxide radicals and also it has many role important and significant to working antioxidant enzymes and to prevention many diseases like rheumatoid arthritis, heart diseases and other illness. Superoxide radicals are generated at various sites inside the cell, like ER, ETC in mitochondria, as well as various enzymatic sources, like NOX and XO, all of which contributing to the make of reactive oxygen species after ionizing radiation. Superoxide radicals are detoxified by SOD to shape hydrogen peroxide, which is in addition detoxified via (GPx or Prx). GR is used to regeneration of the GSH.

Endoplasmic reticulum, electron transport chain, glutathione peroxidase, glutathione reductase, GSH reduced glutathione, NOS, NADPH oxidase, peroxiredoxin, superoxide dismutase, xanthine Oxidase (Holley et al., 2014) (Figure 2.9).

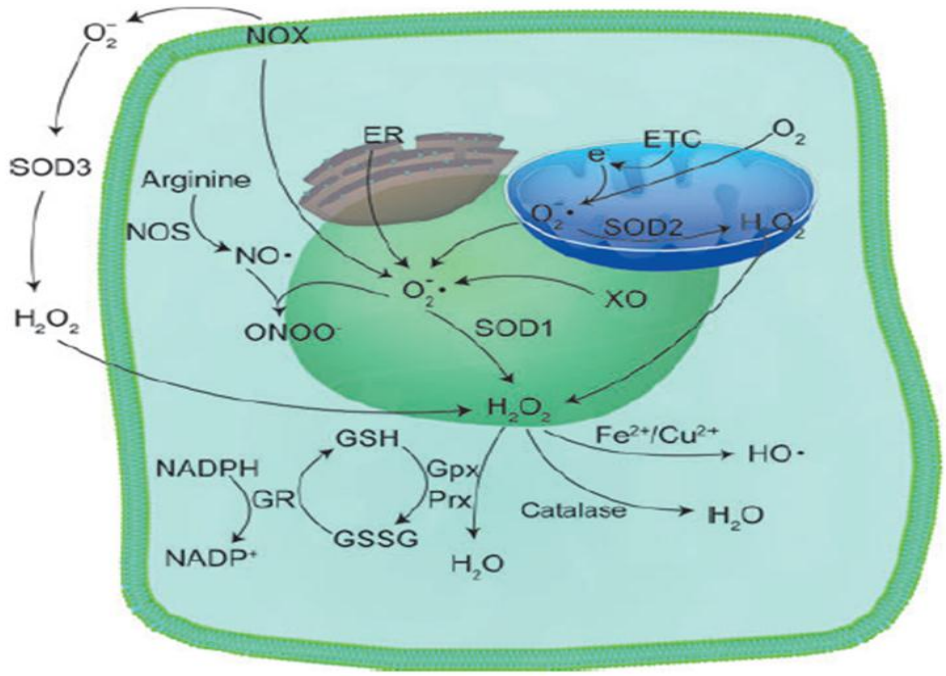

Figure 2.9. Sources and means of detoxification of ROS in the cell (Holley et al., 2014).

2.3.5.2. Glutathione reducing enzymes

Reductase glutathione that is a family of the enzymatic antioxidant glutathione reductase (GR, EC 1.6.4.2) is a ubiquitous enzyme, which catalyzes the reduction of oxidized glutathione (GSSG) to glutathione (GSH). Glutathione reductase is essential for the glutathione redox cycle that maintains adequate levels of reduced cellular GSH. GSH serves as an antioxidant, reacting with free radicals and organic peroxides, in amino acid transport. This enzyme is a family member of flavoprotein disulfide oxidoreductases. GR is that recognize NADPH depended oxidoreductase that alternate GSSG to GSH via using the pentose phosphate reaction pathways.

$$GSSG + NADPH + GR \rightarrow 2GSH + NADP^+$$

The function of glutathione reductase (GSH) that within the chain of the cellular comprise amino acid its operating the transport. Making the nucleic acid and protein the enzymes activity modulation of carcinogen xenobiotic and reactive oxygen species the biochemical function of the flavoprotein.

GSH is an important antioxidant. Hydrogen peroxide, generated as a result of aerobic metabolism, may be metabolized by mean of GSH peroxidase inside the cytosol and mitochondria, and via catalase within the peroxisome. GSSG this is formed is reduced retrace to GSH with the aid of GSSG reductase at the expense of NADPH, thereby forming a redox cycle. Organic peroxides (ROOH) can be reducing by other GSH peroxidase or GSH S-transferase. Beneath a cute oxidative stress, the ability of the cell to reduce GSSG to GSH can be defeat, due to an accumulation of GSSG. To prevent a shift in the redox equilibrium, GSSG can either be actively transported out of the cell or react with a protein sulfhydryl (PSH) to form a mixed disulfide (PSSG).

GR is catalysis of the NADPH-dependent reduction of oxidized glutathione. The glutathione is a tripeptide widely distributed within plant and animal. As a result the enzyme is found in a lot of tissues, allowing the cells to maintain adequate stage of reduced glutathione. Reducing glutathione is a significant cellular antioxidant and likewise a substrate for the glutathione peroxidases, which provide a mechanism for the detoxification of xenobiotic. Oxidative stress has been implicated in ageing and within the pathogenesis of a number of problems. The extent of damage is typically related to an increase or decrease of one or more free radical scavenging enzymes. Excessive range of GR activity had been found in erythrocytes from patients with rheumatoid arthritis.

Reductase glutathione content to responsive the environmental factor many distinction condition are known to change intracellular GSH content excessive glucose concentration, heavy metal, exposure to ROS and RNS specially hydrogen peroxide and nitric oxide.

Mammalian glutathione reductase activity is present in both the cytosol and mitochondria. However recommending that both are encoded by a single nuclear gene. Both the human and the mouse GR genes consist of many exons, and both sequences code for an N-terminal mitochondrial-targeting sequence (Kelner et al., 2000). Due to of the essential role of GSH within removal of deleterious reactive oxygen species and

preservation of the protein thiol redox state, GR is crucial to the cell's antioxidant defense mechanism and maintenance of enzyme activities and protein roles.

Synthesis of GSH happens though a two-step ATP-requiring enzymatic process. Steps one is catalyzed by means of glutamate-cysteine ligase (GCL), which consist of catalytic and modifier subunits (GCLC and GCLM). This is step conjugates cysteine with glutamate, generating γ-glutamylcysteine. The second one step is catalyzed via GSH synthase, which add glycine to γ-glutamylcysteine to shape γ-glutamylcysteinylglycine or GSH. GSH exerts a terrible remarks inhibition on GCL. (Figure 2.10)

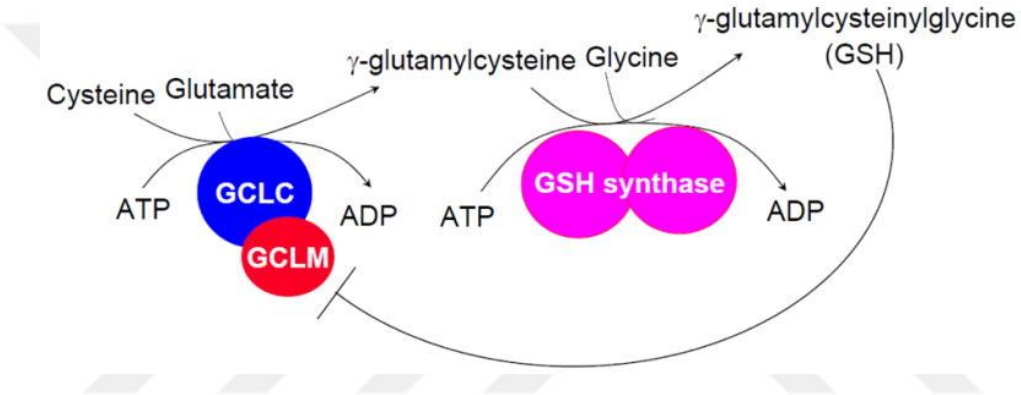

Figure 2.10. Synthesis of glutathione reductase (Lu, 2012).

2.3.5.3. Catalase enzymes

Enzymes are very important molecules located in every cell. Enzymes normally act as catalysts that increase the speed rate at which substances in a cell get transformed into other substances. Without enzymes, some reactions might take place much slowly or might not take location in any respects. Each enzymes has a different job and plenty of enzymes must work together to protection an organism alive and healthful

Catalases are enzymes that catalyze the convert hydrogen peroxide to water and oxygen, using both manganese with iron cofactor. This is present in peroxisomes at most eukaryotic cells. It's just substrate is hydrogen peroxide. Its cofactor is oxidized by one molecule of hydrogen peroxide after which regenerated via transferring the bound oxygen to a second molecule of substrate.

Catalases are ubiquitous enzymes. It is every aerobic organism, whether prokaryotic or eukaryotic have at the least one enzyme with catalytic activity. Catalases are organized into three main groups based on the variety of subunit sizes, different heme prosthetic groups, that is numerous of sequence group. no functional heme-containing enzymes, the catalase-peroxidases, and the non-heme or Mn-containing catalases. And also diverse group of heme-containing proteins, consist of chloroperoxidase, plant peroxidases, myoglobin and catalatic activity. This may be attributable to the found of heme which alone can exhibit a very low catalase activity (Nicholls et al., 2001).

Catalases catalyze decomposition of hydrogen peroxide to water and oxygen and the overall response may be surly represented as in reaction.

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

Catalases shapes three groups observe essentially two stage process for the decomposition of hydrogen peroxide. H_2O_2 reduction accompanied with the aid of H_2O_2 oxidation. There are two distinct stages inside reaction pathway for the heme and non-heme catalases, however the mechanism involved in these two stages differ considerably for classic catalases versus that for non-heme catalases. This section will limit itself to a consideration of heme containing proteins with catalatic activity. In heme catalases, the first level involves oxidation of heme iron using H_2O_2 as substrate a good way to form a ferryl porphyrin cation radical intermediate water (Nicholls et al., 2001). Within to the second stage acts as two electron oxidant, generating oxygen and regenerating the ferric shape the enzyme .The catalase interest is commonly monitored via both one common methods: observing the di-oxygen at the some point of catalysis using an electrode or monitoring the decrease in hydrogen peroxide by lower in absorbance at 240 nm.

In eukaryotes, catalases are present in the peroxisomes, where H_2O_2 is generated via oxidative processes β-oxidation of long chain fatty acids. (Press et al., 1995). (H_2O_2) is able of initiating damage cellular with the aid of direct reaction with sure bimolecular components, but greater often reactive species derived from H_2O_2 include hydroxyl

radical are the true causing agent. For this reason catalases get rid of hydrogen peroxide and avoid the cells from encountering harmful reactive species.

2.3.6. Non enzymatic antioxidant

2.3.6.1. Glutathione

Glutathione (γ-glutamylcysteineylglycine) system could be a kind of non-enzymatic antioxidant which enzymes are a tripeptide comprising of three amino such as glycine, glutamic acid, and cysteine. Glutathione synthesis includes two enzymes that need to ATP. The primary reaction is the condensation within cysteine and glutamate via Y-glutamyl cysteinyl synthetase. The type two glutathione synthetase catalyses the response between (gamma-glutamylcysteine) with glycine to create GSH. The major thiol (-SH) antioxidant is the glutathione a tripeptide .which is considered as the foremost vital intracellular defense against ROS-induced oxidative harm. Glutathione is present inside eukaryotic cells and may be non-enzymatic antioxidant multi-functional intracellular within the body, since of its increases intracellular concentration. It is recognized the major thiol-disulphide redox of the cell. Glutathione could be a major hydrophilic antioxidant and existing within cytosol, nuclei, and mitochondria of the cell (Masella et al., 2005).

The availability of cysteine within the arrangement is ordinary rate-limiting as well. It had been recommended that Glutathione may be a useful capacity for cysteine. Glutathione is easily oxidized under normal condition oxidative stress. Two molecules of oxidized GSH can react with each other forming a glutathione disulphide (GSSG) linked by a disulfide bridge. This sulphur bridge can be broken upon reduction. The reduction of GSSG is catalyzed by glutathione reductase (GR) utilizing (NADPH) to reconvert glutathione disulfide (GSSG) to two molecules of glutathione (Lu, 1998).

As well high a concentration of Glutathione disulfide (GSSG) may harm numerous enzymes oxidative. GSSG can response with protein sulfhydryl groups to produce protein-glutathione-mixed disulfides. Following reaction:

GSSG + protein-SH ↔ protein-SSG + GSH

The blended disulphide (protein-SSG) has a longer half-life than GSSG; most may be due to protein collapsing (Masella et al., 2005). Glutathione has various roles inside the cell, such as scavenge hydroxyl radicals and single oxygen ($^1O_2$), and remove toxic substances like hydrogen peroxide and lipid peroxides. Glutathione is capable of regenerating and protect damage to cellular compounds.

2.3.6.2. Vitamin E

Vitamin E consist of two families of compounds, the refer tocopherols and tocotrienols, their common characteristic inside presence of a 6-chromanol ring and an isoprenoid aspect chain. the contributors of every family are particular for tocopherols with tocotrienols in eight shapes compound antioxidant for each (alpha, beta, gamma, delta) (figure 2.11) that is the alpha-tocopherols has a highest biological activity also vitamin E in fat soluble antioxidant that could improve immune function. Number of important sources of vitamin-E such as tomatoes, sweet potatoes, spinach, Brussels, sprout, blackberries, mangoes, olive oil, sunflower oil, mackerel, and salmon. Also vitamin-E it has a significant role as antioxidant it able to capacity prevent of the chronic diseases. Especially those believed to have an oxidative stress factor consisting of rheumatoid arthritis, cardiovascular diseases, atherosclerosis, and cancer.

Vitamin-E is good antioxidant that assists to keeps cells from damage via free radicals. Free radicals may be damage tissues and organs within the body. A specific role for vitamin-E as functions need for metabolic processes vitamin E principle function seems to be as a non-specific chain-breaking antioxidant that avoids the stage propagation of free-radical reactions. The vitamin-E is a scavenger of peroxyl radical and especially keeps polyunsaturated fatty acids within membrane phospholipids and in plasma lipoproteins. Vitamin-E is good diagnosis for its role in maintaining membrane integrity and preserving from RNS and ROS. Vitamin-E can be interference in signal transduction by modifying specific enzymes such as protein kinase C protein kinase B, protein tyrosine phosphatase, phospholipase A2, cyclooxygenase-2 (COX-2), and the mitogen-activated protein kinase signal transduction pathway. Modulation of these proteins happens through direct binding or by the intervention with enzyme activation and enzyme redox regulation.

Vitamin E deficiency take place rarely in humans and over deficiency symptoms in normal individuals low consuming diets in vitamin-E have never been showed Vitamin E deficiency occurs just as a result of genetic abnormalities in alpha tocopherol transfer protein, as a result of numbers fat malabsorption syndromes, also as a result protein-energy malnutrition. Deficiency can of course result from insufficient dietary intake of the vitamin. Numerous other dietary factors impact the need for vitamin E. Two are most important in this regard selenium and polyunsaturated fatty acids Se spares the need for vitamin E and therefore, adequate intake of vitamin E becomes even more important individuals taking low Se-diets (IOM, 2000).

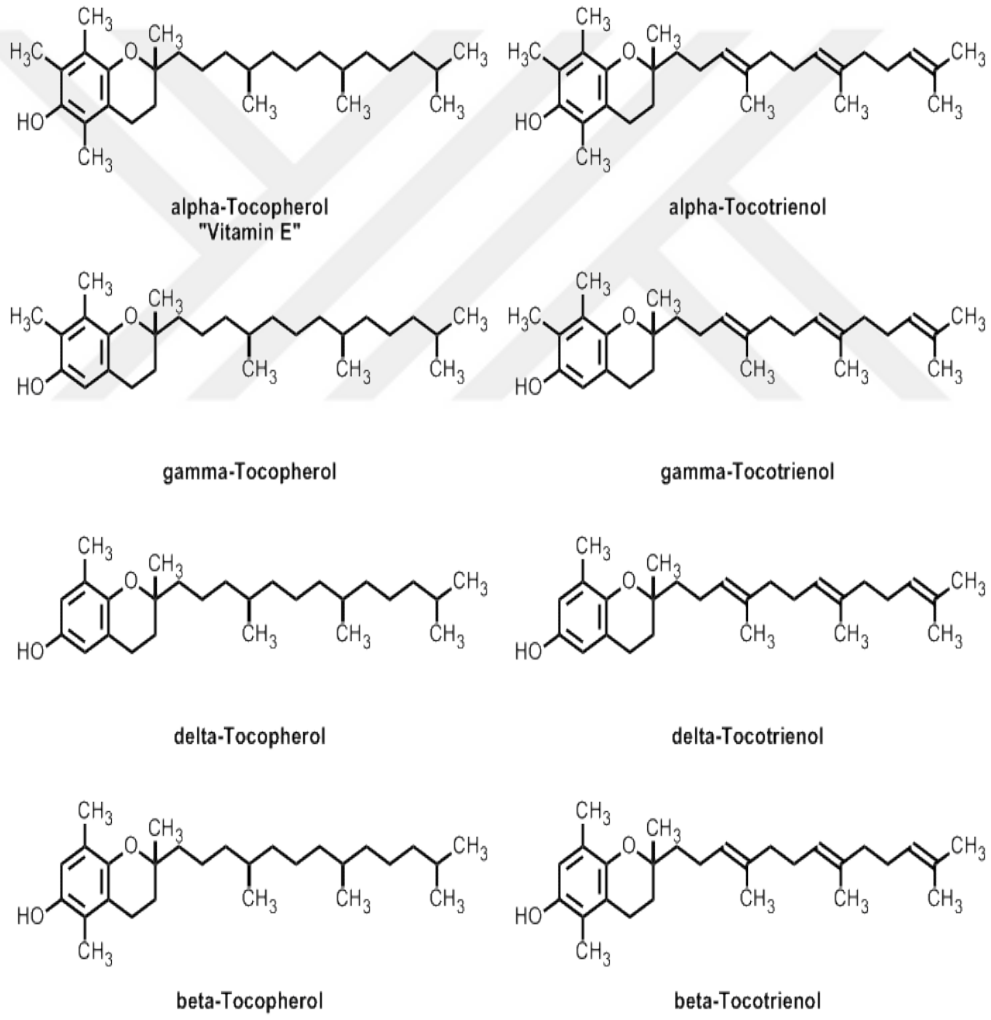

Figure 2.11. Structures of the tocopherols and tocotrienols (IOM, 2000).

2.3.6.3. Vitamin C

Ascorbic acid that is need for the human body additionally referred as to ascorbic acid or ascorbate. (Figure 2.12). Vitamin-C is micronutrient that is essential for normal metabolic functioning of the body. Vitamin-C is plentiful especially in citric fruits and vegetables (Bendich, 1997). Also low vitamin-C in the diet due to the deficiency disease scurvy. Vitamin-C, can be water soluble is an antioxidant inside biological fluids. An antioxidant had been defining as most substance. When found at low concentrations compared to those of an oxidizable substrate like proteins, lipids, carbohydrates and nucleic acids. Important lack or avoid oxidation on the substrate (Halliwell, 1996). Vitamin-C conveniently scavenges reactive oxygen and nitrogen species, like superoxide and hydroperoxyl radicals, aqueous peroxyl radicals, singlet oxygen, ozone, peroxynitrite, nitrogen dioxide, nitroxide radicals, and hypochlorous acid (Halliwell, 1996). As a result effectively protection any substrates from oxidative damage.

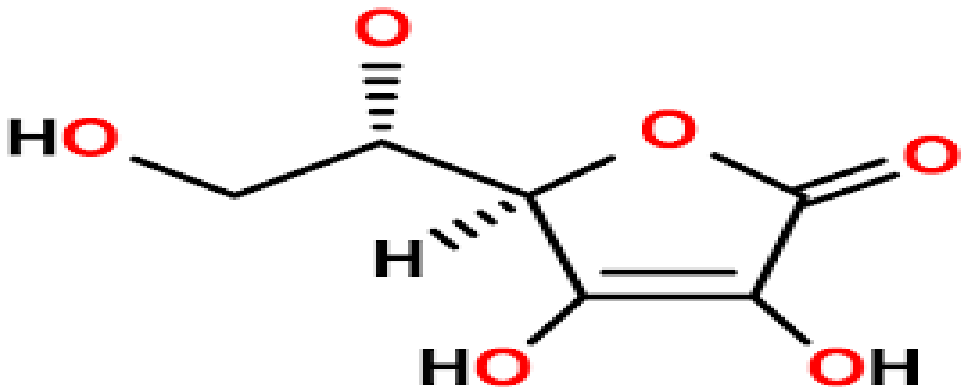

Figure 2.12. Structure vitamin C (Halliwell, 1996).

As Biological functions Vitamin C acts as a toxic pro-oxidant and this activity is transition metal-dependent due to its reduce capacity. In the absence of enzymes, vitamin C reduces free Fe^{+3} and Cu^{+2} to reactive forms, Fe^{+2} and Cu^{+1} which then participate in the radical-producing Fenton response. Vitamin C also assists in avoid and treatment of non-scurvy diseases in human. Clinical trials of vitamin C supplementation have demonstrated a keep effect of vitamin C in cardiovascular diseases. In fact, no

other antioxidant vitamin supplementation had been shown to affect the risk of cardiovascular diseases (Kris-Etherton et al., 2004). Vitamin-C is effective in protection against oxidative damage in tissues, and also suppresses the formation of carcinogens like nitrosamines. Many studies have shown that vitamin-C intake is inversely related to incidence of cancer, due to its protective effects on the lung, breast, pancreas, stomach, cervix, rectum and oral cavity. (Simon et al., 2001). Vitamin-C is involved in synthesis of corticosteroids and also in collagen and bone formation. It is a part of many metabolisms namely. Iron and hemoglobin, trytophan, tyrosine, folic acid and cholesterol. It is a component of the electron transport chain; also it enhances the synthesis of immunoglobulin and increases the phagocytic activity of white blood cells.

Deficiency clearing low levels of vitamin-C are seen in numerous conditions like increased oxidative stress, such as cancer, diabetes mellitus, cataract, HIV infection, smoking habits had been reported that diabetic individual have low levels of vitamin-C in the plasma and in the white blood cells, which rebuild our immune defense (Simon et al., 2001).

2.4. Oxidative stress

There are two primary types of free radicals produced as part of metabolic functions. These are reactive oxygen and nitrogen species. The metabolism counters these reactive species with a series of endogenous and exogenous antioxidants, which can be stored at cellular level. In normal cases, these antioxidants and the reactive species produced are in a balance. This balance can be lost, however, when free radicals are in excessive amounts, or antioxidant stores are lower than normal. This loss of balance starts what is known as the oxidative stress.

Oxidative stress is therefore an ever present phenomenon in human body resulting of normal cellular processes. As discussed before, there is normally a balance in metabolism between ROS and antioxidant production (figure 2.13) (Rahal et al., 2014). Cells are usually capable of resisting a certain degree of oxidative stress thanks to the antioxidant defenses they have in store, or due to their capability to repair minor oxidative damages they suffer (Zadák et al., 2009). This balance can be lost due to

several reasons however, and reactive oxygen species production can exceed normal physiological levels, which in turn can lead to cellular damage (Saral et al., 2005).

The sources for oxidative stress can be internal or external. Internal sources include enzymatic actions and oxygen radical generating cellular and metabolic processes. Pollutants in the air, radiation, and consumption of certain foods are amongst the primary sources of external oxidative stress. When the oxidative stress reaches a threshold that overcomes the antioxidant defenses of the metabolism, lipids and proteins in cellular structures become oxidized, which changes their functionality and reactions they can participate in, often leading to cellular and tissue damage. Oxidative damage can also damage the nucleic acids of the cell (Mc Cord, 2000).

Infections, trauma, certain toxins and overworking can lead to short term oxidative stress, all of which lead to minor injuries to various tissues. When damaged, these tissues generate certain enzymes that contribute to radical production xanthine oxidase, lipogenase, cyclooxygenase. They also activate phagocytes, cause more iron and copper ions to be released into circulation, and disrupt electron transport chains. When these effects last for a long period of time, the ROS can be antioxidant balance can be lost, which in turn can lead to development various types of cancer, or cause the existing cancer to spread easily. Furthermore, a relationship between reactive oxygen species and diabetes mellitus and various age-related diseases like the Parkinson's disease were also shown in various studies (Rao et al., 2006). Oxidative stress may also lead to atherosclerosis, malaria, chronic fatigue, Alzheimer's disease (Figure 2.14) (Chaitanya et al., 2010).

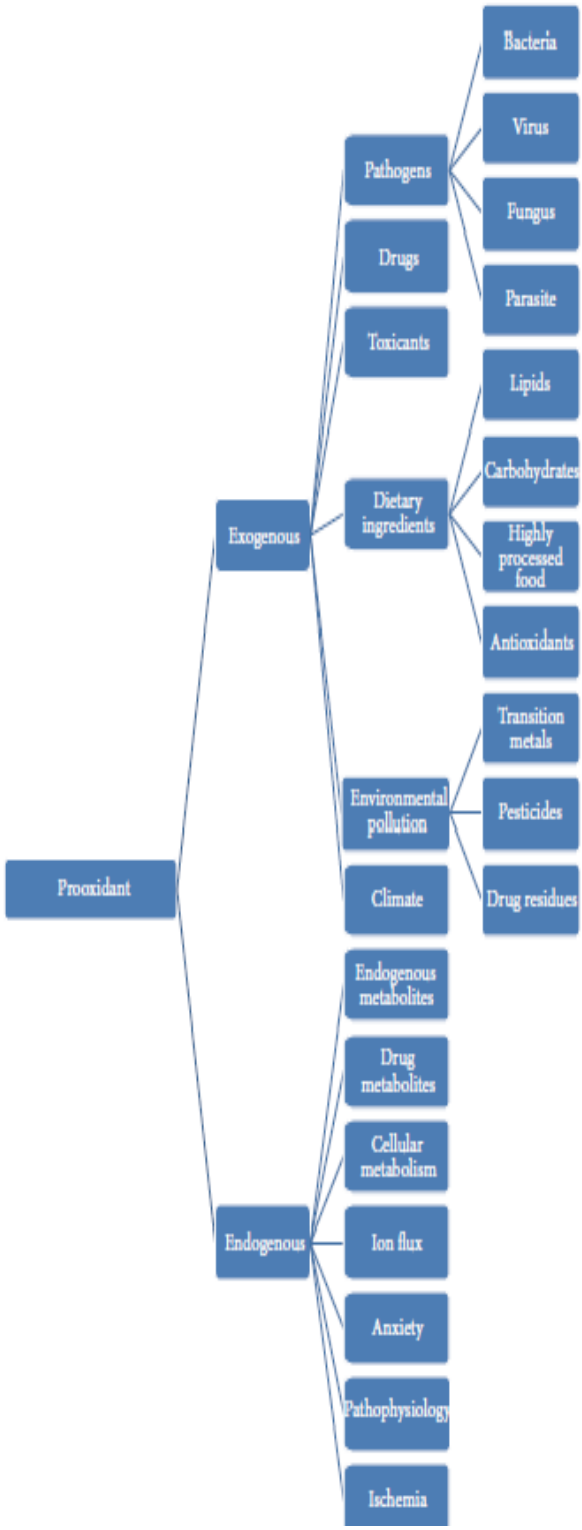

Figure 2.13. General classification of antioxidants (Rahal et al., 2014).

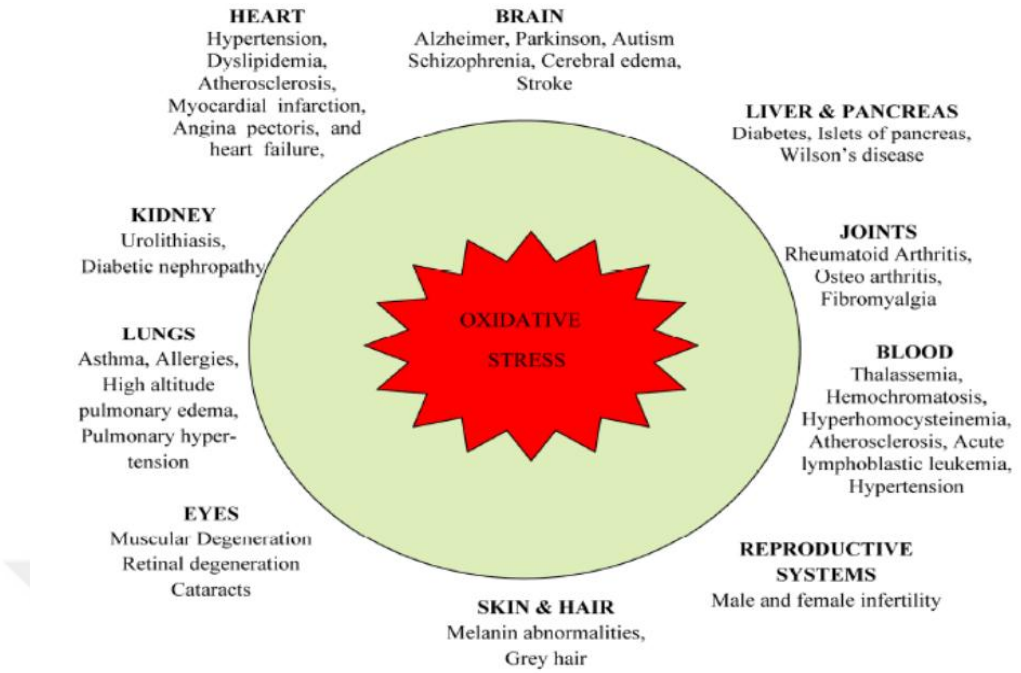

Figure 2.14. Deleterious effects of oxidative stress on human health environmental Pollution, viral, bacteria, infection hyperthermia and UV radiation show To have correlation with oxidative stress

The reactive oxygen species and reactive nitrogen species respectively cause oxidative and nitrosative stress (Kovacic et al., 2001). Reactive oxygen refers to all unstable metabolic oxygen (O_2) metabolites like superoxide radical ($O_2\cdot$) and hydroxyl radical ($HO\cdot$), which are more reactive than molecular oxygen O_2. Certain non-radicals like the hydrogen peroxide (H_2O_2) are also amongst the ROS.

Mitochondria are believed to be the cell organelle that produces the most ROS (Liu et al., 2002). This is due to the fact that they are tasked with providing ATP through oxidative phosphorylation processes. This process involves one- or two-electron reduction, compared to the normal electron reduction of four, from the O_2. This difference is what leads to the productions of the mentioned $O2\cdot$ and H_2O_2, which are capable of being transformed into other reactive oxygen species as well. The damage caused by ROS or RNS occurs as a result of complex processes, and the extent and type of the damage is related to the type of the oxidant, the location of the damage, the intensity of the imbalance between ROS and RNS and antioxidant stores, the composition and amount of the available antioxidants, and the capabilities of the repair systems of the affected tissue.

2.4.1. Lipid peroxidation and malondialdehyde

Malondialdehyde It is considered to be the terminal compound and the very important marker for monitoring lipid peroxidation of polyunsaturated lipid and oxidative damage induced by reactive oxygen species and reactive nitrogen species (Kose et al., 2001) It is also considered as a thiobarbituric acid reactive substance (MDA). Is one of many low molecular weight end-products of lipid hydoperoxide decomposition and is the most often measured as an index of lipid peroxidation. (Hong et al., 2000). Lipid peroxidation is a chain reaction occurring during oxidative stress also the determination of oxidative stress in human body is very difficult directly during the reactive oxygen species and free radicals are very short life. Lipid peroxidation has been implicated in the pathogenesis of rheumatoid arthritis, cancer, atherosclerosis, degenerative diseases and inflammatory arthritis.

During lipid peroxidation, polyunsaturated fatty acids are oxidized to produce lipid peroxyl radicals that in turn lead to further oxidation of polyunsaturated fatty acids in a perpetuating chain reaction that can lead to cell membrane damage. This peroxidation is determination as MDA that is detected in serum and used as an oxidative stress.

Oxidative stress correlated with an elevation in MDA and to decreases the antioxidant and the started in many disease such as stomach diseases, kidney illness, rheumatoid arthritis, breast and lung cancer according to our study was proof the ratio of MDA level are the diseases increase but other enzymes decreases such as CAT. Lipid Peroxidation Products Lipid peroxidation or reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products. The main primary products of lipid peroxidation are lipid hydoperoxide (LOOH). Among the many different aldehydes which can be formed as secondary products during lipid peroxidation, MDA is very important as biomarker for oxidative stress and lipid oxidation also MDA many thing causes of high like all types of alcohol, smoking the hydrogen peroxide more affect the MDA according this study. MDA can be found in most biological samples including foodstuffs, serum, plasma, tissues and urine, as a result of lipid peroxidation, and has become one of the most widely reported analyses for the purpose of estimating oxidative stress effects on lipids.

The principle Serum lipid peroxidation in the form of lipid peroxidation product malondialdehyde is determined by the colorimetric thiobarbituric acid (TBA) method. Lipid peroxides break down to form MDA under acidic and heating conditions. The MDA reacts with TBA to form pink MDA-TBA adduct which absorbs at 532 nm. (Menter et al., 2009).

2.4.2. Oxidative Stress and human Diseases

Today the world is experiencing a rise in age related chronic health diseases like rheumatoid arthritis, cardiovascular disorders, cancer, and so forth and their associated negative health impacts and mortality casualty (Racek et al., 1995; Rahman., 1992). Some metabolic diseases like diabetes are also associated with an enhanced level of lipoperoxidation (Figure 2.15).

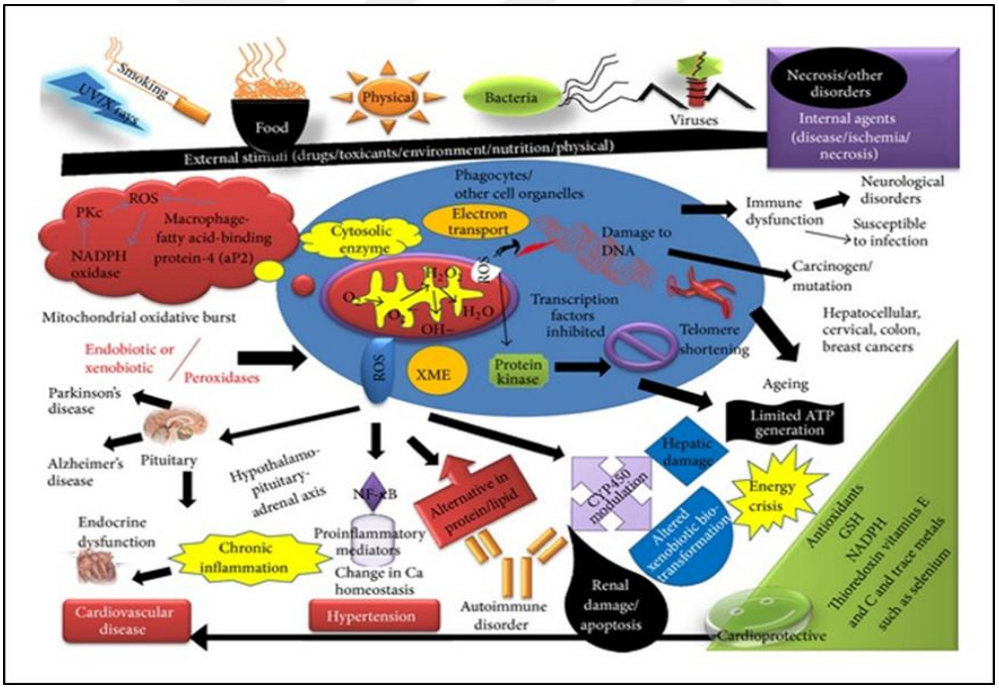

Figure 2.15. Oxidative stress and development diseases (poljsak., 2011).

The central nervous system is extremely sensitive to free radical damage because of a relatively small total antioxidant capacity. The reactive oxygen agent produced in the tissues can inflict direct damage to macromolecules, such as lipids, nucleic acids,

and proteins (Cherubini et al., 2005). The polyunsaturated fatty acids are one of the favored oxidation targets for ROS. Oxygen free radicals, particularly superoxide anion radical (O_2·), hydroxyl radical (OH·), alkylperoxyl radical (·OOCR), are potent initiators of lipid peroxidation, the role of which is well established in the pathogenesis of a wide range of diseases. Once lipid peroxidation is initiated, a propagation of chain reactions will take place until termination products are produced. Therefore, end products of lipid peroxidation, such as malondialdehyde (MDA), 4-hydroxy-2-nonenol (4-HNE), isoprostanes, are accumulated in biological systems. DNA bases are also very susceptible to ROS oxidation, and the predominant detectable oxidation product of DNA bases in vivo is 8-hydroxy-2- deoxyguanosine. Oxidation of DNA bases can cause mutations and deletions in both nuclear and mitochondrial DNA. Mitochondrial DNA is especially prone to oxidative damage due to its proximity to a primary source of ROS and its deficient repair capacity compared with nuclear DNA. These oxidative modifications lead to functional changes in various types of proteins .which can have substantial physiological impact. Similarly, redox modulation of transcription factors produces an increase or decrease in their specific DNA binding activities, thus modifying the gene expression.

Among different markers of oxidative stress, malondialdehyde (MDA) and the natural antioxidants, metalloenzymes Cu, Zn-superoxide dismutase Cu, Zn-SOD, and selenium dependent glutathione peroxidase are currently considered to be the most important markers (Singh et al., 2006). Malondialdehyde is a three-carbon compound formed from per oxidized polyunsaturated fatty acids, mainly arachidonic acid. It is one of the end products of membrane lipid peroxidation. Since MDA levels are increased in various diseases with excess of oxygen free radicals, many relationships with free radical damage were observed. Cu, Zn-SOD is an intracellular enzyme present in all oxygen-metabolizing cells, which dismutase’s the extremely toxic superoxide radical into potentially less toxic hydrogen peroxide. Cu, Zn-SOD is widespread in nature, but being a metallo-enzyme, its activity depends upon the free copper and zinc reserves in the tissues. GSH, an intracellular enzyme, belongs to several proteins in mammalian cells that can metabolize hydrogen peroxide and lipid hydro peroxides. The no specificity of these oxidants is an advantage since they take care of all the antigenic components of the pathogenic cell (Rice's et al., 1995).

Several studies have demonstrated the interdependency of oxidative stress, immune system, and inflammation. Increased expression of NO has been documented in dengue and in monocyte cultures infected with different types of viral infections. Increased production of NO has also been accompanied with enhancement in oxidative markers like lipid peroxidation and an altered enzymatic and non-enzymatic antioxidative response in dengue infected monocyte cultures (Valero et al., 2013) more specifically, the oxygen stress related to immune system dysfunction seems to have a key role in senescence, in agreement with the oxidation inflammation theory of aging. Moreover, it has been revealed that reduced NADPH oxidase is present in the pollen grains and can lead to induction of airway associated oxidative stress. Such oxidative insult is responsible for developing allergic inflammation in sensitized animals. There is triggering of production of interleukin (IL-8) along with proinflammatory cytokines, that is called tumor necrosis factor (TNF)-alpha and IL-6. There is initiation of dendritic cell .The immune status directly interplays with disease production process. The role of physical and psychological stressors contributes to incidences and severity of various viral and bacterial infections. Both innate as well as acquired immune responses are affected by the altered IFN-γ secretion, expression of CD14, production of the acute-phase proteins, and induction of TNF-α. Fatal viral diseases produce severe oxidative stress causing to rigorous cellular damage.

However, initiation, progress, and reduction of damages are governed by the redox balance of oxidation and antioxidation. The major pathway of pathogenesis for cell damage is via lipid peroxidation particularly in microsomes, mitochondria, and endoplasmic reticulum due to OS and free radicals (Stehbens ., 2004; Hodgson et al., 2012). All the factors responsible for the oxidative stress directly or indirectly participate in immune system defense mechanism. Any alteration leading to immune suppression can trigger the disease production (Table. 2). The many of pathway of cell and tissue damages through the lipid peroxidation in microsomes, endoplasmic and mitochondria causes free radicals and oxidative stress (Rahal et al., 2014).

2.4.3. Oxidative damage to DNA

At high concentrations, reactive oxygen species can be important mediators of damage to cell structures, nucleic acids, lipids and proteins (Valko et al., 2006) The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone. Permanent modification of genetic material resulting from these oxidative damage incidents represents the first step involved in rheumatoid arthritis carcinogenesis and ageing.

Mitochondria are unique organelles, as they are the main site of oxygen metabolism, accounting for approximately 85 to 90 % of the oxygen consumed by the cell. Incomplete processing of oxygen and release of free electrons results in the production of oxygen radicals. Mitochondria constantly metabolize oxygen thereby producing ROS as a byproduct. These organelles have their own ROS scavenging mechanisms that are required for cell survival. It has been shown, however, that mitochondria produce ROS at a rate higher than their scavenging capacity, resulting in the incomplete metabolism of approximately 1 to 3 % of the consumed oxygen. The byproducts of incomplete oxygen metabolism are superoxide, hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). The formation of superoxide occurs via the transfer of a free electron to molecular oxygen. This reaction occurs at specific sites of the electron transport chain which resides in the inner mitochondrial membrane (Figure 2.16). Electron transport chain complexes I (NADH dehydrogenase) and III (ubisemiquinone) produce most of the superoxide, which is then scavenged by the mitochondrial enzyme manganese superoxide dismutase (MnSOD) to produce hydrogen peroxide.

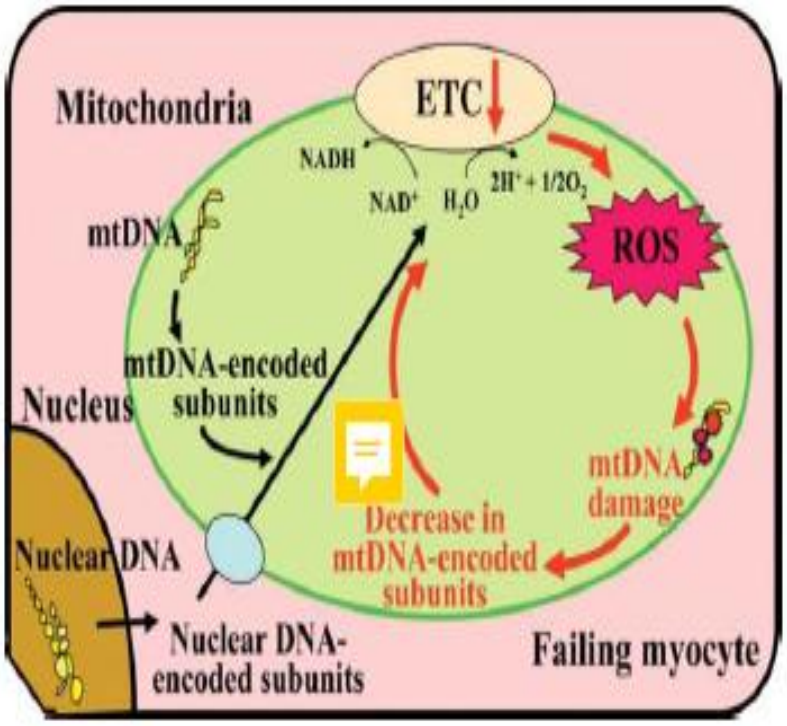

Figure 2.16. Generation of ROS in electron transport chain (Valko et al., 2006).

2.4.4. Oxidative damage to Proteins

Proteins are resistant to damage by hydrogen peroxide and simple oxidants unless transition metals are present. Metal catalyzed damage to proteins involves oxidative scission, loss of histidine residues, bityrosine cross links, the introduction of carbonyl groups, and the formation of protein-centered alkyl (R•), alkoxyl (RO•), and alkylperoxyl (ROO•), radicals (Eaton et al., 2002). Studies on the metal-induced protein denaturation lead to the discovery that degradation occurs when the protein has been oxidized. Iron-mediated oxidation of a protein may be a site specific process as proline, histidine, and arginine, lysine, and cysteine residues in proteins which are highly sensitive to oxidation by iron (Stadtman, 1990). The Fe^{2+} protein complex reacts with hydrogen peroxide via the Fenton reaction to yield an active oxygen species (•OH) at the site, which represents the major species responsible for the oxidation of protein.

2.5. Rheumatoid Arthritis

Rheumatoid arthritis is chronic systemic auto-immune inflammatory disease characterized by articular and extra-articular manifestation, with symptoms that can be caused in part human body by inflammation. RA symptoms can vary from person to person, including .Joint pain, swelling around the joint, making it tender and warm and stiffness and muscle pain, weight loss, fatigue, malaise and anemia Extra-articular manifestations of RA occur in about 40 % of patients, either in the beginning or during the course of their disease patients with RA, who have high titers of rheumatoid factor, are most likely to have extra-articular manifestations of their disease (Turesson et al., 2004). Patients with RA may present with hematological abnormalities either at the time of diagnosis, or during the course of their illness. Hematological manifestations in RA can be broadly categorized into areas of anemia, thrombocytosis, neutropenia, thrombocytopenia, eosinophilia, and hematological malignancies (Bowman, 2002). Anemia is by far, one of the most common extra-articular manifestations of RA. The cause of anemia in RA is multifactorial including disease activity, drug induced, nutritional, gastrointestinal bleeding, bone marrow suppression, and ineffective erythropoiesis (Agrawal et al., 2006). The types of anemia in RA may include anemia of chronic disease, iron-deficiency anemia, vitamin deficiency anemia, aplastic anemia, or hemolytic anemia (Turesson et al., 2008).

Rheumatoid arthritis begins with the inflammation of the synovial membrane of the joints, especially in the small ones of the fingers and feet and is mostly bilateral. The inflammatory cells, if in inappropriate large amount, destroy body tissue. The synovial fluid accumulates and the joints swell in time and thicken into a pannus (abnormal tissue). Over time the pannus erodes the joint’s cartilage and, possibly, scar tissue will be formed, connecting bone ends. Later this scar tissue can ossify wherewith the joints get immobilized and deformed. The surrounding structures of the inflammatory joints, as tendon sheaths, bursas and origins of muscles are often involved supporting joint deformations as well (Marieb et al., 2006).

RA is an autoimmune disease with greater incidence among women. The pathologic immune response is not only affecting joints and bone but it is causing a systemic syndrome involving many other organs causing, either systemic disease like

vasculitis, lung fibrosis and increasing gene expression related to cancer development (Omode et al., 2009). Heart conditions such as ischemic heart disease and cardiac failure have been shown to be more common in RA. Reduced mobility and inflammation are concurrent causes of osteoporosis. Unfortunately, the causes of this disease are still unknown.

Inflamed synovial tissue from RA patients is characterized by the presence of inappropriately activated and interacting immune cells (Klareskog et al., 2009). In short, antigen-presenting cells communicate with T-cells through T-cell receptor-MHC interaction inducing T-cell activation in the presence of co-stimulatory signals mediated by the CD28-B7 receptor family. The antigen-presenting B-cells also function as antibody-producing cells and the produced anti-bodies lead to immune complex formation in the joints. Mono-cytes and macrophages activated by T-cell signaling and immune complexes produce pro-inflammatory cytokines such as tumor necrosis factor alpha (TNF), interleukin 1 (IL-1) and interleukin 6 (IL-6), all of which stimulate further cytokine production and expression of cell-adhesion molecules. This inflammatory cascade results in synovial hyperplasia, increased synovial vascularity and fibrous deposits (Klareskog et al., 2009). The inflammatory process affects all joint structures, including the bone at the joint margins, the articular cartilage and the perarticular bone.

A joint is the part of the body where two or more bones meet, e.g. hip or knee. The bones of a joint are covered with a smooth, elastic material called cartilage. The cartilage acts as cushion between the bones in the joint, which enables the joint to move without pain. The joint is also lined by a thin film tissue called synovium that produces a slippery fluid called synovial fluid. This fluid helps to reduce friction between the bones. Arthritis pain and swelling occur when any area in and around joint becomes inflamed. Synovitis is an inflammation of the synovial membrane-soft tissue that lines the joints. The synovial membrane produces lubricant called synovial fluid which helps bones to glide over each other when we move. With RA, body's immune system mistakes the cells of the synovial membrane as foreign invaders. RA can affect every joint where cartilage over line bone and also those with a joint cavity lines by synovial membrane containing synovial fluid. Chronic inflammation can be a persistent acute inflammation due to non-degradable pathogens, persistent foreign bodies and auto immune reaction Major cells are involved mononuclear cells (Monocytes, macrophages,

lymphocytes and plasma cells). Fibroblasts, primary mediators being IFN-gamma and other cytokines, growth factors, reactive oxygen species and hydrolytic enzymes. Here can the explain the normal and rheumatoid arthritis joint show in (figure 2.17)

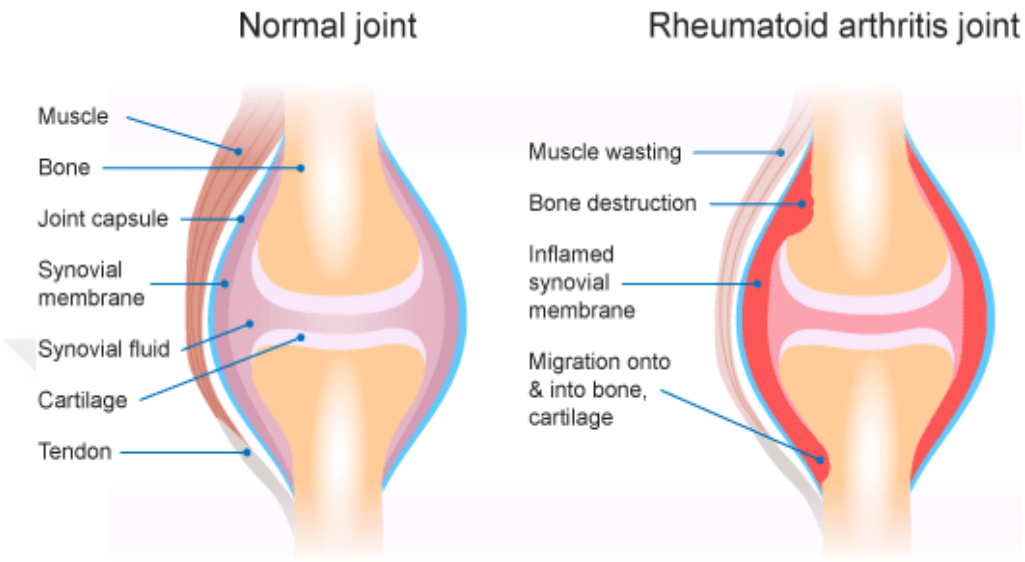

Figure 2.17. Structure of a normal and inflamed joint (Klareskog et al., 2009).

2.5.1. Epidemiology

RA affects approximately 0.5 to 1% of the general population of the wide world (Holmdahl et al., 2014) populations suffer from RA. The disease is 2 to 3 times more frequent in females compared to males. Incidence of RA ranges from 5 to 50 per 100 000 adults in developed countries and increases with any age. The most often onset of RA is in the age ranging between 40 and 60 years, but it can develop either earlier or later.

2.5.2. Etiology

The etiology of RA isn't always absolutely understood regardless of considerable take a look at of metabolic and nutritional factors, the endocrine system, and geographic, psychological, and occupational information it now appears that an unknown antigen initiates the autoimmune response resulting in RA. This response

supports the suspicion of an infectious origin of the disease process, which includes various bacteria and viruses, but without evidence of precipitating events. Even without this specific knowledge, treatment modalities have been developed that, while not curing the disease, can provide relief from the symptoms of the diseases proof points to a complex interaction between environmental and genetic elements. In monozygotic twins, there's a greater than 30 percent concordance price for rheumatoid arthritis improvement, and 80 percentages of whites with rheumatoid arthritis express the HLA-DR1 or -DR4 subtypes. These and different areas of the predominant Histocompatibility complicated may confer susceptibility to extra intense disorder via inflicting a selected arthrogenic peptide to be presented to $CD4^+$ T-cells. Scientists are actually focusing on the concept that it's miles a T-cellular-mediated autosomal disease precipitated with the aid of both genetic and environmental factors. (Goldring, 2005).

2.5.3. Pathophysiology

The joint capsule is lined with a type of tissue called synovium, which produces synovial fluid. The synovial fluid secreted by the synovium is thought to serve two main purposes, lubrication of the joint and provision of nutrients to the avascular articular cartilage. The attack on a joint by the disease usually begins with the synovium (Firestein, 2005). Joint damage in rheumatoid arthritis begins with the proliferation of synovial macrophages and fibroblasts after a triggering incident, either autoimmune or infectious. White blood cells that are part of the normal immune system travel to the synovium and cause a reaction. This reaction, or inflammation, is called synovitis, and it results in warmth, redness, swelling, and pain that are typical symptoms of RA. Lymphocytes infiltrate the perivascular regions, endothelial cells proliferate and these result in neovascularization. Thus early in the disease, edema begins to be seen in cells in the synovium and multiplication of synovial lining cells occur (Alamanoos et al., 2006). During the inflammation process, the cells of the synovium grow and divide abnormally, making the normally thin synovium thick and resulting in a joint that is swollen and puffy to the touch. Blood vessels in the affected joint become occluded with small clots or inflammatory cells. As the disease progresses, inflamed synovial tissue begins to grow considerably and irregularly, forming invasive pannus tissue. The

pannus is a sheet of inflammatory granulation tissue that spreads from the synovial membrane and invades the joint in rheumatoid arthritis and destroys cartilage and bone ultimately leading to fibrous ankylosis. Pannus can be considered the most destructive element affecting joints in the patient with rheumatoid arthritis. Pannus can attack articular cartilage and destroy it. Further, pannus can destroy the soft subchondral bone once the protective articular cartilage is gone. There is chronic inflammation with lymphocytes and plasma cells that produce the blue areas beneath the nodular proliferations (Alamanoos et al., 2006). Multiple cytokines, interleukins, proteinases, and growth factors are released, causing further joint destruction and the development of systemic complications. (Greenland et al., 1988).

In this disease process, an interaction between antibodies and antigens occurs, and causes alterations in the composition of the synovial fluid. Ultimately, digest are formed in the fluid that attacks the surrounding tissue. Once the composition of this fluid is altered, it is less able to perform the normal functions noted above, and more likely to become destructive (Breman et al., 1997). The changes in the synovium and synovial fluid are responsible for a large amount of joint and soft tissue destruction. The destruction of bone eventually leads to laxity in tendons and ligaments. Under the strain of daily activities and other forces, these alterations in bone and joint structure result in the deformities frequently seen in patients with rheumatoid arthritis. Considerable destruction of the joint can occur with pannus invading the subchondral bone (Cartte et al., 2000). Bone destruction occurs at areas where the hyaline cartilage and the synovial lining do not adequately cover the bone. If the disease progresses to a more advanced stage, the articular cartilage may lose its structure and density resulting in an inability to withstand the normal forces placed on the joint. In such advanced cases, muscle activity causes the involved ends of the bones to be compressed together causing further bone destruction. Further, the disease can irreversibly change the structure and function of a joint to a degree that other degenerative changes may occur, especially in the weight bearing joints of the body. Thus, joint destruction can progress to the degree that joint motion is significantly limited and joints can become markedly unstable.

2.5.4. Genetic factors

Rheumatoid arthritis is a chronic and inflammatory disease that targets synovial membranes, bones and joints. It's a many factor illness and a variety of things contribute to the development and pathogenesis of the disease. Contribution of genetic factors towards RA ranges from 50 to 60 % Human leukocyte Antigen (HLA) locus is the most important genetic risk factor in RA. The most genetic risk factor associated with RA is found at the 6p21 cytoband. This region comprises 3.6 Mb and is split into completely different class I, II and III HLA genes HLA class III genes don't seem to be concerned in antigen presentation (Balsa et al., 2010). HLA-II has been documented to contribute to up to one third part of the genetic component associated with susceptibility to RA. Recent studies suggest that this percentage is overestimated. Data indicate that HLA-DRB1 contributes only by 11%. HLA-I and II genes are highly polymorphic and encoding for cell-surface heterodimer proteins and have as primary role binding to own or foreign short peptides and presenting them to $CD8^+$ and $CD4^+$ T-cells, respectively (Taylor et al., 2009).

Genome Wide Association Studies have emphasized on the association of single nucleotide polymorphisms with RA. Single nucleotide polymorphisms are particularly useful as every individual is uniquely characterized by a set of at least three million common SNPs that are distributed across the genome. SNPs, in turn, can determine the response of an individual towards differ environmental factors.

Cytokines are significant component of the immune system. These cytokines integrate the immune regulatory pathways that ultimately due to different autoimmune diseases such as RA. T-helper cell (TH-cell) cytokines are the most important of all the cytokines that might play a role in RA (Firestein, 2003). It's primarily secreted by mononuclear phagocytes. It regulates T-cell and natural killer cell activation and proliferation.

The levels of IL-15 had been monitored to be increased in RA as compared to different rheumatic diseases. It is secreted by fibro-blasts and macrophages in the inflamed synovial membrane during RA. Increased levels of this cytokine may lead to the recruitment and activation of synovial T-cells. It results to the differentiation of the

osteoclast progenitors which is the key step that causes towards the bone deterioration (Ogata et al., 2009).

2.5.5. Environmental factors

The main were well investigated environmental risk factor for the development of rheumatoid arthritis such as cigarette smoking (Figure 2.18). There may be is a positive association among cigarette smoking, the RF and ACPA with positive RA (Klareskog et al., 2006). Furthermore, the doses of cigarettes in keep with day and the years of smoking had been present to have the greatest damage with the fact that smoking and HLA-DRB1 alleles synergistically high the risk of having ACPA. Moreover, it it's only active, however also previous cigarette smoking represents an independent risk factor for the development of RA its severe (Baka et al., 2009). A mechanistic link has be proposed for carrying unique HLA-DRB1 alleles encoding the shared epitope and smoking, which results into ACPA positive with RA (Klareskog et al 2006). An increasing speed of lung cancer observed in rheumatoid arthritis patients can also according to defined by the association among rheumatoid arthritis and smoking (Mcinnes, 2011).

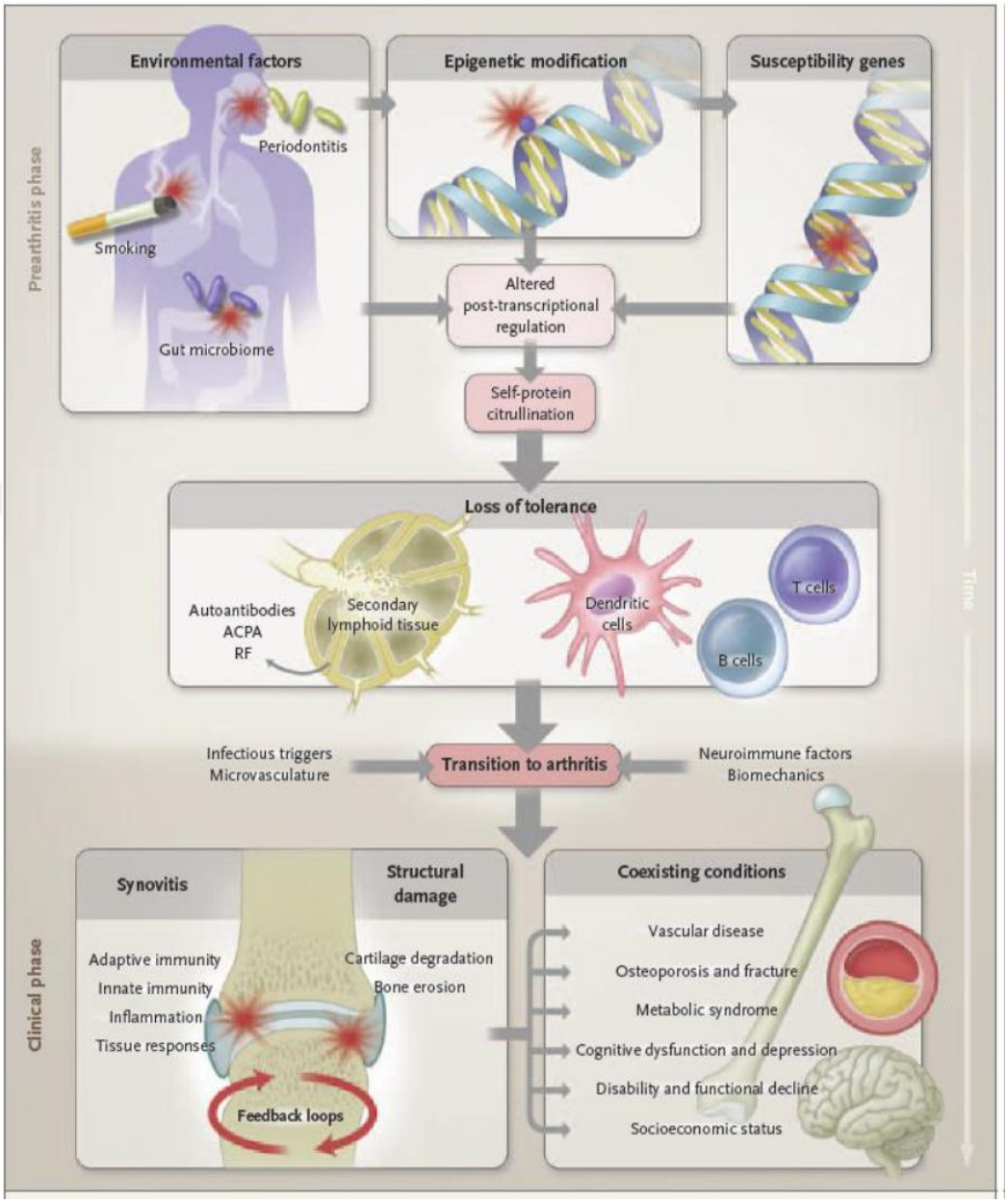

Figure 2.18. Environmental gene interactions that are suggested to play a significant Role significant role in rheumatoid arthritis development (Mcinnes et al., 2011).

The association among RA risk and different factors consist of alcohol, diet, caffeine intake and body mass index are weak or that are not proven. consistent with a Danish study have a look at comparing RA patients who drink alcohol and those who do now not drink found out that alcohol can also lower the risk factor of RA, mainly in ACPA effective main group (Liao et al., 2009). This become supported by way of

meta-analysis of prospective studies demonstrating that low to mild alcohol intake inversely associates with the development of RA (Jin et al., 2013).

Vitamin-D, it is an important hormone for both bone and mineral homeostasis had been hypothesized to have a significant role in RA development. It is involved in both adaptive and innate immune systems by way of vitamin-D receptor as a suppressor in pro-inflammatory reactions. There's a few evidence that vitamin-D deficiency play a function inside the development of a few autoimmune diseases, consist of rheumatoid arthritis (Song et al., 2012).

2.5.6. Clinical symptoms

The onset of rheumatoid arthritis and the course of the disease may be extremely variable. The main features of RA are defined as joint pain, tenderness, swelling and morning stiffness that can lead to subsequent development of joint deformities (Figure 2.19). These are the common physical symptoms and signs that define RA as a chronic inflammatory disorder. The chronicity of the disease may be finally followed by severe disability, increased morbidity and premature mortality.

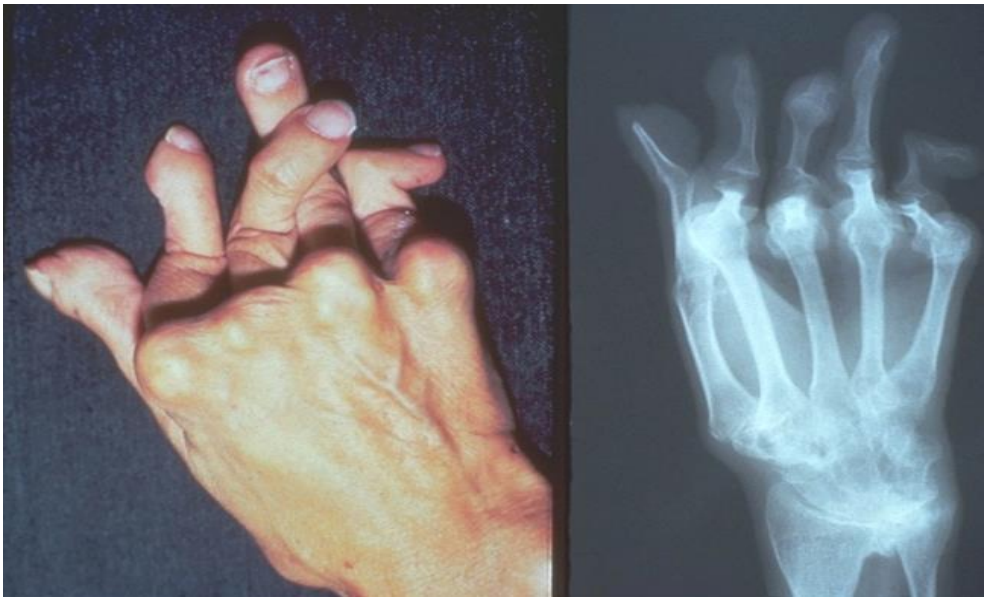

Figure 2.19. The hand joints in early and longstanding rheumatoid arthritis (Mcinnes et al., 2011).

The clinical presentation of rheumatoid arthritis does not show similar patterns in affected patients. Most common finding is long-lasting symmetric arthritis accompanied by tenderness, pain and swelling of the hand and feet joints, wrists and ankles, sometimes also knees, elbows and shoulder joints. RA is often associated with systematic features such as fatigue, fever, and in severe cases also anorexia and weight loss. The onset of the disease is variable, ranging from one single affected joint monoarthritis to progression to severely polyarticular involvement. Subcutaneous granulomatous lesions, called rheumatoid nodules, are late symptoms presented in few patients. Parenchymal lung disease, ocular involvement, including secondary Sjögren's syndrome, cutaneous vasculitis and pericartidis represent further extra-articular manifestations of rheumatoid arthritis (Mcinnes et al., 2011).The main characteristics of the disease are given in (Table 2.2) (Assil et al., 2008).

Table 2.2. Characteristic of rheumatoid arthritis

Characteristic of rheumatoid arthritis	
Clinical	Female more than male two for three times more, morning stiffness more than one hour, fatigue, fever.
Laboratory	Rheumatoid factor, anti citrullinated peptide antibodies, elevated inflammatory markers including erythrocyte, sedimentation rate and C-reactive protein.
Radiographic	Erosions, perarticular osteopenia, joint space loss.
Musculoskeletal	Symmetric, polyarticular involvement, most commonly affects metatarsophalangeal, proximal interphalangeal joints, wrists, metatarsophalangeal joints. Sometimes cervical spine spares thoracolumbar spine.
Extra-articular	Cardiovascular disease such as atherosclerosis, rheumatoid nodules, ocular involvement episcleritis, pulmonary fibrosis and nodules, renal manifestations, AA amyloidosis, sicca symptoms dry eyes and mouth, hematologic anaemia of chronic diseases.

2.5.7. Classification and diagnosis of rheumatoid arthritis

Many biological markers are used in clinical care of patients with RA as a means of providing further information for diagnostic and prognostic purposes. Four of the most commonly used biomarkers are erythrocyte sedimentation rate (ESR), CRP, rheumatoid factor (RhF) and anti-cyclic citrullinated peptide (anti-CCP). ESR and CRP are markers of inflammation and are elevated in the majority of patients experiencing active disease.

In 2010, the ACR and the European league against rheumatism (EULAR) developed new classification criteria for RA (Aletaha et al., 2010). These criteria required the presence of synovitis in at least one joint and the absence of an alternative diagnosis that offered a more suitable explanation for the synovitis. As outlined in (Table 2.3). A scoring system was developed where a total of 6 or greater out of 10 is required for classification as RA.

Table 2.3. The 2010 American College of Rheumatology European League against Rheumatism classification criteria for rheumatoid arthritis

Criterion	Score
Joint involvement	
large joint	0
2 – 10 Large joint	1
1 – 3 small joint (with or without involvement of large joints)	2
4 – 10 small joint (with or without involvement of large joints)	3
> 10 joints (at least 1 small joint)	5
Serology (at least 1 test result is needed for classification)	
Negative RhF and negative ACPA	0
Low-positive RhF or low-positive ACPA	2
High-positive RhF or high-positive ACPA	3
Acute-phase reactants (at least 1 test result is needed for classification)	
Normal CRP and normal ESR	0
Abnormal CRP or abnormal ESR	
Duration of Symptoms	1
< 6 weeks	0
≥ 6 weeks	1

2.5.8. Treatment of rheumatoid arthritis

There is no therapy for rheumatoid arthritis. But recent discoveries indicate that remission of signs is more likely when treatment starts early with strong medication drug referred to as diseases modifying anti-rheumatoid drugs (DMARDs). Many medication alternatives exist for the treatment of patients with RA, with the closing purpose of minimizing disease activity. In addition to drug remedy, non-pharmacological treatment, which including patient education, physiotherapy, occupational therapy, orthotics, and surgery are they have got many ways used to control for rheumatoid arthritis.

2.5.8.1. Disease-Modifying Antirheumatic Drugs

Disease-modifying anti-rheumatic drugs are representing the current front line treatment for RA. Folic acid and Methotrexate analogue are recognized because the first line DMARD for RA because of its protection, efficacy and fee effectiveness (Parida., 2015) Methotrexate it is specifically polyglutamated metabolite, inhibits the enzyme aminoimidazole carboxamide ribonucleotide, transformylase (Chatham., 2003) this inhibition causes to a high concentration in adenosine in the extracellular space. It is hypothesized that the anti-inflammatory effects of Methotrexate are mediated through reduced leukocyte adhesion to endothelial cells caused by the accumulation of adenosine. At the same time as this anti-inflammatory mechanism is supported in the literature, several other pathways have been proposed which including reduction of antigen-dependent T-cell proliferation, increases of T cell apoptosis and manipulation of cytokine production. Methotrexate is usually administered oral however also can give by subcutaneous and intramuscular on a weekly basis, with doses various from 7.5 to 25 mg (Tavares, 2012). Folic acid is prescribed to assist offset folate deficiency caused by Methotrexate. Administration and regular laboratory testing is recommended to reveal liver and kidney function (Tavares, 2012).

Leflunomide is every another DMARD and shares many similarities with MTX. The therapeutic movement of leflunomide is mediated through a metabolite (Tavares, 2012). Leflunomide disrupts nucleic acid synthesis by inhibiting an enzyme responsible

for the formation of pyrimidine nitrogenous bases (Tavares, 2012). This inhibition leads to a reduced proliferation of lymphocytes making it a beneficial immunosuppressant for the therapy for RA. Leflunomide is gave orally (10-20 mg/day) and patients should be monitored closely for potential side effects via laboratory testing, similar to MTX (Tavares, 2012).

2.5.8.2 Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs is inhibiting cyclooxygenase enzyme, which reasons a reducing prostaglandin mediated inflammatory activity (Tavares, 2012). A ramification of NSAID is available for clinical use in RA, including celecoxib, diclofenac and naproxen, among others.

These drugs are normally taken daily with doses varying according to brand (Tavares, 2012). Similar to NSAIDs corticosteroids offer anti-inflammatory useful to patients with rheumatoid arthritis. Corticosteroids, such as prednisone, represent a class of adrenal hormones that provide symptomatic relief and can be given orally or intra-particularly (Tavares, 2012). Also this therapy can reduce the pain, inflammation, slow joint, damage.

2.5.8.3. Biologic treatment of rheumatoid arthritis

Biological response modifiers are the newest class of drugs used to treat rheumatoid arthritis. Biologics work by interrupting immune system signals involved in the harm of joint tissue. Any newer drugs target protein referred to as tumor necrosis element (TNF). That is medication called anti-TNF biologics. Like other DMARDs, biologics affect immune system function. Abatacept works by crippling a type of white blood cell called T-cells. T-cells play a role in the inflammation that causes RA. Many biologics work by interfering with the activity of tumor necrosis factor. This is a key immune system protein. These drugs include adalimumab, etanercept, infliximab people who don’t respond to older RA drugs, such as methotrexate, may benefit from treatment with a biologic. Sometimes biologics may be

given alone. Other times they may be given in combination with another type of drug. Taking a biologic drug with methotrexate is effective for general public with RA.

Subsequently, physiotherapy and physical exercising could be very vital in many Sufferers with rheumatoid arthritis. Supervised training can be useful to exercising the muscles and joints in a gentle way without unnecessarily teat at the joints. Physiotherapy, occupational remedy and rehabilitation play essential roles inside the control of rheumatoid arthritis, especially in patients with extensive disease related incapacity and pain.

3. MATERIALS AND METHODS

3.1. Materials

In our investigation, we took blood from 30 healthy individuals, and 30 patients with rheumatoid arthritis in both male and female. From each of healthy and patient individuals, we took 4ml of blood from an antecubital venous vein and added 2ml to the biochemistry tube and the other 2ml to the EDTA tube.

3.1.1. Tools and Materials

Vortex
Deep Freezing Tubes
Serum Storage Tubes
Spectrophotometer
Adjustable Automotive Pipettes
Thermo stated Water Bath
Glass pipette
Cooled centrifuge
Deep freeze
Oven
Stopwatch
Sensitive balance
Spectrophotometer cuvette
Automated Pipette Tip
PH-meter
Magnet
Beaker
Flask
Incubator
Test tube rack
Volumetric flask

Cylinder

Watch glass

Spatula

Funnel

Filter paper

3.1.2. Reagents and chemicals

Potassium hydroxide

Hydrogen peroxide

Monopotassium phosphate

Disodium phosphate

Sodium hydroxide

Sodium citrate

Elman’s reagent (5,5'-dithiobis-(2-nitrobenzoic acid).

Xanthine

Ethylene demines tetra acidic acid.

Sodium bicarbonate

Bovine serum albumin

Xanthine oxidase

Ammonium sulfate

Cupper chloride

Ethylene diaminetetra acetic acid disodium

Butyl hydroxyl toluene

Thiobarbituric acid

Trichoroacetic acid

Sodium chloride

Water

Methanol

Nitroblue tetrazolium

3.2 Method

The total population of the study that diagnosed and monitored was ranging from 21-84 years of age which included 30 patients who had been diagnosed with rheumatoid arthritis and 30 healthy individuals. Biochemical parameters were determined by serum samples. Before the collection of blood samples for this study, local ethical committee approval was obtained from koya university faculty of science and healthy educational research and rizgary training hospital department of rheumatology and diseases clinic and laboratory research center. From selected healthy and sick individuals 4 ml blood was taken from venous conveniently as the study subject and centrifuged with 2000 r/min for 3 minutes and then the serums were separated from plasma. The separated serums were used to determine the superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels.

3.3. Analysis Method

3.3.1. Determination of superoxide dismutase (SOD) activity

Superoxide dismutase activity was determined by using the proposed method of (Popov et al., 2004). SOD accelerates the dismutation of hydrogen peroxide and molecular oxygen of superoxide radicals ($O_2^{\bullet -}$) formed during the oxidative energy production. This method is based on the reading of optic density resulted from using of xanthine and xanthine oxidase in which superoxide radicals that generated from the blue colored formazan dye of the nitro blue tetrazolium (N.B.T) in the optical density wavelength of 560 nm. The SOD that exists in the sample serum inhibits the formazan reaction by excluding superoxide radicals from the environment. Under the experimental conditions, 1 unit of SOD is the % 50 inhibition of N.B.T reduction rate.

$$\% \text{ inhibition} = (\text{blank OD} - \text{sample OD}) / \text{blank OD} \times 100$$

3.3.2. Determination of reduced glutathione (GSH) activity

All the proteins that don't carry sulfhydryl (SH) group are precipitated with precipitation solution. The glutathione (GSH) level was measured as the final product of the reaction was achieved. That was the formation of the yellow color, of obtained clear liquid of sulfhydryl groups and DTNB (5 ',5'- (dithiobis 2-nitrobenzoic acid). Measurement of the reduced glutathione level in the EDTA blood was done in 412 nm wavelength in the spectrophotometer within 24 hours (Beutler et al., 1963).

Activity (mg / dl) = [(OD2 – OD1) / 13600 x E1 1.25] x 1000

OD1 = First absorbance before addition of DTNB at 412 nm.

OD2 = Second absorbance after addition of DTNB at 412nm.

E1 = 1 in the calculations.

13600 is the molar extinction coefficient of the yellow color that formed during the interaction of GSH and DTNB.

3.3.3. Determination of catalase (CAT) activity

One empty tube and one sample tube were achieved for measurement of catalase activity. 2.8 ml of 30 mM hydrogen peroxide (H_2O_2) was placed into the empty tube and 0.2 ml of phosphate buffer was adding. The blend was shaken quickly and spectrophotometric measured (Ati Unicam UV/VIS-UV2-100, England) two times at 240 nm with thirty-second intervals. Then again, 2.8 ml of 30 mM hydrogen peroxide (H2O2) was placed into the sample tube and 0.2 ml of serum was adding. The combination was shaken quickly and absorbance was read at 240 nm in Hitachi U-2900 (Aebi, 1984).

Activity = (2,3 / Δx) x [(log A1 / log A2)];

Activity; Calculated as in U / L.

Δx = 30 seconds

2,3 = 1 μmol optical density of H_2O_2 in 1 cm light path.

3.3.4. Determination of malondialdehyde (MDA) level

The reaction of fatty acids with free radicals result in malondialdehyde, which is the final product of lipid peroxidation, is measured with thiobarbituric acid that gives a colored form (JM, 1995). 200ml from the blood is taken and put into 1 tube. 800ml phosphate buffer, 25ml BHT solution, and 500ml of %30 TCA were added. The tubes were stirred with vortex and kept on ice for 2 hours. Then centrifuged at 2000 rpm for 15 minutes. 1 ml from the supernatant was taken and transferred to other tubes. Then 75 ml of EDTA and 250 ml of TBA were added. Tubes were mixed in the vortex and kept in a hot water bath for 15 minutes. Then, they were brought to room temperature and their absorbance was read at UV / Vis spectrophotometer at 532 nm.

$$C = F * 6.41 * A$$

C: Concentration.

F: Dilution factor.

A: Absorbance.

3.4. Statistical Data Analysis

The defining statistics for the studied parameters were expressed in standard deviation. In paired group comparisons, T-test was utilized where normal deviation was achieved, and Mann-Whitney U statistics was utilized where it wasn’t. The significance level was assumed to be 5%, and all calculations were made with SPSS statistics package software.

4. RESULTS

The finding of the current study showed that 30 subjects out of 60 were healthy controls whereas the other 30 were rheumatoid arthritis cases. The results suggested a statistical significant difference in the mean of MDA, CAT, SOD and GSH levels in the rheumatoid arthritis compared with the healthy controls.

When malondialdehyde (MDA) level was examined (Table 3.1), It was proved that there was a statistically significant relationship ($p<0.05$) between control and patient group levels (0.2313 ± 0.008 µmol/L and 0.6923 ± 0.054 µmol/L, respectively).

In contrast, the relationship between control and patient group glutathione (GSH) reducing enzyme activity results (0.025 ± 0.002 µmol/L and 0.0032 ± 0.0009 µmol/L, respectively) (Table 3.1), were found to be statistically insignificant ($p<0.05$).

Furthermore, as superoxide dismutase (SOD) enzyme activity was determined (Table 3.1), the correlation between control and patient group level (0.017 ± 0.0006 U/L and 0.0016 ± 0.00003 U/L, respectively), was also found to be statistically insignificant ($p<0.05$).

On the other hand, the results of the catalase (CAT) enzyme test (Table 3.1), indicate that control and patient group levels (1216.7 ± 24.45 U/L and 330.8 ±71.60 µmol/L, respectively) were statistically significant ($p<0.05$).

Table 3.1 Comparison of control and patient group rheumatoid arthritis markers

Parameters	Controls (n= 30) **Mean±SD**	Patients (n=30) **Mean±SD**
MDA (µmol/L)	0.2313 ± 0.008	0.6923 ± 0.054**
GSH (µmol/L)	0.025 ± 0.002	0.0032 ± 0.00009 ^
SOD (U/L)	0.017 ± 0.0006	0.0016 ± 0.00003^
CAT (U/L)	1216.7 ± 24.45	330.8 ± 71.60^

$p<0.05$ The P-Value for all parameters.

The comparison of GSH, SOD, and CAT enzyme activities and serum MDA level

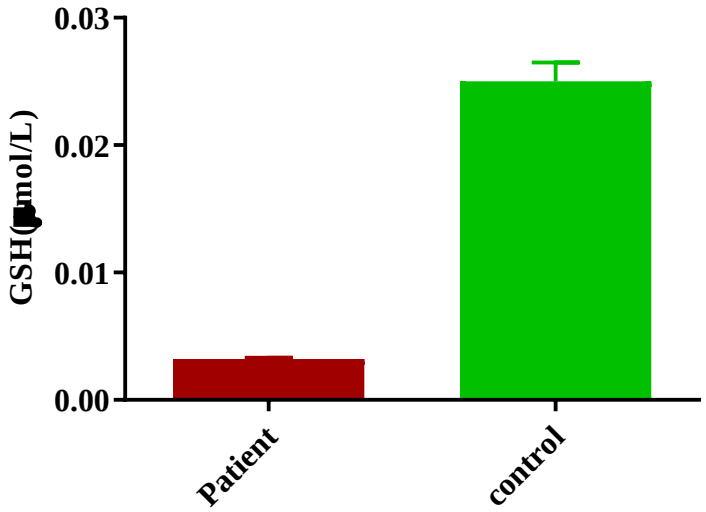

Figure 3.1. The level of GSH enzyme compared between control and patient has RA.

Nonetheless, it was found that the relationship between control and patient group GSH enzyme activities (0.025 ± 0.002 µmol/L and 0.0032 ± 0.00009 µmol/L, respectively) had a statistically insignificant relationship ($p<0.05$) (Table 3.1).

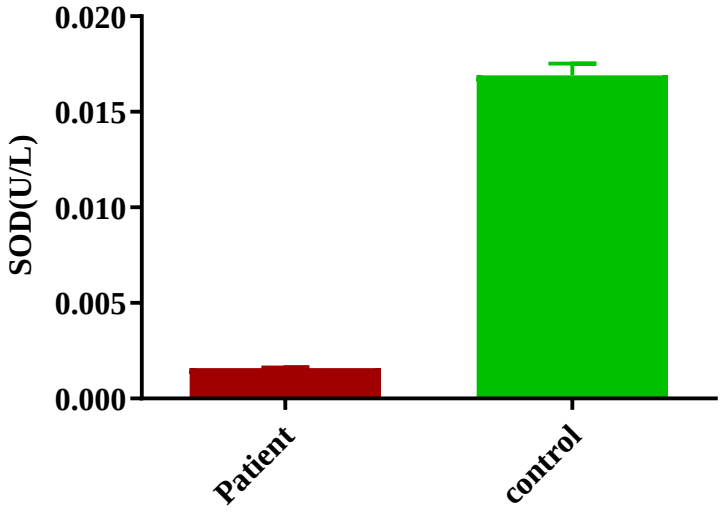

Figure 3.2. The level of SOD enzyme compared between control and patient has RA.

Moreover, the comparison of superoxide dismutase (SOD) enzyme (Table 3.1), reveals that the relationship between control and patient group levels (0.017 ± 0.0006 U/L and 0.0016 ± 0.00003 U/L, respectively) is statistically insignificant ($p<0.05$).

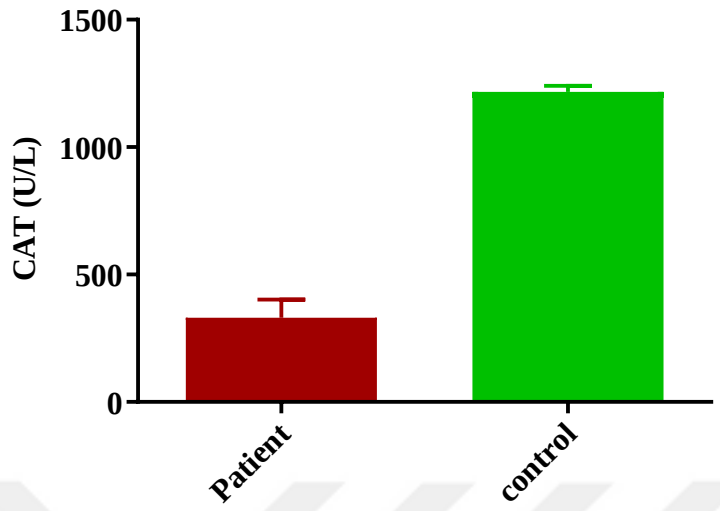

Figure 3.3. The level of CAT enzyme compared between control and patient has RA.

In addition, catalase (CAT) enzyme activity was analyzed, as shown in (Table 3.1). The results showed that the relationship ($p<0.05$) between control group levels (1216.7 ± 24.45 U/L) and patient group levels (330.8 ± 71.60 U/L) was statistically significant.

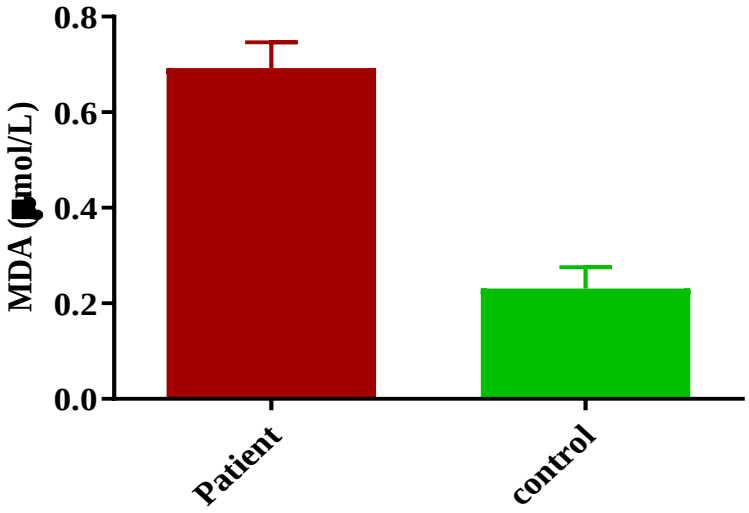

Figure 3.4. The level of MDA compared between control and patient has RA.

Finally, the comparison of MDA (malondialdehyde) activities of control and patient group (0.2313 ± 0.008 µmol/L and 0.6923 ± 0.054 µmol/L, respectively). (Table 3.1) indicates a statistically significant relationship ($p < 0.05$) between control group levels.

5. DISCUSSION AND CONCLUSION

It has been recently concluded that one of the most common diseases all around the world is rheumatoid arthritis. It is a chronic and system autoimmune inflammation disease. This disease makes the body's immune system mistakenly attack its own cells and tissues. In most countries, the affected percentage of this disease is between 0.5 to 1%. Women were shown to be three times more susceptible to the disease compared to men. Further, age also plays a role in being affected by rheumatoid arthritis. For instance, rheumatoid arthritis mostly occurs between ages 40 to 60 (Doherty et al., 2006).

The characteristic effects of the disease are swelling, stiffness, articular tissue destruction, and joint deformities, which mostly occur in peripheral joints, and through non-specific inflammatory reactions. Various physiological systems like cardiovascular, respiratory, and hematopoietic systems may also be involved in the development and course of the disease. That being said, typically the main lesion of RA is synovitis that acts a basis for pannus to form within the inflammatory synovial membrane, which may lead to complete joint dysfunctionality (Zhang et al., 2015).

It is this inflammation that causes the tendon sheathing, which is what the symptoms of the RA, shows itself with. Due to this sheathing, and the inflammation itself, the joint becomes swollen, painful to touch, warm, and stiff, particularly in the mornings. Over time RA usually spreads to other joints as well, thereafter which it is called polyarthritis. It is the small joints in hands and feet that are usually the first to be affected, although there are individual variations. Synovitis may cause the dislocations and erosion of the joint surface, and is responsible for deformity and loss of function. Many other factors such as genetic and environmental aspects, viruses, UV, and many chemical substances are also cited amongst the most common causes for diseases like rheumatoid arthritis. That being said, the main cause of rheumatoid arthritis is still not exactly clear to this day. Many risk factors were identified and numerous advancements in the understanding of the underlying disease mechanism were accomplished. RA is considered to be a multifactor disease (Alamanos et al., 2015).

Interaction between environmental and genetic factors that control the progression, degree, and inflammation strength of RA may also occur.

Furthermore, genetic, environmental, age, and gender factors seem to have a strong influence on the development of rheumatoid arthritis. The environmental risk factor involved the RA are bacteria, virus, smoking, stress, female hormones, and infection (Karlson et al., 2012).

Furthermore, family history is another significant factor that has to develop rheumatoid arthritis. In addition to the fact that RA affects the joints, people with RA may also suffer from conditions such as heart diseases and anemia, which increases the risk factor for rheumatoid arthritis (Weinblatt et al., 2007).

In the present study, the malondialdehyde level (MDA), which stands out as one of the indicators of the oxidative stress in rheumatoid arthritis and presence of other antioxidant enzymes such as glutathione reductase, catalase enzymes, superoxide dismutase, was investigated and the results between RA patients and healthy individuals were compared.

Findings of the study show that the lipid-peroxidation, in terms of MDA production in patients with RA when compared to the controls group, was statistically significantly increased ($p<0.05$). In RA patients, oxidants in high concentrations, which cause oxidative, are released by stress-activated macrophages and neutrophils. This can lead to the damage of lipids, proteins, carbohydrates, and DNA. The unsaturated fatty acids of cell membranes that react with lipid peroxidation and MDA are released. It acts as oxidative stress marker and reacts with lysine residues that lead to further production of immunogenic molecules that increase the effects of the inflammation (Hagfors et al., 2003; Walwadkar et al., 2006). The increased formation of ROS which might extend to great spread as the chronic inflammation continues may also lead to unnecessary damage to tissues. This, in turn, may result in the rise in lipid peroxidation product.

ROS attacks occur through the polyunsaturated fatty acids in the membrane lipids and might disorganize the function and structure of the cell. It may cause lipid peroxidation. Furthermore, a wide variety of end-products, such as malondialdehyde will be produced by the decomposition of per-oxidized lipids (Mishra et al., 2012). One of the most important lipid peroxides which are most common among RA patients is malondialdehyde (Mishra et al., 2012). MDA measurement is commonly used as an indicator of lipid peroxidation.

Lipid peroxidation occurs as a chain reaction in a way that involves the oxidation of polyunsaturated fatty acids in the membrane lipids stimulated by free radicals. Malondialdehyde (MDA) which is a marker for oxidative stress is one of the final products of lipid peroxidation (Moncada et al., 1991).

Reactive oxygen agents and oxidative stress take a significant part in the pathogenesis of RA. ROS comes up with a great number of pathology and many potential follow-up diseases which may lead to death. These include long inflammation and autoimmune illness such as diabetes mellitus, rheumatoid arthritis, and cardiovascular diseases like hypertension, atherosclerosis, and ischemia. Oxidative stress is also found to have a strong influence over many kinds of cancer, including lung, renal, and breast cancers (Briege et al., 2012; Salman et al., 2012).

Reactive O_2 species and free radicals which might be called reactive oxygen agents and various nitrogen species plays a role in initiating and promoting multistep carcinogenesis (Sun., 1990) states that sulfur, oxygen, and nitrogen molecules generate free radicals in the living cell. During cellular normal function, oxygen reactive agent is a product similar to hydrogen peroxide, superoxide anion, hydroxyl radical's ozone, oxygen singlet, and hydrogen peroxide. As can be seen there are numerous reactive oxygen radicals and other free radicals, which are generated from inflammatory cells inside the synovium, and contribute to pannus formation and affect joint (Halliwell et al., 2006). These are normally harmless radicals. That being said, ROS and RNS are two active and prominent sources of DNA damage when their regulation is malfunctioning. According to (Mate’s et al., 2000). These are very transient species owing to their high chemical reactivity which causes lipid per-oxidation, massive protein oxidation, degradation, and oxidation of some enzymes.

ROS develops as a typical result of aerobic metabolism. Aerobic metabolism is essential to sustain oxygen homeostasis of tissues. If the tissues fail to sustain their oxygen homeostasis, oxidative stress at the cellular level environment increases. Such as hydroxyl radicals, superoxide, and hydrogen peroxide which are constantly produced by the mitochondria of the changing cells are regular metabolic by-products. Other prominent intracellular sources of ROS include microsomal cytochrome P_{450} enzymes, peroxisomal enzymes and flavoprotein oxidases (Seifried et al., 2007).

Nitrogen species and reactive oxygen remarkably have an effect over both normal medical condition and various diseases. ROS also have a significant effect on numerous physiologic processes including normal vascular cell functioning and continuous vascular diameter regulation. This function is based on the immune response increase to limits towards ROS, and regulation of glucose uptake by skeletal muscles (Salman et al., 2012). They participate in the control of inflammatory responses and growth factor stimulation along with proliferation and apoptosis. Finally, they are reported to have influence over regulation of cytoskeleton formation, differentiation, contraction, and migration (Seifried et al., 2007).

Oxidative stress occurs as a result of the loss of balance between oxidants and antioxidant production. It results in the increased production and accumulation of oxidants in the body. It seems to have a significant effect on carcinogenesis. It has been proven that oxidative stress plays an important role in several human pathological situations like cancer, atherosclerosis, rheumatoid arthritis, malaria, smoking, diabetes, aging, with chronic inflammatory diseases (Salman et al., 2013).

Previous studies have confirmed that RA patients have increased MDA level in their serum, plasma and synovial fluid, and diminished antioxidant status. Inflammatory cells release a number of reactive species at the site of inflammation which are leading to inflated oxidative stress, whereas reactive species may urge changes in transcription factors, as well as trigger intracellular signaling cascades which encourage pro-inflammatory gene expression. When oxidative stress recurs as the primary abnormality, inflammation appears and further accentuates oxidative stress (Vaziri et al., 2006).

MDA has an impact on the pathogenesis of RA. There is an increasing concern towards the subject as reactive oxygen agent and free radicals are now being recognized for their influence over cellular injuries and tissue damage in RA cases. The current study registers the increased level of MDA in RA cases compared to controls group and to the findings of studies on RA (Hassan et al., 2010; Mateen et al., 2016), and reports that the autoimmune responses are characteristic to the rheumatoid arthritis.

The findings of this study confirmed that the major consequence of oxidative stress is lipid peroxidation. Oxidative stress in chronic inflammatory diseases can cause oxidation of biomolecules such as lipids. Lipid peroxidation is very damaging as it decreases membrane fluidity, increases membrane permeability, damages the membrane

proteins, and impairs the activity of membrane receptors, enzymes, and ion channels. Lipid peroxidation is the source for the production of numerous bioactive aldehydes like MDA.

In our study, rheumatoid arthritis that is chronic inflammation disease is another kind of inflammation disorders. In our investigation, it is clear that patients of rheumatoid arthritis and the oxidative stress were affected by many factors such as environmental factor, since MDA levels were significantly increased ($p<0.05$) in the study group as compared to healthy controls. Besides, the low level of non-enzymatic antioxidants such as vitamin A is also signaled. Vitamin A indicates the relationship between the serum antioxidant and inflammation in the rheumatoid arthritis patients.

In addition many researches showed that vitamin-D affects the pathogeneses of autoimmune diseases especially in RA vitamin D is known as a steroid hormone that takes part in physiological events related to bones and calcium metabolism, as it manipulates the gastrointestinal system to determine the absorption level of calcium-vitamin D has extra-skeletal roles as well, since the Vitamin-D deficiency was shown to have correlation with certain autoimmune diseases like RA. Some researchers acknowledged that small levels of Vitamin-D are because of the inflammatory process however, this was not always the case. Fortunately, this study clarified this. As age increases, a negative correlation is observed in which Vitamin-D levels were decreased. Significantly, lower 25-hydroxy vitamin D levels were observed between cases RA and control group ($p<0.001$) and the affected ones were mostly females, and most of the cases of rheumatoid arthritis are reported three times s more in female (Onur et al., 2016).

The mean level of SOD activity showed a statistically significant decrease in RA cases when compared to controls group in rheumatoid arthritis ($P<0.05$). The findings of the current study comply with the results of the study by (Bae et al., 2008). The decreased SOD activity may be the consequence of SOD degradation as part of the detoxifying process due to the influence of ROS. That being said (Jira et al., 2007) some studies suggested that lowered SOD activity may be caused by inhibitory effects of hydrogen peroxide. This would demonstrate that increased production of hydrogen peroxide during the dismutation reaction has influence over the process (Kalpakcioglu et al., 2008). Such a result would also be in concordance with other researches

performed on the subject (Mohamad et al., 2011). Whereas, possible increased activity of SOD (Vijayakumar et al., 2006) might be attributed to higher (O_2-) production by hyperactive cells, causing SOD to trigger. Immoderate free radicals produced by the (xanthine, xanthine oxidase) path may also be effective over the process and even be the primary cause in RA, instead of a function antioxidant metabolism (Cimen et al., 2000). In other high-SOD activity cases, the cause might be the difficulty in nullification of the excessive amounts of free radicals produced. The antioxidants-treatment after the fact may lead to decreased plasma MDA activity and higher total antioxidant capacity (Nourmohammadi et al., 2010).

Superoxides anion (O_2-) has a great effect on the pathogenesis of many diseases (Henrotin et al., 2005). When it enters a reaction with SOD, it is reduced to H_2O_2, which can further be satisfied by catalase and glutathione peroxidase interaction. When (O_2-) is thusly reduced into H_2O_2, overly-active compounds like (ONOO-) and ($^{\cdot}OH$) is also suppressed. That would mean that various radicals, including the superoxide anion, are not duly detoxified in RA cases due to deficiencies in enzymatic and non-enzymatic antioxidant systems working as mediators for tissue and cell damage. As was discussed before, neutrophils create large volumes of superoxide anion along with H_2O_2 as part of their increased activities, which are then consumed by erythrocytes in circulation.

In addition, SOD can be considered as the initial defense against free radical creation. SOD is well necessary for the alteration of superoxide radicals into H_2O_2 and O_2. If this was not the case, it would have led to the deactivation of catalase, along with glutathione peroxidase. The reduced SOD activity may also occur as a result of the ROS related downgrade of SOD as detoxification occurs. That being said (Jira et al., 2007) certain studies have confirmed that reduced SOD activity may also occur as a consequence of H_2O_2 related enzyme suppression. Such a phenomenon would demonstrate the increased H_2O_2 generation as part of the dismutation reaction (Kalpakcioglu et al., 2008). The reduced erythrocyte-SOD activity also agrees with other research (Mohamad et al., 2011). On the other hand, reduced SOD activity was revealed in RA patient groups on MTX treatment, compared to the patients that are not (Youzbaki et al., 2013). Moreover, MTX direct or indirect suppression over active oxygen metabolite production was revealed, which was initiated by the presence of IL-6. IL-6 is created in return to TNF-α stimulation in RA influenced synovial tissues (Sung et

al., 2000), along with polymorph nuclear cells. (Cu/ZnSOD) activity is found to be elevated on these patients, which is in line with the radical-related injury theory.

Increased SOD presence causes superoxide dis-mutation which is resulting in the accumulation of H_2O_2. The analysis of H_2O_2 in different settings directs the writer to come to the conclusion that more SOD does not mean more H_2O_2 (Lin et al., 2006). Superoxide amount is the limiting factor for its dismutation, and as such, the formation of hydrogen peroxide increases. Superoxide creation is slower compared to transformation into H_2O_2. When the superoxide accumulates, it promotes the oxidation of NO, which causes peroxynitrite formation. That is even higher hydrogen peroxide levels may be non-toxic, as only a meager cytokine (H_2O_2) would match a potent (peroxynitrite) (Mohamad et al., 2011).

The involvement of both, the environmental and genetic factors in the etiology of rheumatoid arthritis (Rahman et al., 2006) is crystal clear. The immune regulatory role of vitamin D and its relationship with auto-immunity confirms that vitamin D may be a great environmental factor that involves the self-tolerance control in autoimmune rheumatic

Nevertheless, there are other studies that have conducted after treatment of rheumatoid arthritis that distinguish between the antioxidant enzyme levels and MDA in smoking patients with RA. In view of the fact that when the process of inflammation expands, the level of oxidants and antioxidants changes; in accordance with the phase of rheumatoid arthritis. To begin with, the level of antioxidants increases and later decreases in the current research, which might lead to the lack of noticeable differences between antioxidants and oxidants in smoking patients with rheumatoid arthritis.

The antioxidants in tissues can be summarized in two main groups as enzymatic and non-enzymatic antioxidants. The enzymatic antioxidants can further be classified into primary and secondary enzymatic defenses. These preserve the cell and tissue from free radicals and oxidative stress (Carocho et al., 2013). The primary enzymatic defense consists of three important enzymes that suppress the free radical generation and neutralize them. Glutathione peroxidase in one of them, and it offers two electrons which decrease peroxides by the formation of selenium. In addition, it terminates peroxides by including them in the Fenton reaction substrate catalases. Catalase transforms H_2O_2 into H_2O and molecular oxygen, and as such is the source of a very

potent antioxidant. A single molecule of catalase is capable of converting quite number of H_2O_2 molecules (Rahman et al., 2007). Finally, superoxide dismutase transforms superoxide anions into H_2O_2 to be used in various catalytic reactions as a substrate.

The secondary line of defense covers glucose-6-phosphate dehydrogenase enzyme, known as the reductase Glutathione which lowers glutathione antioxidants from their oxidized states reduced forms. This is a recycling process, similar to a chain reaction which keeps neutralizing even more free radicals (Rahman et al., 2006). Glucose-6-phosphate restores NADPH, which contributes towards an environment with reduction capabilities. Though these two enzymes don't directly neutralize free radicals, they support the primary enzymatic defense antioxidant. Non-enzymatic antioxidants line includes several types of molecules like enzyme cofactors, minerals (zinc, selenium, and Q10) and also vitamin (C and E) (Carocho et al., 2013).

There are several researches that show a strong synergism which is available between GSH, vitamin (C and E). Vitamin C is a water-soluble and also well-functioning antioxidant. It especially proceeds as a scavenger for the free radicals. Its major role in action is extracellular fluid and water compartments. It has a great role in protecting many different diseases like rheumatoid arthritis, coronary, artery disease, cancer, and aging (Padayatty et al., 2003).

Vitamin E. is a water-soluble vitamin with high antioxidant potential. It exists in the cell membranes of the body which is usually saved in the form of adipose tissue. The cell membranes are vitamin E's main site of action, where it stops the damage by free radicals by preventing the lipid peroxidation. It is thought that Vitamin E is very effective against many kinds of cancers diseases example Brest cancer, colon cancer. Also protects many different degenerative diseases like rheumatoid arthritis, cardiovascular diseases and neurological disorders (Mayo., 2005).

H_2O_2 is generated as a result of superoxide dismutase interactions but needs to be inactivated by glutathione peroxidase and catalase interaction. This interaction has a significant role as a barrier between the ROS-mediated damage by using hydrogen peroxide. Rheumatoid arthritis patients have significantly reduced catalase activity as compared to healthy individuals. This reduced catalase activity might be a result of the interaction of catalase with hydrogen peroxide (Mohamad et al., 2011). Lowered activities of their enzymes may turn H_2O_2 inside radical hydroxyl when the iron

molecule is set free from the hemoglobin of the destroyed erythrocytes (Taysi et al., 2002).

In this study, the resulting level of catalase activity was statistically and significantly decreased when it is compared to the controls group in rheumatoid arthritis ($P<0.05$). This decreased catalase activity in RA group may have occurred due to catalase being inactivated by H_2O_2. Both of which also show reduced catalase activity RA patients serum. Catalase expression influences the expression of genes which affect inflammation (Vasanthi et al., 2004). The reduced catalase level of RA patient may be explained with increased inflammation. Catalase causes of the changes H_2O_2 into H_2O andO_2. Consequently, it preserves the cells from the harmful effects of accumulated hydrogen peroxide. This result is in accordance with other findings (Mohamad et al., 2011).

It is also acknowledged that catalase activity is reduced in the liver and brain of rats with RA (Cimen et al., 2002). Two enzymes catalase and GPx use hydrogen peroxide as a substrate. Catalase takes effect when a high volume of the substrate is present, but GPx works at low amounts of substrate presence. It is also believed that hydrogen peroxide focus might be lower compared to other long inflammatory diseases and that it is possible that the oxidative damage is regulated by hydroxyl radicals which contributes to maintaining homeostasis in aerobic organisms. The loss of this balance in enzymatic antioxidant causes oxidative damage that leads to inflammation on the hand and feet joint; they cannot work properly, and they will be deformed. This is known as oxidative stress. It has been identified as decreased levels of other antioxidant status markers such as catalase in rheumatoid arthritis (Desai et al., 2010; El-Barbary et al., 2011).

In this study, our result level of GSH activity was statistically and significantly decreased when it is compared to the controls group in rheumatoid arthritis ($P<0.05$). Reducing glutathione is a flavoenzyme and it depends on NADPH which takes part in reducing for GSSH into GSH provided by glucose-6-phosphate dehydrogenase. Riboflavin has a great significance for NADP-NADPH cycling (Feijoo et al., 2010). Glutathione reductase also takes part as a peroxyl scavenging mechanism. GSH is a non-protein sulfhydryl molecule and is considered as a very essential antioxidant defense system for body metabolism. The molecule acts as an intra-cellular reluctant in

redox reactions by keeping the cellular element protected against potential damaging ROS. Low concentration of GSH reported in the plasma of RA patients is in line with some other studies as well (Renke et al., 2000; Jaswal et al., 2003).

The balance of ROS molecules against antioxidant molecules is fragile but important to illustrate, in humans, loss of this equilibrium can lead to serious medical problems, such as rheumatoid arthritis, cardiovascular and cancer (Valko et al., 2007).

Reduced SOD and GSH activity levels in patients with RA may result in a degradation of these antioxidant enzymes by free radicals such as superoxide radicals and hydrogen peroxide during detoxification processes.

These antioxidant processes may occur as a result of the decrease in antioxidant vitamin levels. This is known in rheumatoid diseases, especially in RA. Reduced SOD activity levels may take place owing to the disturbance in Cu and Zn levels. Another study by (Jalili et al., 2014) acknowledged that antioxidants could help reduce the disease activity, even though they may fail to help with the aching and swollen joint (Jalili et al., 2014). In that regard, antioxidant is candidates for support agents that may influence the outcomes and help alleviate the oxidative stress of individuals with RA. To conclude, RA patients may receive support from antioxidant therapy along with DMARD. Combined with catalase and GPx antioxidants helpful effects might be elevated.

Many risk factor for formation rheumatoid arthritis in many country of the world is the most prevalent risk factors favoring lithogenesis according to the results of the study were age, sex, metabolic syndromes, marital status, smoking, family history, physical activity, some biochemical parameters and some oxidative stress markers in the study. Some studies have shown that after radiotherapy of patients with lips-oral cavity cancer will come down the elevated of lipid peroxidation and increase the levels of antioxidant.

In the present study, it can be understood that antioxidants level like SOD, CAT, and GSH are reduced, and oxidative stress is enhanced as evidenced by elevated levels of lipid peroxidation like malondialdehyde (MDA) in patients with RA, compared to control group. The results indicate that oxidative stress is related to rheumatoid arthritis, and can increase the adverse effects of the disease. The majority of malignant rheumatoid arthritis cases consist of squamous cell carcinomas. Genetic and

environmental factors such as smoking, air pollution with lifestyle are the main carcinogens that are related to rheumatoid arthritis, and the human papillomavirus has a small but important part as well. ROS are formed as part of normal metabolic processes. They act as signaling agents that regulate the responses of living cells. When ROS level in the cell increases beyond a certain limit, it may lead to oxidative damage to DNA, and other proteins and lipids in the cell. Nowadays, it is accepted that ROS generated in the cells are involved in the redox regulation of signal transduction pathways.

In conclusion, our results also support all the previous literature findings and understanding that oxidative stress is influential in rheumatoid arthritis, antioxidant supplementation can help alleviate the adverse effects of the oxidative stress, and can help reduce the disease activity in RA patients. However, our findings also suggest that more randomized-controlled clinical trials are needed to evaluate the true influence level of antioxidant therapy for different stages RA.

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EXTENDED TURKISH SUMMARY (GENİŞLETİLMİŞ TÜRKÇE ÖZET)

ROMATOİD ARTRİTLERDE OKSİDATİF STRES SEVİYELERİNİN VE BAZI ANTİOKSİDAN ENZİM FAALİYETLERİNİN BELİRLENMESİ

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Temmuz 2019, 139 Sayfa

Öz

Oksidatif stresin ve antioksidan durumunun Romatoid artritte çok önemli bir rol oynadığı iyi bilinmektedir. Oksidatif stres düzeyleri ve antioksidan parametreler hakkında daha fazla araştırma yapmak için, bu çalışmada 60 denek çalışılmış, Romatoid artrit şikayeti olan 30 hasta ve bunlardan 30'u kontrol grubu olarak herhangi bir hastalığı bulunmayan sağlıklı deneklerdir. Bu çalışmada, Kuzey Irak Bölgesi Rizgary Eğitim Hastanesi Romatoloji Bölümü ve CMC özel hastanesinde kan örnekleri toplandı. Hasta ve sağlıklı bireylerden kan örnekleri alındı. Her iki grubun serumları, lipit peroksidasyonunun son ürünü olan malondialdehit (MDA) seviyesi ve antioksidan enzim aktivitelerinden süperoksit dismutaz (SOD), glutatyon (GSH) ve katalaz enzim aktivitesi belirlendi. Veriler, hasta grubundaki ortalama serum MDA düzeyinin kontrol grubuna göre anlamlı derecede yüksek olduğunu göstermektedir ($p < 0.05$). Hasta grubunun ortalama süperoksit dismutaz SOD aktivitesi romatoid artrit grubu sağlıklı kontrol grubundan anlamlı derecede düşük bulundu ($p < 0.05$) (Tablo 3.1). Hasta grubunun ortalama serum GSH ve CAT düzeylerinde kontrol grubuna göre anlamlı bir fark bulundu ($p < 0.05$). Bu çalışmada, oksidatif stresin, Romatoid artrit (RA) hastalarında dokunun hücresel hasarını çok iyi etkilediğini göstermektedir.

1. GİRİŞ

Romatoid artrit kronik bir oto-bağışıklık hastalığıdır ve tüm dünyada bu hastalık mevcuttur (Doherty ve ark., 2006). T hücrelerinin ve sitokinlerin RA gelişiminde etkili olmasına rağmen, reaksiyonun bir parçası olarak aktif olan makrofajlar da oksijen radikalleri oluşturarak yer alır. Bu enflamatuar hücreler, sinoviyuma taşınır ve etkilerini, özellikle bazı enflamatuvar yanlısı mediatörleri serbest bırakarak oluştururlar (Edwards ve ark., 1997; Bellucci ve ark., 2016). Bu değişiklikler sinovyal hiperplaziye neden olur ve uzun süre boyunca kalıcı olarak etkilenen kemiklere ve kıkırdaklara zarar verir. RA'nın görülme oranı dünya nüfusunun% 0,5 ila %1 inde yaygın olduğuna inanılmaktadır (Senna ve ark., 2004). Kadınların, erkeklere kıyasla RA'ya karşı üç kat daha duyarlı oldukları gösterilmiştir. Benzer şekilde, RA tüm yaş gruplarında görülebilirken, 40 ve 50 yaşları hastalığın en sık görüldüğü yerlerdir. RA'nın kesin nedeni hala bilinmemektedir (Haris ve ark., 1989; Krane ve ark., 1989). Ayrıca, yapılan çok sayıda çalışma, çevre koşullarına benzer enfeksiyonlar ve sigara ile tetiklenen genetik faktörlerin, immün sistemi ile ilgili problemlerinin ve eklem dokusu hasarında bulunan inflamatuar sistemlerin RA' nın altında yatan nedenler olabileceğini öne sürülmüştür (Özkan ve ark., 2007).

RA'da iltihaplanmayı başlatan ajanlar açıkça tanımlanmıştır ve ayrıca, sitokinler, büyüme faktörleri, kemokinin, yapışma molekülleri ve matris metaloproteinazdan meydana gelmiştir. Bu ajanlar yukarıda belirtilen hücreleri dolaşımdan çeker ve aktive eder ve sinoviyositlerin çoğalmasına ve aktive olmasına neden olur. Özellikle proteazların neden olduğu genel değişim, eklem kıkırdaklarına, kemiklere, tendonlara ve ligamentlere zarar verebilecek lokalize bir tümöre oldukça benzerdir (Sommer ve ark., 2005). Çeşitli çalışmalar bu iddiayı desteklemekte ve RA'nın patofizyolojisinde RA'nın etkili olduğunu bildirmektedir (Öztürk ve ark., 1999).

RA deney hayvanlarında ROS oluşumunu ve lipit ve protein oksidasyon göstergelerini arttırdığı gösterilmiştir. RA hastalarının ayrıca serumlarında, beyinlerinde, karaciğerlerinde ve damar dokularında oksidatif durumlarında değişiklik olduğu gösterilmiştir.

Bu çalışmanın temel amacı, bazı antioksidan enzimlerin yanı sıra malondialdehit

(MDA) gibi lipid peroksidasyon göstergelerinin varlığını ve etkisini ortaya çıkarmak için RA hastalarındaki GSH, CAT ve SOD enzim aktivitelerini ölçmektir.

2. Materyal ve Yöntem

2.1. Materyal

Bu Araştırmada sağlıklı 30 erkek ve kadın ve 30 romatoid artrit hastasından kan alındı. Sağlıklı ve hasta bireylerin her birinden, antekubital venöz venden 4 ml kan alındı ve biyokimya tüpüne 2 ml, serum tüpüne ise 2 ml ilave edildi.

2.1.1. Cihazlar ve malzemeler

Vortex

Derin Dondurucu Tüpler

Serum Tüpleri

Spektrofotometre

Ayarlanabilir Otomotiv Pipetleri

Thermo ile belirtilen Su Banyosu

Cam pipet

Soğutmalı santrifüj

Derin dondurucu

Fırın

Kronometre

Hassas terazi

Spektrofotometre küveti

Otomatik Pipet Ucu

pH ölçer

deney şişesi

kuvvartz

Test tüpü

Cam şişe

Spatula

Huni

Filtre kağıdı

2.1.2. Reaktifler ve kimyasallar

Potasyum hidroksit

Hidrojen peroksit

Potasyum mono fosfat

Disodyum fosfat

Sodyum hidroksit

Sodyum sitrat

Elman’ın reaktifi (5,5'-ditiobis- (2-nitrobenzoik asit).

Ksantin

Etilen tetra asidik asit

Sodyum bikarbonat

Sığır serum albumini

Ksantin oksidaz

Amonyum sülfat

Bakır klorür

Etilen diaminetetra asetik asit disodyum

Butil hidroksil toluen

Tiyobarbitürik asit

Trikoasetik asit

Sodyum klorit

Su

Metanol

Nitroblue tetrazolyum

2.2.Yöntem

Bu çalışmada, romatoid artrit teşhisi konulmuş 30 hasta ve 30 sağlıklı bireyden oluşan ve yaşları 21-84 arası bireylerden seçildi. Biyokimyasal parametreler serum örnekleri ile belirlendi. Bu çalışma için kan örneklerinin alınmasından önce, Irak Koya Üniversitesi Sağlık Eğitim Araştırmaları ile romatoloji hastalıklar kliniği ve laboratuvar araştırma merkezindeki Rizgary Eğitim Hastanesi bölümünden yerel etik kurul onayı alındı. Seçilmiş sağlıklı ve hasta bireylerden, usulüne uygun olarak venözden 4 ml kan alındı ve 2000 rpm/dk ile 5 dakika santrifüj edildi ve ardından serumlar plazmadan ayrıldı. Ayrılan serumlar, süperoksit dismutaz (SOD), redükte glutatyon (GSH), glutatyon peroksidaz (GPx) ve malondialdehit (MDA) seviyelerini belirlemek için kullanıldı.

Süperoksit dismutaz (SOD) aktivitesi tayini

A-) (Manuel Yöntem)

Reaktif Çözeltisinin Hazırlanışı:

1. 0.3 mM Ksantin: 4.56 mg ksantin (Sigma X7375) önce birkaç damla 1N NaOH de çözüldü ve 100 ml bidistile suda çözüldü.
2. 0.6 mM EDTA: 4.46 mg EDTA 20 ml bidistile suda çözüldü.
3. 150 mg/L NBT: 12.3 mg NBT (Sigma N6876) 100 ml bidistile suda çözüldü.
4. 400 mM Na_2CO_3: 2.544 g Na_2CO_3 60 ml bidistile suda çözüldü.
5. Sığır serum albümin (1g/L): 12 mg BSA (Sigma A2153) 12 ml bidistile suda çözüldü.

Reaktif çözeltinin hazırlanışı: 40 ml ksantin çözeltisi, 20 ml EDTA çözeltisi, 20 ml NBT çözeltisi, 12 ml Na_2CO_3 çözeltisi, 6 ml BSA'yı karıştırıldı.(Koyu renkli bir şişede saklayınız)

- Ksantin oksidaz (167 u/L)(Sigma X1875) enziminden 16 µl alınıp, 1 ml 2 M $(NH_4)_2SO_4$ da çözüldü.
- 2M $(NH_4)_2SO_4$: 2.643 g $(NH_4)_2SO_4$ 10 ml'ye saf su ile tamamlandı (+4 ˚C'de muhafaza edildi).
- 0,8 mM $CuCl_2.2H_2O$ 13.6 mg $CuCl_2.2H_2O$ hazırlandı, 100 ml'ye saf su ile tamamlandı.

Çizelge 1.1. SOD aktivitesi tayin yöntemi

Reaktif	Kör	Örnek
Reaktif	1.425µl	1.425 µl
Örnek	50 µl	50 µl
Bidistile	100 µl	100 µl
Ksantin Oksidaz	25 µl	25 µl
Cu cl_2	50 µl	50 µl

Çizelge 1.1.'de belirtildiği gibi pipetlemeler yapıldıktan sonra, kör ve örnek tüpleri 560 nm'de bidistile suya karşı okundu.

Aktivite Hesabı:

% inhibisyon: [(Kör OD – Numune OD) / Kör OD] x 100

1 Ünite SOD: NBT redüksiyonunu %50 inhibe eden enzim aktivitesidir.

Aktivite= (% inhibisyon) / (50 x 0.1)

Aktivite; U/ml cinsinden hesaplandı.

Katalaz (CAT) aktivitesi tayini

Hidrojen peroksidin substrat olarak kullanılan bu çalışmada Aeibi yöntemine göre katalaz aktivitesi belirlendi. Aktivite şu şekilde yapıldı önce iki tüp alındı kör tüpüne 1.4 ml 30 mM'lık H_2O_2 ilave edilir ve üzerine 0.1 ml fosfat tamponu eklenir. Numune tübüne ise 1.4 ml 30 mM'lık H_2O_2 ilave edilir. Üzerine 0.1 ml enzim eklenerek vortexle karıştırıldı. 30 saniye aralıklarla iki defa 240 nm'de absorbanslar okundu ve böylece aktivite tayin edildi (Aeibi., 1984).

Kullanılan çözeltiler:

1. 30 mM H_2O_2'nin hazırlanışı: 10 ml bidistile suyun içine, % 30'lik H_2O_2'den 34 µl alınarak konuldu (% 35'lik H_2O_2'den 25,8 µl alınarak konuldu).
2. 50 mM Fosfat Tamponunun hazırlanışı: 6.81 ″ g ″ KH_2PO_4 ve 7.1″ g ″ Na_2HPO_4 bidistile suda çözülerek, tamponun Ph'ı 1N NaOH ile 7.4'e ayarlandı ve hacim 1 litreye tamamlandı.

Aktivite Hesabı:

E.Ü.= (2,3 / Δx) x [(log A_1 / log A_2)] Aktivite; U/L cinsinden hesaplandı.

Δx= 30 saniye

2,3= 1 µmol H_2O_2'nin 1 cm'lik ışık yolunda verdiği optik dansisite

Malondialdehit (MDA) düzeyi tayini

Kullanılan çözeltiler:

1-) 0.1 M EDTA çözeltisi (Etilen diamin tetra asetik asit disodyum): 37.224 gr EDTA-Na_2H_2O 1 litre bidistile suda eritildi.

2-) % 88'lik BHT çözeltisi (Bütil hidroksi toluen): 0.220 ″ g ″ BHT, 25 ml saf alkolde çözüldü.

3-) 0.05 N NaOH çözeltisi (Sodyum hidroksit): 2 ″ g ″ gr NaOH, 1 lt bidistile suda eritildi.

4-) % 1'lik TBA çözeltisi (Tiobarbitürik asit) : 1 ″ g ″ TBA 100 ml'ye 0.05 N NaOH ile tamamlandı.

5-) % 30'luk TCA çözeltisi (trikloroasetik asit) : 30 ″ g ″ TCA, 100 ml distile suda eritildi.

6-) Fosfat Tamponu: 8.1 ″ g ″ NaCl, 2.302 ″ g ″ Na_2HPO_4, 0.194 gr NaH_2PO_4 bidistile suda eritilerek 1 lt'ye tamalandı. pH'sı 1N NaOH ile 7.4'e ayarlandı.

Deneyin yapılışı:

Bir tüpe serumdan 200 µl alındı. Üzerine 800 µl fosfat tamponu ve 25 µl BHT çözeltisi ve 500 µl % 30' luk TCA eklendi. Tüpler vortekste karıştırıldı, kapakları kapatıldıktan sonra 2 saat buz banyosunda tutuldu. Tüpler oda sıcaklığına getirildi. Daha sonra, tüplerin kapakları çıkartıldıktan sonra, 15 dk 2000 rpm'de santrifüj edildi. Santrifüjden elde edilen süpernatantın (süzüntünün) 1 ml'si alınarak başka tüplere aktarıldı. 1 ml'si alınan süzüntülerin üzerine 75 µl EDTA, 25 µl TBA eklendi. Tüpler vortekste karıştırıldı ve 15 dk (70˚C) sıcak su banyosunda tutuldu. Sonra oda ısısına getirilerek 532 nm'de UV/Vis spektrofotometrede absorbansları okundu.

Malondialdehit düzeyi hesaplaması:

C= konsantrasyon

F=Seyreltme faktörü

A=Absorbans

C= F x 6.41 x A

Düzey hesabı; µmol/L olarak hesaplandı.

Redükte glutatyon (GSH) tayini

Kullanılan çözeltiler:

1. Fosfat tamponu: 0.3 M disodyum fosfat bidistile su ile hazırlanır.

2. Ellman's ayıracı:; %1 sodyum sitrat, 100 ml'ye bidistile su içinde eritilir. İçerisine 40 mg DTNB (5',5'-(2-ditiobis nitrobenzoik asit) eklendi.

GSH tayin yöntemi:

1-) 200 µl serum üzerine 800 µl fosfat tamponu eklendi. 412 nm'de ilk absorbans (OD_1) kaydedildi. Aynı tübe 100 µl Ellman's ayıracı ilave edildi, 2.absorbans (OD_2) kaydedildi.

Hesaplama:

Glutatyon derişimi mmol/g protein biriminden hesaplandı.

$C / 1000 = (OD_2\text{-}OD_1) / 13600 \times E_1 \times 5/2 \times ½$

13600: GSH ile DTNB etkileşimi sırasında oluşan sarı rengin molar ekstinksiyon katsayısı.

E_1: Eni 6 nm'den büyük olan bant kullanılırsa hem ışık yolu hem de bant genişliği farklarını düzelten bir türev ekstrinksiyon katsayısı kullanılır. Bizim kullandığımız bantın eni 2 nm'dir. Hesaplamalarda E1=1 olarak alındı.

1000: mmol'e dönüşüm katsayısı.

C: mmol / glutatyon (mg/dl)

OD_1: DTNB ilave edilmeden önce 412 nm dalga boyunda ölçülecek optik dansite.

OD_2: DTNB ilave edildikten sonra 412 nm dalga boyunda ölçülecek optik dansite.

İstatistiksel Analiz

Çalışılan parametreler için tanımlayıcı istatistikler standart sapmada ifade edildi. Eşleştirilmiş grup karşılaştırmalarında normal sapmanın sağlandığı yerde T testi, olmadığı yerlerde Mann-Whitney U istatistikleri kullanılmıştır. Anlamlılık düzeyi% 5 olarak kabul edildi ve tüm hesaplamalar SPSS istatistik paket yazılımı ile yapıldı.

3. SONUÇLAR

Romatoid artritte MDA, CAT, SOD ve GSH düzeylerinin sağlıklı kontrollerle karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık gösterdi. Malondialdehit (MDA) düzeyi incelendiğinde (Tablo 3.1), kontrol grubu ile hasta grubu düzeyleri arasında sırasıyla (0.2313 ± 0.008 µmol/L ve 0.6923 ± 0.054 µmol/L) istatistiksel olarak anlamlı bir ilişki olduğu saptandı ($p<0.05$).

Buna karşılık, kontrol grubu ile hasta grubu arasında redükte glutatyon (GSH) arasında (sırasıyla 0.025 ± 0.002 µmol/L ve 0.0032 ± 0.0009 µmol/L) (Tablo 3.1) istatistiksel olarak anlamlı bulundu ($p<0.05$).

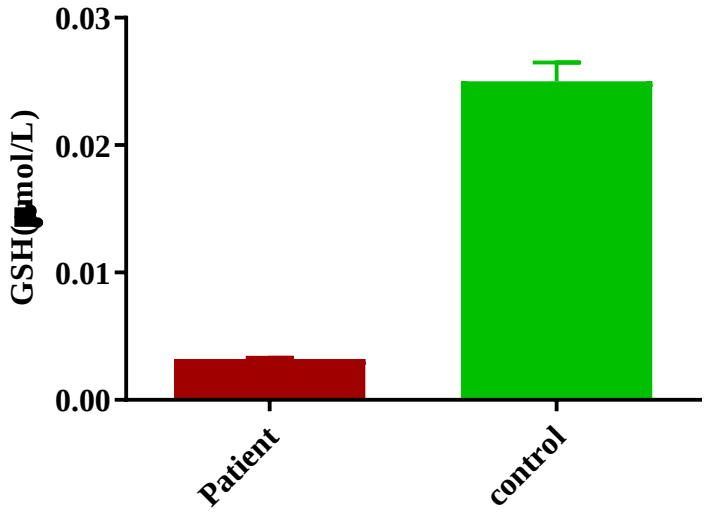
Ayrıca, süperoksit dismutaz (SOD) enzim aktivitesi (Tablo 3.1), kontrol grubu ile hasta grubu aktivitesi(sırasıyla 0.017 ± 0.0006 U/L ve 0.0016 ± 0.00003 U/L) arasındaki korelasyonun da istatistiksel olarak önemli olduğu bulundu ($p<0.05$).

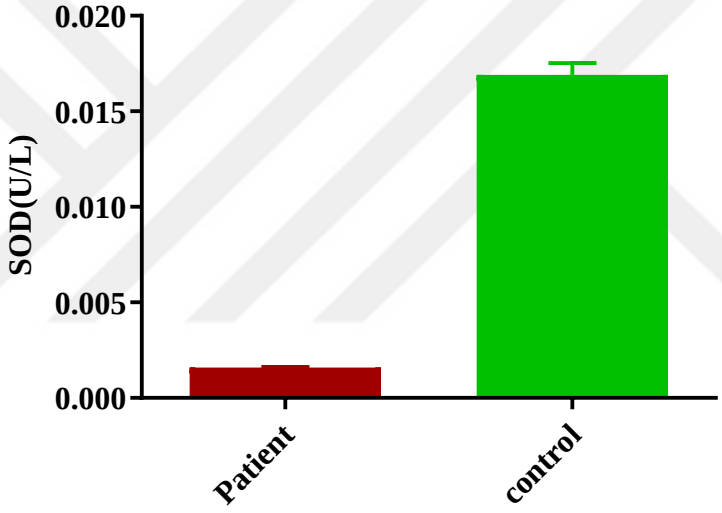
Öte yandan, katalaz (CAT) enzim aktivitesi (Tablo 3.1), kontrol ve hasta grubu seviyelerinin (sırasıyla 1216.7 ± 24.45 U/L ve 330.8 ± 71.60 µmol/L) istatistiksel olarak anlamlı olduğunu göstermiştir ($p< 0.05$).

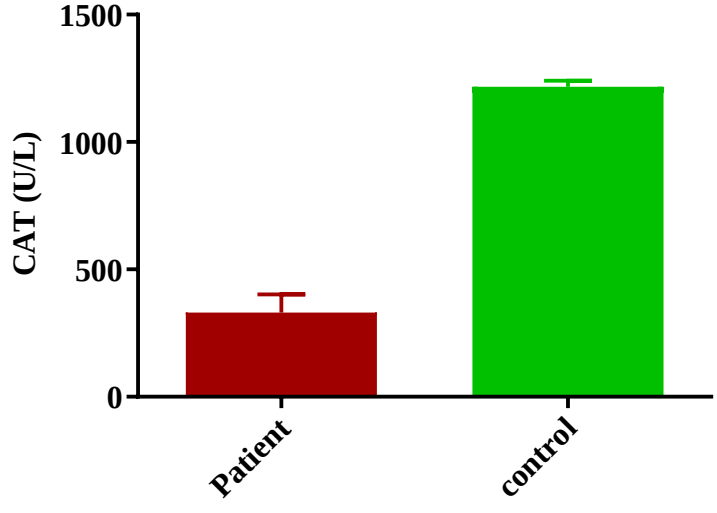
Tablo 3.1 Kontrol ve hasta grubu romatoid artrit belirteçlerinin karşılaştırılması:

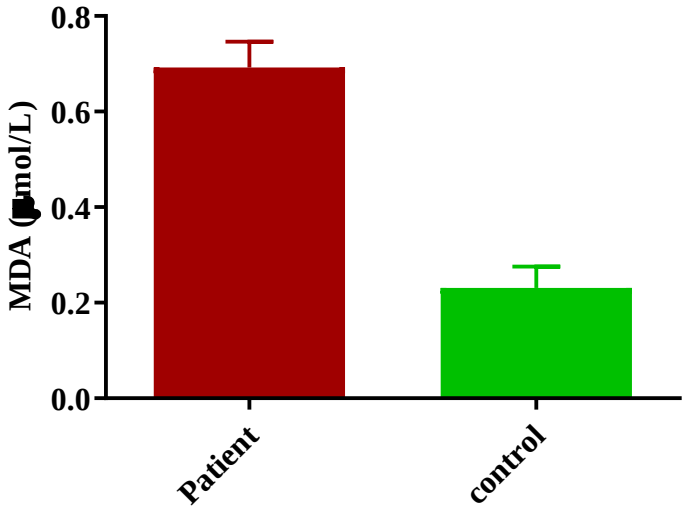
Parametreler	Kontrol grubu (n= 30) Mean±SD	Hasta grubu (n=30) Mean±SD
MDA (µmol/L)	0.2313 ± 0.008	0.6923 ± 0.054**
GSH (µmol/L)	0.025 ± 0.002	0.0032 ± 0.00009 ^
SOD (U/L)	0.017 ± 0.0006	0.0016 ± 0.00003^
CAT (U/L)	1216.7 ± 24.45	330.8 ± 71.60^

$p<0.05$: anlamlı kabul edildi


0.03
0.02
0.01
0.00
Patient
control


SOD(U/L)
0.020
0.015
0.010
0.005
0.000
Patient
control


CAT (U/L)
1500
1000
500
0
Patient
control



4. TARTIŞMA VE SONUÇ

Son zamanlarda tüm dünyada en yaygın hastalıklardan birinin romatoid artrit olduğu ortaya konulmuştur. Bu hastalık aynı zamanda, kronik ve multisistemik bir otoimmün enflamasyon hastalığıdır. Bu hastalık vücudun bağışıklık sisteminin yanlışlıkla kendi hücrelerine ve dokularına saldırmasını sağlar. Çoğu ülkede, bu hastalıktan etkilen etkilenen % 0.5 ile % 1 arasındadır. Kadınların, erkeklerle karşılaştırıldığında hastalığa üç kat daha duyarlı oldukları görülmüştür. Ayrıca, yaş faktörü bu hastalığı etkilenmede rol oynamaktadır. Örneğin, romatoid artrit daha çok 40 ila 60 yaşları arasında görülür (Doherty ve ark., 2006).

Bu çalışmada, romatoid artrit hastalığında oksidatif stresin göstergelerinden biri olan malondialdehit seviyesi (MDA) araştırılmış ve redükte glutatyon, süperoksit dismutaz ve katalaz gibi antioksidan enzimlerin RA daki hastaları ile sağlıklı bireyler karşılaştırıldı.

Çalışmanın bulguları, lipid-peroksidasyonunun bir götergesi olan MDA'nın, RA'lı hastalarda kontrol grubuna kıyasla MDA üretimi açısından istatistiksel olarak anlamlı şekilde arttığını göstermiştir ($p<0.05$). RA hastalarında, oksidatif olan yüksek konsantrasyonlardaki oksidanlar, stresle aktive olan makrofajlar ve nötrofiller tarafından salınır. Bu, lipidlerin, proteinlerin, karbonhidratların ve DNA'nın zarar görmesine neden

olabilir. Lipid peroksidasyonu ve MDA ile reaksiyona giren hücre zarlarının doymamış yağ asitleri serbest bırakılır. Oksidatif stres belirteci olarak işlev görür ve iltihaplanma etkilerini artıran immünojenik moleküllerin daha fazla üretimine yol açan lisin kalıntılarıyla reaksiyona girer (Hagfors ve diğerleri., 2003; Walwadkar ve diğerleri, 2006). Kronik iltihap devam ettikçe büyük yayılım gösterebilecek ROS oluşumunun artması da dokulara gereksiz yere zarar verebilir. Bu da, lipid peroksidasyon ürününde artışa neden olabilir. ROS saldırıları, membran lipidlerinde çoklu doymamış yağ asitleri vasıtasıyla meydana gelir ve hücrenin işlevini bozabilir, sonuçta lipit peroksidasyonuna neden olabilir. Ayrıca, malondialdehit gibi lipid peroksidasyonun son ürünü, oksitlenmiş lipidlerin ayrıştırılmasıyla üretilecektir (Mishra ve diğerleri., 2012). RA hastalarında en sık görülen lipit peroksitlerden biri malondialdehit'tir (Mishra ve ark., 2012). MDA ölçümü yaygın olarak lipit peroksidasyonunun bir göstergesi olarak kullanılır. Yapılan çalışmalarda RA hastalarının serum, plazma ve sinovyal sıvılarında MDA düzeyinin arttığını ve antioksidan durumunun ise azaldığını doğrulyan araştırmalar vardır (Vaziri ve ark., 2006). Çalışmamızda kronik inflamasyon hastalığı olan romatoid artrit, başka bir inflamasyon bozukluğu türüdür. Araştırmamızda, romatoid artrit hastalarında MDA seviyeleri sağlıklı kontrollere kıyasla anlamlı derecede artmıştır ($p<0.05$).

Ortalama SOD aktivitesi düzeyi, RA vakalarında, romatoid artritte kontrol grubuyla karşılaştırıldığında istatistiksel olarak anlamlı bir düşüş gösterdi ($P<0.05$). Mevcut çalışmanın bulguları, literatür verileriyle uyum içerisindedir (Bae ve diğerleri., 2008). Azalan SOD aktivitesi, ROS etkisine bağlı olarak detoksifikasyon işleminin bir parçası olarak SOD bozulmasının bir sonucu olabilir.

Bu çalışmada CAT aktivitesi, romatoid artritte kontrol grubuyla karşılaştırıldığında istatistiksel olarak ve anlamlı bir şekilde azaldı ($P<0.05$). RA grubundaki bu azalmış katalaz aktivitesi, katalazın H_20_2 ile etkisizleştirilmesinden dolayı meydana gelmiş olabilir. Her ikisi de azalmış katalaz aktivitesi gösterir (Vasanthi ve ark., 2004). RA hastasının azalmış katalaz seviyesi, artan inflamasyonla açıklanabilir. Katalaz H_20_2 'nin H_2O ve O_2'ye değişmesine neden olur. Sonuç olarak, hücreleri biriken hidrojen peroksitin zararlı etkilerinden korur. Bu sonuç diğer yapılan litaratür bulgularla uyumludur (Mohamad ve ark., 2011).

Bu çalışmada GSH aktivitesi romatoid artritte kontrol grubuyla karşılaştırıldığında istatistiksel olarak ve anlamlı derecede azaldı ($P < 0.05$). Glutatyonun azaltılması bir flavoenzimdir ve GSSH'nin glukoz-6-fosfat dehidrojenaz tarafından sağlanan GSH'ye indirgenmesinde rol oynayan NADPH'ye bağlıdır. (Feijoo ve ark., 2010).

Sonuç olarak, Bu çalışmada, SOD, CAT ve GSH gibi antioksidant enzimlerin azaldığı ve RA'lı hastalarda malondialdehit (MDA) gibi yüksek lipid peroksidasyon seviyesinin arttığı görülmüştür. Sonuçlar, oksidatif stresin romatoid artrit ile ilgili olarak hastalığın olumsuz yönde etkilerini artırabileceğini göstermektedir.

Bu çalışmada, oksidatif stresin, Romatoid artrit (RA) hastalarında dokunun hücresel hasarını çok iyi etkilediğini göstermektedir.

CURRICULUM VITAE

Darya Assi YOUNUS was born in 1988, Koya / Taq Taq / Erbil / Iraq. He was finished secondary and high school education from Taq Taq in 2009. The same year, got accepted in Chemistry Department of College Science of healthy at Koya University. In 2013, he was graduated from Chemistry Department. At February, 2017 started his graduate study in the department of Chemistry (Biochemistry), Institute of Science of Van Yüzüncü Yıl University.

UNIVERSITY OF VAN YUZUNCU YIL
THE ISTITUTE OF NATURAL AND APPLIED SCIENCES
THESIS ORIGINALITY REPORT

Date: 02/07/2019

Thesis Title: **EFFECT OF ORGANIC FERTILIZER AND INTERCROPPING ON GROWTH AND YIELD OF SWEET CORN AND FRESH BEAN**

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