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

DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER

M.Sc. THESIS

PREPARED BY: Amani Tahsin YASIN
SUPERVISOR: Prof. Dr. Halit DEMIR

Van-2017

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ACCEPTANCE AND APPROVAL PAGE

This study named "**DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN CHILDREN WITH FAMILIALMEDITERRANEAN FEVER**" and prepared by Amani Tahsin YASINunder consultation of Prof. Dr. Halit Demir in Department of Chemistry,on date of --/--/2017 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.

Supervisor: Prof. Dr. Halit DEMİR Signature:

Member: Signature:

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The Board of Directors of the Institute of Science:...../...../..... date and is approved by decision No.....

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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

Amani Tahsin YASIN

ÖZET

AİLEVİ AKDENİZ ATEŞİ ÇOCUKLARDA OKSİDATİF STRES DÜZEYİ VE BAZI ANTİOKSİDANT ENZİM AKTİVİTELERİN BELİRLENMESİ

Tahsin YASIN, Amani
Yüksek Lisans Tez, Kimya Anabilim Dalı
Danışman: Prof. Dr. Halit DEMİR
Ocak 2017, 80Sayfa

Bu tez çalışmasında, Yüzüncü Yıl Üniversitesi Tıp Fakültesi Çocuk sağlığı ve Hastalıkları Anabilim Dalında Ailevi Akdeniz ateşin tanısı konulan ve alınan kan serum örneklerinden antioksidan enzim aktiviteleri olan SOD, GSH ve GPx ve lipid peroksidasyonun son ürünü olan malondialdehit (MDA) seviyeleri ölçülmüştür. Ailevi Akdeniz ateşi olan hastalarda, hasta gruplarının SOD aktivitesi sağlıklı kontrol grubuna göre anlamlı olarak düşük bulundu ($p<0.001$) (Çizelge 3.1.). Hasta gruplarınserumlarında GSH aktivitesinin değerlendirdiğinde, sağlıklı kontrol gruplara göre anlamlı bir fark gözlenmedi. Ayrıca, serum GPx aktivitesi için hasta grubu kontrol grubu ile kıyaslandırıldığında ise anlamlı bir fark bulunamadı. Serum MDA düzeyi hasta grubu sağlıklı kontrol grubuna karşılaştırıldığında ise anlamlı derecede yüksek bulunmuştur. GSH ve GPx ortalamaları arasındaki fark önemsiz bulunurken, hasta ve kontrol gruplarında ise SOD ve MDA ortalamaları arasındaki farkın anlamlı olduğu bulunmuştur ($p<0.005$). Sonuç olarak, Ailevi Akdeniz ateşi olan hastalarda MDA düzeyleri artmıştır. Bununla birlikte, öncelikle SOD ve GSH antioksidan enzimlerin aktivitesi düşmüştür. Bu nedenlerden dolayı, AAA'i olan hastalarda klinik olarak yeni yaklaşımlar sergilenmeli ve antioksidan enzimler hastalığın etyopatogenezini etkileyebilir. Böylece, Ailevi Akdeniz ateşi hastalığı gelişimin nedeni antioksidanlar ve oksidatif stres arasındaki dengesizliğin sonucu olabileceği sonucuna varılabilir.

Anahtar kelimeler: Ailevi Akdeniz ateşi, GPx, GSH, MDA, SOD.

ABSTRACT

DETERMINATION OF OXIDATIVE STRESS LEVELS AND SOME ANTIOXIDANT ENZYME ACTIVITIES IN CHILDREN WITH FAMILIAL MEDITERRANEAN FEVER

Tahsin YASIN, Amani
M.Sc. Thesis, Department of Chemistry
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January 2017, 80Pages

In this thesis study, the antioxidant enzyme activities such as (SOD, GSH, and GPx) and malondialdehyde (MDA) level which is the end product of lipid peroxidation, were determined from the serum samples taken from patients diagnosed with familial Mediterranean fever in Pediatrics Department of Medical Faculty of Yüzüncü Yıl University. The SOD activity of patient groups was found significantly lower than the healthy control group in patients with familial Mediterranean fever ($p<0.001$) (Table 3. 1.). In theevaluation of GSH activity for patient serums, no significant difference observed when compared to control groups. Also for serum GPx activity, no significant difference was noticed when compared to control group. Serum MDA level is found significantly high when compared to control groups. The difference between GSH and GPx averages were found to be insignificant, while the difference of SOD and MDA averages for patient and control groups were found to be relevant ($p<0.05$). As a result, MDA levels increased in patients that suffer familial Mediterranean fever disorder. Whereas, firstly antioxidant enzymes activity of SOD and GSH have been decreased. Due to these reasons, new approaches should be exhibited clinically for FMF patients and antioxidant enzymes can affect the etiopathogenesis of the disease. Thus, we concluded that the cause of development of Familial Mediterranean Fever disease may be the result of an imbalance between the antioxidants and oxidative stress.

Keywords: Familial Mediterranean fever, GPx, GSH, MDA, SOD.

PREFACE

This study was intended to determine the levels of certain antioxidant enzymes in patientsencountered with familial Mediterranean fever in Van region and to indicate the oxidative stress level and the benefit of basic and clinical diagnosis in children that face this autoinflammatory disorder.

I would like to appreciate my supervisor Prof.Dr.Halit DEMIR for encouraging and guiding me during the experimental methods and executing of my study in thelaboratory and all of his supports and information that gave me. Also with all my regards and respect to Prof.Dr.Suat EKIN during the studying courses. Furthermore, I'd like to express my thanks to Eren SARIKAYA for his supports in the laboratory, the academic staff of biochemistry laboratory and special thanks to Lect.Canan DEMIR for making the statistical part of this thesis. I wish to express my appreciation to Asst.Prof.Dr.Lokman Üstyol and Dr.Şükran AKGEYİK in Van Regional Training and Research Hospital (Dursun Odabaş Tıp Merkezi) in Pediatrics Department, for theirsupport in the sample gathering during the entire project**.**Additionally,my thanks to my classmate Rabia AKGEYIK for her supports.

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January 2017

Amani Tahsin YASIN

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SYMBOLS AND ABBREVIATIONS

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	Millimolar
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**
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

RNSReactive 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

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)

1. INTRODUCTION AND LITERATURE REVIEW

Free radicals are reactive chemicals in an outer orbit with an unpaired electron(Poljsak et al., 2013). In the biology system, the free radicals are derived commonly from oxygen, nitrogen and sulfur molecules. These freeradicals are parts of groups of molecules called reactive oxygen species (ROS), reactive nitrogen species (RNS) and reactive sulfur species (RSS)(Lü et al., 2010). Oxygen-free radicals ROS and RNS are admitted for having a dual character as both deleterious and beneficial species(Valko et al., 2005).

Reactive oxygen species (ROS) comprehendfree radical and non-free radical oxygen-containing molecules such as hydrogen peroxide (H_2O_2), superoxide($O_2^{\cdot-}$), singlet oxygen ($1/2O_2$), and the hydroxyl radical (·OH). There are also reactive nitrogen, iron, copper, and sulfur species (Poljsak et al., 2013). RNS are derived from nitric oxide through the reaction with $O_2^{-\bullet}$ to form $ONOO^{-\bullet}$. RSS are easily formed from thiols by reaction with ROS(Lü et al., 2010). Which can ascribe formation of high levels of ROS and oxidative stress and lessen the redox balance (Poljsak et al., 2013). The " Two-faced" role of ROS has demonstrated that ROS within cells behave as secondary messengers in intracellular outstanding overflow that generate andprovide the oncogenic phenotype of cancer cells in which ROS can also generate cellular senescence and apoptosis and therefore can act as anti-tumourigenic species(Valko et al., 2005).

Reactive oxygen and nitrogen species (ROS/RNS) that appear as a result of respirative cycle of the oxidative phosphorylation that possibly pushes biological macromolecules (e.g., cellular DNA) , giving rise to single-strand and double-strand splits that finally causes cell aging , mutagenic changes , cardiovascular diseases , and cancerous tumor growth(Çekiç et al., 2013). Free radicals are generated when our cells obtain energy from food and oxygen and while we are revealed to microbial infections, extensive exercise, or pollutants/toxins such as cigarette smoke, ionizing and UV radiations, alcohol, pesticides, and ozone(Poljsak et al., 2013).

All cells contain detailed antioxidant defense systems including low and high molecular weight components to protect against ROS attack. These protective systems are both endogenous (produced in the body) and exogenous (obtained via diet)(Çekiç et al., 2013).

The cumulative formation of ROS/RNS either via endogenous or exogenous contempts is entitled as oxidative stress(Valko et al., 2005).

In the last years, many types of research have indicated the correlation between oxidative stress, cellular senescence, and some diseases. Besides, nowadays our lifestyle give rise to the over-production of free radicals and reactive oxygen species (ROS) in our body organs,expanding levels of oxidative stress while reducing the antioxidant activity(Jimenez-Zamora et al., 2016).Oxidative stress was first described by Sies as“an inconvenience in the prooxidant to antioxidant equilibrium in thebeneficence of the former, affecting potential damage”(Poljsak et al., 2013).Oxidative stress significantly contributes to the pathogenesis of inflammatory disease, cardiovascular disease, cancer, diabetes, Alzheimer’s disease, cataracts, autism and aging(Lü et al., 2010).

Familial Mediterranean fever (FMF) is the most common form of thehereditary auto-inflammatory disease that is characterized by recurrent attacks of fever with serosal or synovial inflammation;the period lasts 12 to 72 hours. It has also been associated with increased risk of secondary amyloidosis, essentially affecting renal and vascular function in untreated or insufficiently treated patients with FMF. Colchicine, the standard of care for patients with FMF, has been considered as safe and effective in the majority of the patients for reducing both the frequency of inflammatory episodes and the risk of developing amyloidosis. However, there are currently no effective and approved alternatives for FMF patients who are intolerant to colchicine, and dose reductions due to adverse effects may result in diminished efficacy. In addition, approximately 5−10 % of patients with FMF continues to have frequent inflammatory episodes despite receiving the highest tolerable doses (1.5 to 2.0 mg/day) of colchicine, which are considered to be within the effective range. The majority of FMF patients have autosomal recessive inheritance associated with mutations in the MEFV gene, which encodes pyrin protein (Gül et al., 2015). Pyrin and various proteins containing the pyrindomain are intracellular modules of inflammatory signaling that are associated with autoinflammatory disorders.Mutations in MEFV, the gene encoding for pyrin, cause familial Mediterranean fever (FMF), a recessive recurrent polyserositisand are associated to a lesser extent with several other autoinflammatory conditions. When combined with a Th1 related autoimmune disease, carrying an MEFV mutation may worsen the disease period, as

displayedfor carriers among patients with multiple sclerosis of non-Ashkenazi origin(Rabinovich et al., 2005).

1.1. Free Radicals and Reactive Oxygens

Free oxygen radicals have been suggested as vital causative agents of aging(Harman, 1981). Harman suggestedthat the theory of aging which damage cells and tissuesis associated with the accumulation of free radicals(Harman, 1988). The univalent decrease of molecular oxygen terminates in reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH_2) (Klaunig and Kamendulis, 2004). These cytotoxic species can affect irreversible oxidative damage to biologic molecules, such as lipids, proteins, and DNA in the cells, which cause enzyme activity and membrane function(Aliahmat et al., 2012).ROS is a collective phrase frequently used to contain oxygen radicals [superoxide ($O_2^{\bullet-}$), hydroxyl ($OH^{\bullet}$) , peroxyl ($RO_2^{\bullet}$) , and alkoxyl ($RO^{\bullet}$)](Figure 1.1) and the non-radicals that are either oxidizing agents or converted into radicals with ease , such as HOCl , ozone (O_3) , peroxynitrite ($ONOO^-$), singlet oxygen ($^1O_2$) , and H_2O_2(Çekiç et al., 2013).

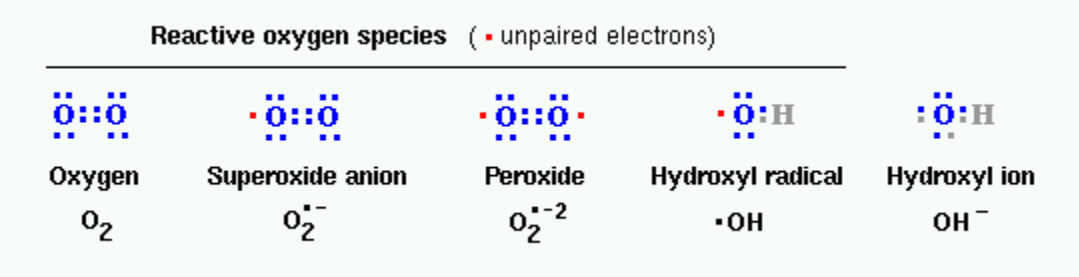


Figure 1.1. Reactive oxygen species(Bowen, 2003).

ROS are generated during cellular metabolism and functional activities, and have vital roles in cell signalling, apoptosis, gene expression and ion transportation (Vajragupta et al., 2004).The aggregation of ROS is providentially controlled in vivo by a broad range of spectrum of non-enzymatic antioxidant systems, such as bilirubin; glutathione (GSH); vitamins A, C, and E; and defense-related enzymes such as superoxide dismutase (SOD),

catalase (CAT), and glutathione peroxidase (GPx)(Artur et al., 1991).High amounts of ROS are able to have damaging impacts on lots of molecules containing protein,lipid,RNA, and DNA, whereas they are tiny and highly reactive. Bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids can be attacked by ROS since·OH is the strongest oxidant (Lü et al., 2010).

ROS and RNS indicate that they own many features of carcinogens. Moreover, functional concentrations of ROS may be necessary for normal cell functioning and ROS are considered to participate in the development of plants and animals. Environmental or behavioral stressors (pollution, sunlight exposure, cigarette smoking, excessive alcohol consumption, etc.) or a tiny malfunction in the generation of antioxidant may cause a free radical excess that is known as " oxidative stress", that the dynamic redox equilibrate among oxidants and antioxidants is shifted toward oxidative potentials (Çekiç et al., 2013)(Figure 1.2).Usually, cells are able to protect themselves against ROS damage by the aid of intracellular enzymes' usage to maintain the ROS homeostasis at alow level. Whereas,in the environmental stress and cell dysfunction periods ROS levels rise and cellular damages occur significantly in the body (Lü et al., 2010).

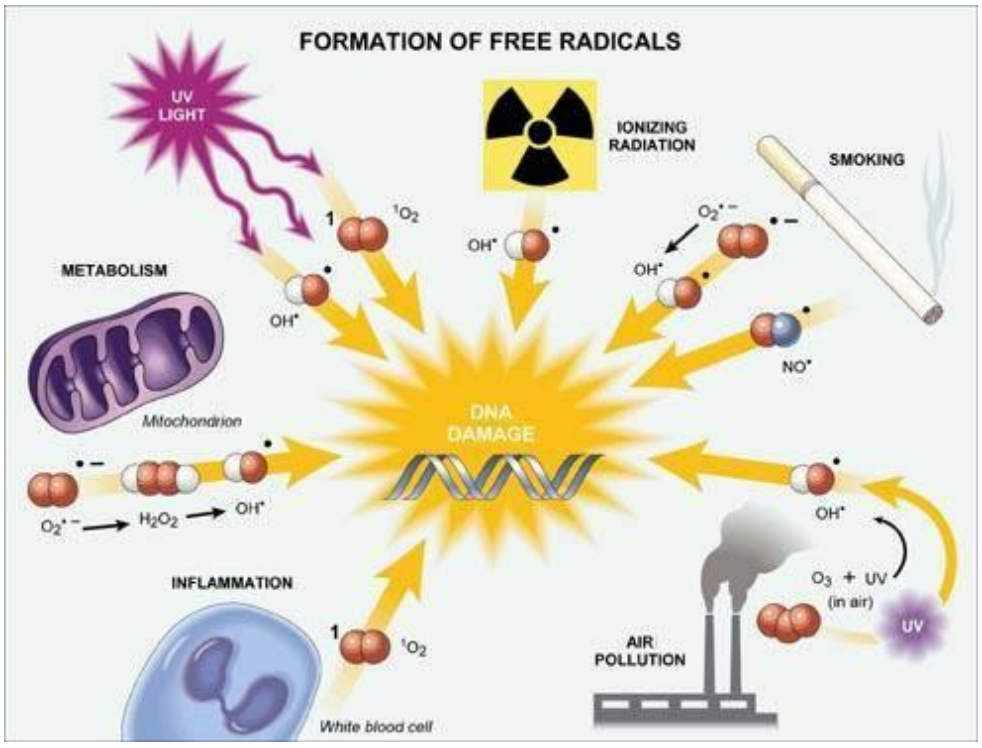


Figure 1.2. Formation of free radicals(Pathology, 2005).

The most vital endogenous sources of oxidizing agents participating in aging are mitochondrial: electron transport chain and nitric oxide synthase reaction. Non-mitochondrial sources of free radicals are Fenton's reaction, microsomal cytochrome P450 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 protection system that contains enzymatic, metal chelating and free radical scavenging activities to neutralize these radicals after they have formed. Additionally, intake of dietary antioxidants can support to maintain a sufficient antioxidant status in the body, in which antioxidant molecules may react directly with the reactive radicals to demolish them. Otherwise, they can turn into less active new free radicals,that live longer,less risky than the radicals that have been neutralized by them (Lü et al., 2010).

When ROS increased, thereaction occurs with polyunsaturated fatty acids to generate the liberation of toxic and reactive aldehyde metabolites, such as malondialdehyde, that is the end product of lipid peroxidation process. Lipid peroxidation is associated with aging and some kinds of chronic health illnesses, such as cancer and atherosclerosis. Many studies have recommended that antioxidants can prohibit the oxidation of different macromolecules, such as DNA,proteins, and lipids, hence preventing the aging process and extending the lifespan of the organisms (Aliahmat et al., 2012).ROS production and the antioxidant defense activityseem to be more or less balanced ***in vivo***. As already mentioned, actually the balance may be slightly tipped in favor of the ROS, therefore, there is continuous ROS formation and low-level oxidative damage in the human body.(Figure 1.3)

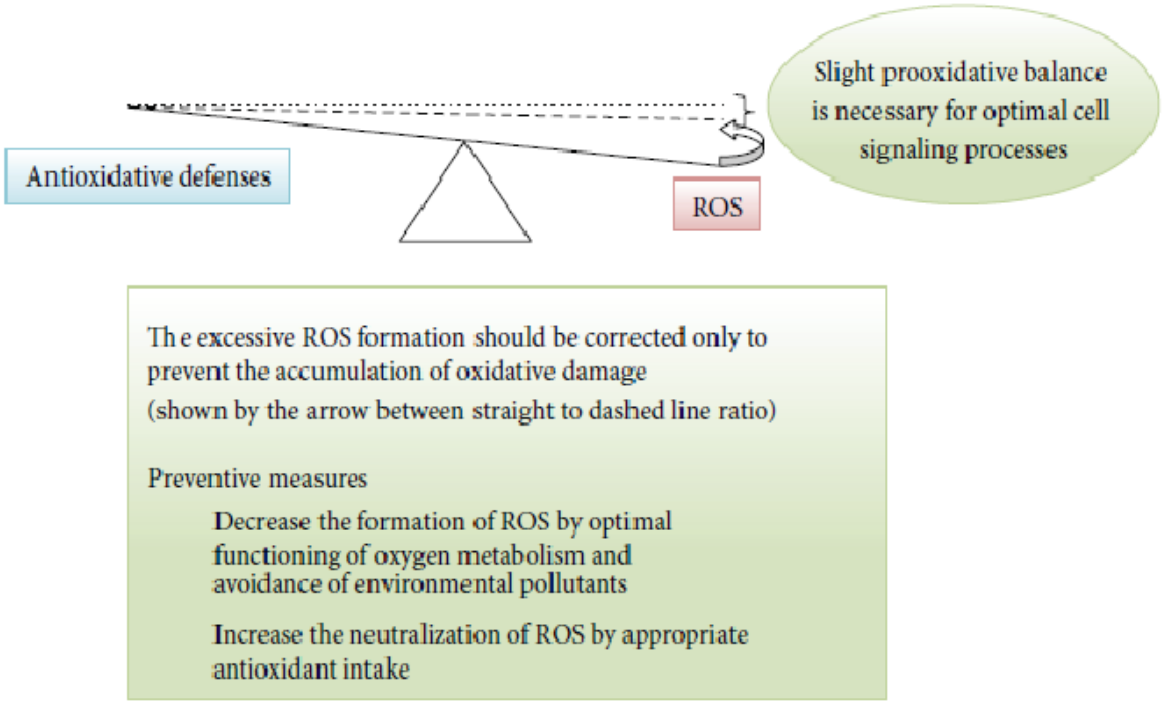


Figure 1.3. Model of antioxidative/oxidative balance of an adult person(Poljsak et al., 2013).

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

Cellular redox homeostasis is cautiously preserved by a detailed endogenous antioxidant defense system, that contains endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), glutathione (GSH), proteins, and low-molecular-weight scavengers, like uric acid, coenzymeQ, and lipoic acid(Halliwell, 2011).It appearsthat defeats of some or all of the repair systems helpmore to 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(Cheeseman and Slater, 1993).

Nucleic acids damaged oxidatively are repaired by specific enzymes, oxidized proteins are removed by proteolytic systems, and oxidized lipids are repaired by phospholipases, peroxidases, and acyltransferases(Poljsak et al., 2013).

1.1.1. Scavenging of free radicals

Exploration and detectionof natural and safe antioxidants, especially of plant origin, have considered beingincreased in the last years. Analyses based on the use of O_2^{-}•, ·OH, DPPH, $ABTS^{-}$·, and N,N-dimethyl-p-phenylenediaminedihydrochloridecation radical ($DMPD^{-}$) are among the most popular spectrophotometric methods for determination of the antioxidant capacity of foods, beverages, and vegetable extracts. DPPH and $ABTS^{-}$· scavenging techniques have been frequently used to assess the antioxidant activity of compounds because of their simple, rapid, sensitive and reproducible procedures. Therefore the researchers usually meditate the radical scavenging analyses for antioxidant studies in the cell-free systems in advance of further studies in cellular lines and/or animal models (Lü et al., 2010).

1.1.1.1. Superoxide scavenging and other ROS

Superoxide (O_2^{-}•), a common cellular free radical that is comprised of a huge number of harmful changes mostly associated with an increase in per oxidative processes and connected to a low antioxidant concentration.However, O_2^{-}• itself is not so reactive to biomolecules but supports the production of more powerful ·OH and $ONOO^{-}$. In phagocytes, O_2^{-}•is generated in large amounts by the enzyme NADPH oxidase for destroying pathogens. O_2^{-}• is also a byproduct of mitochondrial respiration, as well as many other enzymes such as NADH oxidase, XO, mono-oxygenases, and cyclooxygenases. Direct scavenging of O_2^{-}• has been an exemplary for the identification of antioxidant activities. O_2^{-}• can be formed enzymatically or non-enzymatically in the chemical systems from Quinone derivatives, such as 6-anilino-5, 8-quinolinequinone (LY83583), 1,4-benzoquinone, 1,4-naphthaquinone, 2-methyl-1,4-naphthaquinone,Ribo-flavin, etc.(Figure 1.4)

O_2^{-}• is also generated in riboflavin/methionine /illuminate and assayed by the reduction of Nitro blue tetrazolium (NBT) to form blue formazan. Briefly, the reaction mixture is illuminated at 25 ˚Cfor 40 min. and O_2^{-}• generated from the photochemically reduced riboflavin can reduce NBT to form blue formazan which has anabsorbance at 560 nm.This system can be utilized to determine the radical scavenging activity of antioxidants.Antioxidants can be supplemented to the reaction mixture to scavenge O_2^{-}•, thereby preventing the NBT reduction. Decreased absorbance of the reaction mixture shows increased O_2^{-}• scavenging activity. The scavenged percentage of O_2^{-}•is calculated by the alterations in the absorption. NBT salt and other tetrazolium salts are chromogenic probes useful for O_2^{-}• determination. These probes are also widely used for detecting redox potential of cells for viability, proliferation and cytotoxicity assays(Lü et al., 2010).

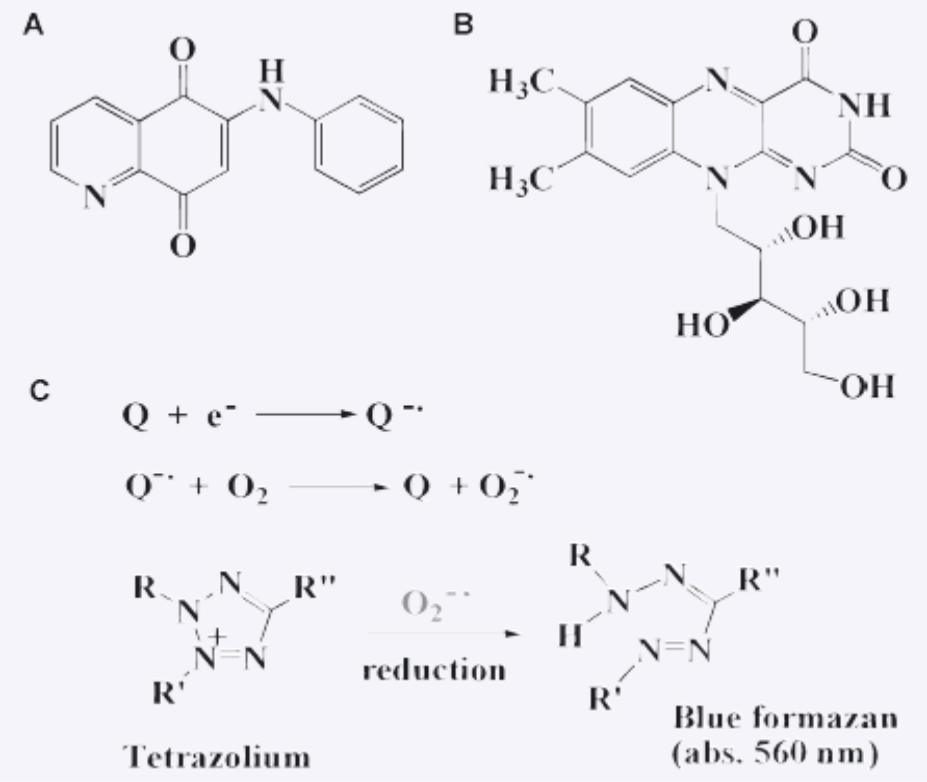


Figure 1.4. Structure of Quinone's.

(Q): LY83583 (A), riboflavin (B) and the formation of superoxide and its reaction with NBT (C), where R, R' and R'' represent p-nitrophenyl, o-methoxyphenyl, and phenyl groups, respectively(Lü et al., 2010).

1.1.1.2. Hydroxyl radical scavenging and other ROS

Hydroxyl radical (·OH) is highly reactive, more toxic than other radical species and can attack biologic molecules such as DNA, proteins, and lipids. It is admitted that ·OH is generated from the Fe^{2+} (or Cu^{+}) / H_2O_2 Fenton reaction system, by simply incubating $FeSO_4$ and H_2O_2 in aqueous solution. Therefore, ·OH scavenging activity of antioxidants can be achieved entirely by direct scavenging or prohibit of the·OH formation through the chelation of free metal ions or convert H_2O_2 to other harmless compounds. The antioxidants' scavenging capability can be determined by Gutteridge method, which is monitored in the Fe^{3+}–EDTA–H_2O_2–deoxyribose system The extent of deoxyribose degradation by the ·OH formed can be measured directly in the aqueous phase by thiobarbituric acid reactive species (TBARS) assay at 532 nm (Figure 1.5).

This method is seton the degradation of deoxyribose phenomenon that ·OH forms areactive genremalondialdehyde, which forms an adduct with athiobarbituric acid (TBA). The adduct, MDA–TBA, has an absorption at 532 nm that can be assayed spectrophotometrically. By thisassay, the ability of many antioxidants to scavenge ·OH has been studied and compared with that of DMTU, uric acid, Trolox, and mannitol.

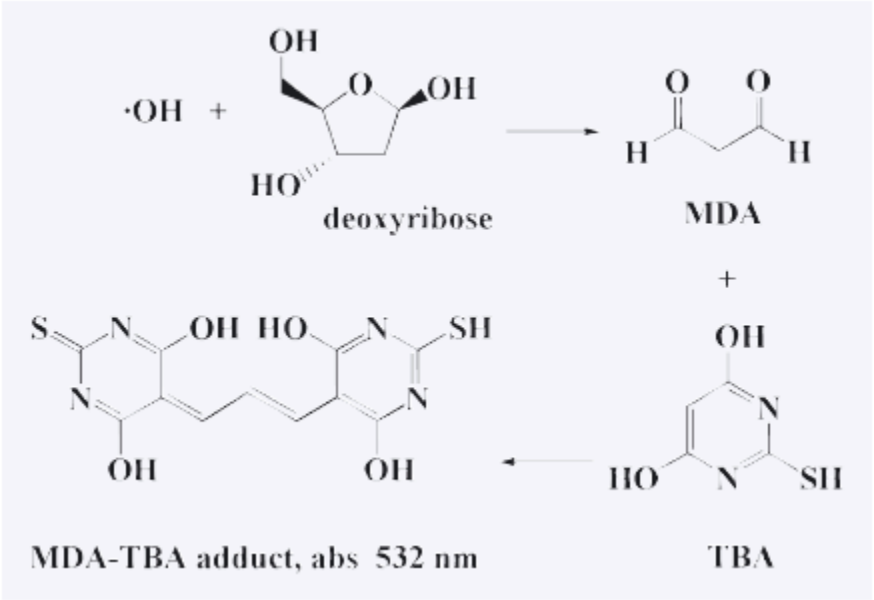


Figure 1.5. Reaction pathway of TBARS assay of •OH.

1.1.2. Sources of ROS generation

High amounts of reactive oxygen species (ROS) balanced against antioxidant defenses are thought to act a basic role in various chronic age-related sicknesses and aging(Poljsak, 2011). Molecular oxygen is comparatively unreactive and not causing damage in its ground state , but is able to undergo partial reduction to generate an amount of ROS containing the superoxide anion and hydrogen peroxide (H_2O_2) , and may subsequently react to generate the highly reactive hydroxyl radical (Figure 1.6)(Morano et al., 2012). There are sub-groups of ROS: free radicals such as superoxide radicals ($O_2^{\cdot-}$) and non-radical ROS such as hydrogen peroxide (H_2O_2) (Rahal et al., 2014).The major mediators that are responsible for the inflammatory response include the free oxygen radicals. They break cell membranes by increasing lipid peroxidation, inhibit ATP synthesis in the mitochondria and may further harm DNA and proteins oxidatively (Aliomrani et al., 2016).Naturally, ROS generation proceeds from environmental causes and side reactions of normal aerobic metabolism are another sources. The main source of ROS in the eukaryotic cells is considered to be mitochondrial respiration through the oxidative phosphorylation process (Murphy, 2009).

The cells of all the aerobic organisms use most of the chemical energy in their mitochondria by consuming oxygen, therefore the main site of intracellular consumption and the primary source of ROS production is mitochondria (Dayem et al., 2010; Morano et al., 2012). Electron transport chain and the reaction of nitric oxide synthase are indicating the mitochondrial ROS sources (Poljsak, 2011).Electrons are transported through protein complexes that are composed of electron transport chain in order to generate ATP, escaping of these electrons from the respiratory chain results in areduction of oxygen and generating ROS (Morano et al., 2012). In the same way, using of oxygen as a terminal electron acceptor during oxidative protein folding indicates that ER is an important source of ROS(Tu and Weissman, 2004). Non-mitochondrial ROS sources include pollutants in food, environmental pollutants, radiation or they are the by-products of other metabolic processes in the organisms (Poljsak, 2011). Furthermore, some cytochrome 450 enzymes are also known to generate ROS (Parke and Sapota, 1996).

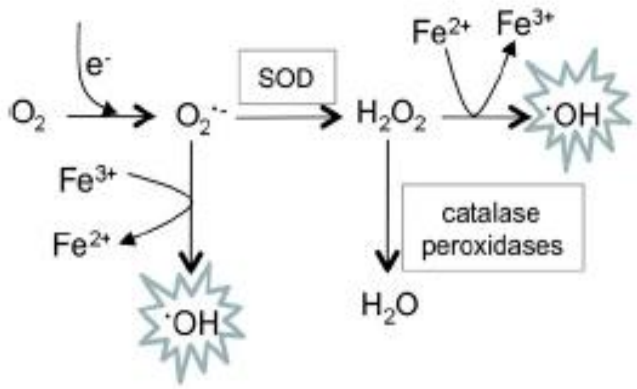


Figure 1.6. Generation of ROS.

The superoxide anion (O_2^-) can be formed via electron leakage to oxygen from electron transport chains. Hydrogen peroxide (H_2O_2) is generated by the breakdown of superoxide catalyzed by superoxide dismutase's (SODs). Hydrogen peroxide can be reduced by iron (Fe^{2+}) in the Fenton reaction to produce the highly reactive hydroxyl radical. In the Haber-Weiss reaction, superoxide can donate an electron to iron (Fe^{3+}), generating the hydroxyl radical and Fe^{2+}, which can further reduce hydrogen peroxide. Various antioxidant enzymes, including catalases and peroxidases, detoxify hydrogen peroxide to prevent such ROS generation(Morano et al., 2012).

1.1.3. Biochemical basis of ROS

ROS are estimated as signaling molecules for arranging a large range of physiology. Firstly in the 1990s, it was suggested that hydrogen peroxide was indicated to be required for cytokine, insulin, growth factor, activator protein-1 (AP-1), and NF-κB signaling. Phosphatase inactivation by cysteine oxidation is supported by hydrogen peroxide, probably supplied a biochemical mechanism by which ROS can influence on signaling pathways. ROS takes part in mediating apoptosis based on signaling cytochrome c. To regulate signaling pathways ROS can rise reversible post-translational protein modifications. A molecule of nitric oxide (NO) is a specific example of the beneficial

physiological role of free radicals. NO is composed from arginine by the action of NO-synthase (NOS). NO is formed by the primary NOS during blood vessel softening processes (eNOS) or during transmission of nerve impulses (nNOS). NO can be reason of damage to proteins , lipids , and DNA either directly or after reaction with superoxide , directing to the composition of the much reactive peroxynitrite anion (nitroperoxide) ONOO-(Rahal et al., 2014). Also there are several endogenous cells and cellular components participate in initiation and propagation of ROS (Table 1.1).

Table 1.1. Endogenous mediators of oxidative stress.

Leakage of free radicals	Membrane-bound enzymes	NADPH oxidase
	Electron transport systems	Mixed function oxidases
Activation of oxygen	Soluble cell constituents	Transition metals, thiol containing proteins, quinine derivatives, epinephrine, metalloproteins, hemeproteins, and flavoproteins
	Xenobiotic metabolizing enzymes	Cyt P_{450}-dependent monooxygenases, Cyt b_5, and NADPH-dependent cytochrome reductases
ROS generation/propagation	Soluble cytosolic enzymes	Xanthine oxidase, superoxide dismutase, catalase
	Phagocytic cells	Neutrophils, macrophages, and monocytes involved in inflammation, respiratory burst, and removal of toxic molecules
	Local ischemia	Damaged blood supply due to injury or surgery

(Rahal et al., 2014).

1.1.4. Physiological role of ROS

Functional role of ROS is incorporated with nearly all the body processes, such as with reproductive processes. Whereas under physiological conditions a particular amount of free radicals and reactive metabolites are needed. Activation of hypothalamic –pituitary-adrenal axis is caused by stress. The increased liberation amount of endogenous catecholamine has been detected in cold environmental circumstances. The succinate dehydrogenase activity also raised pointing out the ROS impact obviously in cold environmental situations. Free radicals act as an irreplaceable role in phagocytosis as one of the major microbial systems, or in various biochemical reactions such as hydroxylating, carboxylate or peroxidation reactions (Figure 1.7), or in the ribonucleotides' reduction(Rahal et al., 2014).

Generation of $O_2 \cdot -$, HOCl, and H_2O_2 by phagocytes is vital for defense against several bacterial and fungal species (Poljsak et al., 2013). Nowadays, the beneficial physiological cellular utilization of ROS is indicated in various fields, including intracellular signaling and redox regulation. It is indicated that low levels of ROS are signaling molecules, modulating cell proliferation, apoptosis, and gene expression through activation of transcription factors such as NF-kappa-B and hypoxia-inducible-factor-1α (HIF). The inducers of NF-kappa-B include also tumor necrosis factor alpha (TNFα) and interleukin 1-beta (IL-1β).ROS can act as signaling intermediates for cytokines, including IL-1 and TNFα. These pro-inflammatory cytokines, tumor necrosis factor (TNF)-α, interleukin-1β (IL-1β), and interferon-γ (IFN-γ), can additionally increase oxidative stress in humans, inducing production of ROS (Poljsak et al., 2013).

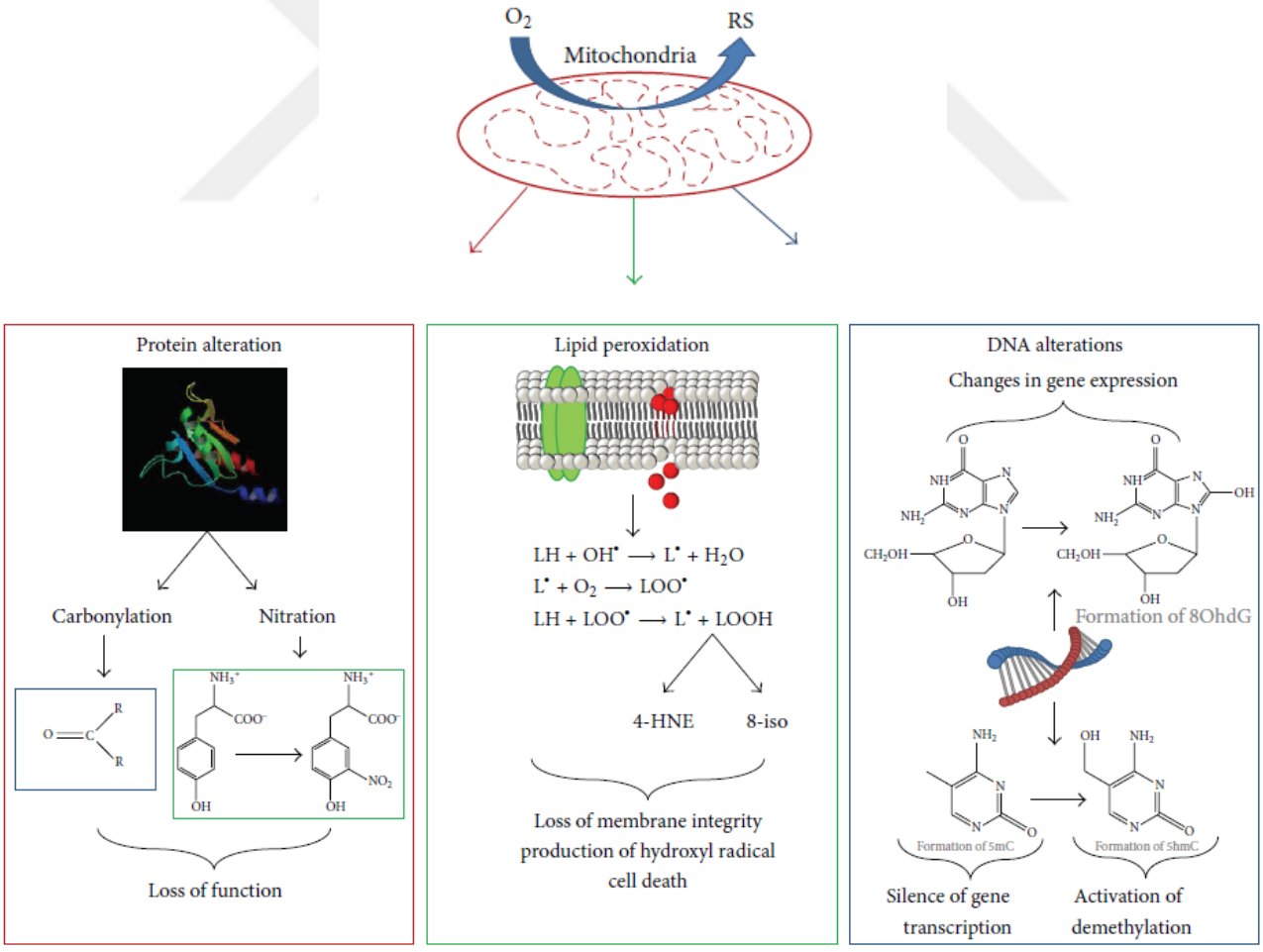


Figure 1.7. Effects of reactive species in the cell(Fraunberger et al., 2016).

1.1.5. ROS determination

Free radicals are hard to measure in the laboratory due to their very short half-life.Though various methods for the measurement of oxidative stress existnowadays, each with their advantages and disadvantages. Several approaches are probable: identification of free radicals either directly by paramagnetic electron resonance (electron spin resonance, ESR), or indirectly by identifying some more stable intermediates:estimation of the traces of radical attack on biological molecules by high-performance liquid chromatography, gas- liquid chromatography, colorimetric tests. The measurement of antioxidant status can be estimated by colorimetric,immune, or enzymatic methods. The direct methods that detect ROS measure superoxide,H_2O_2, and $^{\cdot}OH$. These are very reactive species and detecting their amount is difficult.

*In vivo,*ESR is relatively senseless and needs steady-state concentrations of free radicals in the micromolar range, which restricts its usage in patients for measuring ROS.For *in vivo* samples ESR can be applied only by spin trapping technique. However toxicity is not a crucial case for most traps, there are no efficient spin traps to be directed to humans. In order to overcome these problems, indirect methods are being utilized. Commonly indirect measures measure the changes in endogenous antioxidant defense systems or measure the ROS-induced damage of cellular components. Practically, it is difficult to detect all types of ROS inside the cells or cellular components, in addition to the whole antioxidative protection and repair of cells and organs at any time. Elevation in oxidative stress results in from increased ROS production or from decreased antioxidative defenses(Poljsak et al., 2013).

1.1.6. Inhibition of free radical generating enzymes

NADPH oxidases are a set of plasma membrane incorporated enzymes that shift one electron from the cytosolic NADPH donor to a molecule of extracellular oxygen, and producing $O_2^{-}\bullet$.XO is an enzyme that is related to the generation of uric acid in the body, that catalyzes the hypoxanthine and xanthine oxidation to uric acid resulting $O_2^{-}\bullet$and H_2O_2

and elevates the oxidative level in an organism (Figure 1.8).In phagocytes, O_2^{-}•is produced in huge amounts by NADPH oxidase enzyme for utilization in oxygen-dependent killing mechanisms for attacking pathogens. The controlled formation of reactive oxygen species throughout the respiratory burst is vital for the protection of an organism toward attacking microorganisms without inducing an important damage to tissue functions. Whereas,excessive ROS boost oxidative stress such as low-density lipoprotein (LDL) oxidation (Lü et al., 2010).

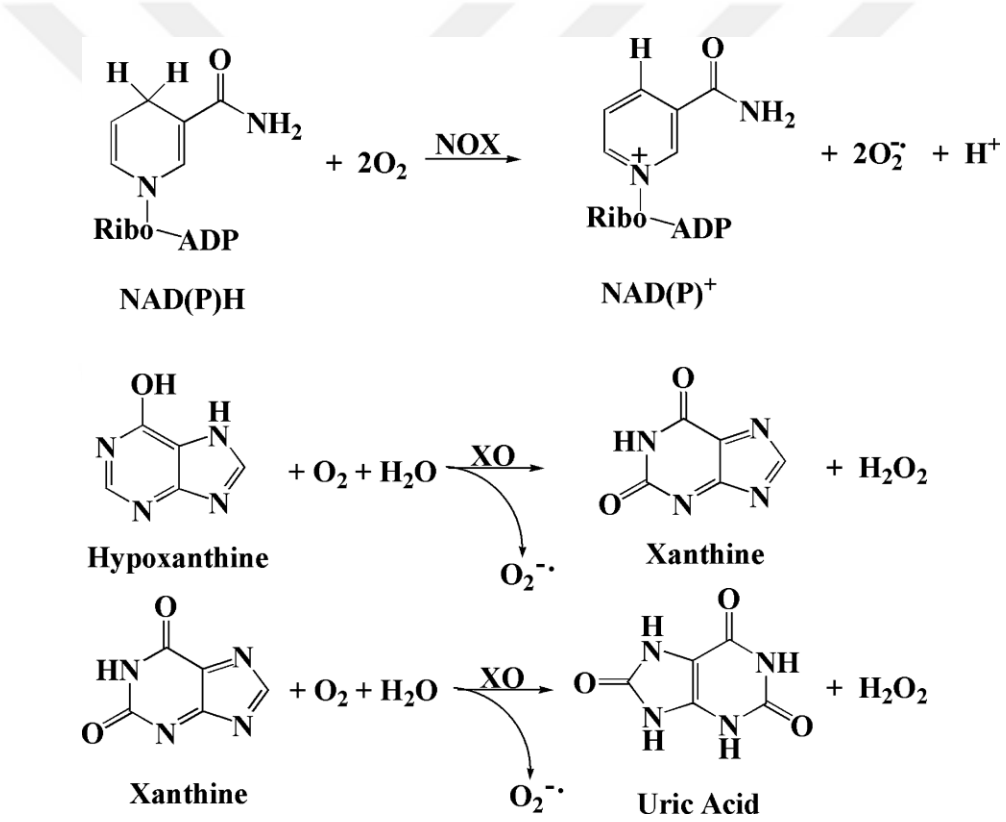


Figure 1.8. Superoxide and hydro peroxide generation(Lü et al., 2010).
(Which induced from NAD(P)H oxidase, (NOX) and XO).

1.2. Antioxidants

Antioxidants as their meaning stand for inhibiting the oxidation of other molecules. They are compoundsthat neutralize reactive species by reducing their activity within the body

(Fraunberger et al., 2016). The body system develops itself with some methods to resist the destructive causes that occur in the cell like prevention of damage, repair mechanism to diminish the oxidative damages, physical protection mechanism against damage and the last most crucial one is the antioxidant defense mechanisms (Rahal et al., 2014).

Antioxidants also act as a significant role in providing cellular functions in the face of redox modulations by a variety of mechanisms. Antioxidants are categorized into two wide parts: endogenous and exogenous. The antioxidants that are found in the body are constituted of antioxidant defense enzymes and other antioxidant components like melatonin and glutathione that are synthesized in the body (Fraunberger et al., 2016). Endogenous antioxidant defenses are sub-divided into enzymatic (superoxide dismutase [Cu, Zn-SOD, Mn-SOD], catalase and glutathione peroxidase) and non-enzymatic antioxidants (vitamin C, Vitamin E, carotenoids, thiol antioxidants(glutathione, thioredoxin and lipoic acid), flavonoids, selenium and others) (Valko et al., 2005).Generally, the enzymic and non-enzymic molecules are distributed in the cytoplasm and different cell organelles. Many basic ubiquitous antioxidant enzymes, such as SOD,catalase, and various peroxidases in eukaryotic organisms catalyze complicated progressive reactionsto convert ROS to more stable molecules such as water and O_2.

In addition to the primary antioxidant enzymes, a large number of secondary enzymes take part in an intimate association with small molecular weight antioxidants to produce redox cycles that maintain essential cofactors for primary antioxidant enzyme functions. Small molecular weight non-enzymatic antioxidants (such as;GSH,NADPH,thioredoxin, vitamins E and C, and trace metals like selenium) also function as direct scavengers of ROS. These enzymatic and non-enzymatic antioxidant systems are required for continuing life by providing a precise intracellular redox balance and diminishing unwanted cellular damage caused by ROS (Duraˇckov´a, 2010). Endogenous and exogenous antioxidants include some high molecular weight (SOD, GPx, Catalase, albumin, transferring, metallothionein) and some low molecular weight substances (uric acid, ascorbic acid, lipoic acid, glutathione, ubiquinol, tocopherol/vitamin E, flavonoids) (Rahal et al., 2014). Outside of the body, antioxidants can be provided by the diet with a broad variety of natural and synthetic compounds found in complex mixtures (like chocolate or olive oil) or isolated to be taken as a supplement (Bouayed and Bohn, 2010).

The mechanism of proceeding of each antioxidant will change depending upon location, chemical structure and bioavailability inside the body in addition to the redox modulation degree experienced by the cell (Fraunberger et al., 2016). If we want to focus on the beneficial utilization of antioxidants, a biological antioxidant has been described as any substance that exists at low concentrations compared to an oxidizable substrate and remarkably postpones or prevents the oxidation of that substrate (John and Halliwell, 1999). An ideal antioxidant should be easily absorbed by thebody and should prohibit free radical formation or chelate redox metals at physiologically proper levels. It should work in aqueous and/or membrane domains and impress gene expression in a positive way (Rahman, 2007). The human antioxidant defense is complicated and must reduce the ROS levels meanwhile permitting beneficial roles of ROS to perform cell signaling and redox regulation (Poljsak et al., 2013).

However recent evidencerecommends that the antioxidant supplements (although highly suggested by the pharmaceutical industry and taken by many individuals) do not supply enough protection against oxidative stress, oxidative damage or increase the lifespan. Many people consume synthetic antioxidants in order to decrease oxidative stress, to modulate the aging process and to extend the health-span.Evidence demonstrate that consumption of fruits and vegetables are better than intake of synthetic antioxidants , people who eat fruits and vegetables have a lower risk of heart disease and some neurological diseases and studies show that some types of vegetables and fruits in general, protect against a number of cancers as epidemiological studies show up, without maintaining the answer whether any specific bioactive molecules within fruit and vegetable have a particular support on lower incidence (Poljsak, 2011). Though, nowadays fruits and vegetables are drained of some necessary micronutrients and antioxidants because of the concentrated type of production or because of postharvest processes,transport, and storage (Tijskens, 2004).

One more vital duty of antioxidants is to regulate ROS-related enzymes and oxidative damage,which can be reduced directly through reacting with free radicals or indirectly by preventing the activity or expression of free radical forming enzymes such as NAD (P) H oxidase or by raising the activity and intracellular antioxidant enzymes’expression like

superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) (Lü et al., 2010).

1.2.1. Endogenous and exogenous enzymes

The response system of endogenous antioxidant is capable of arranginga number of reactive species firmly and reduce the related cellular damage (Fraunberger et al., 2016). The body makes five types of endogenous antioxidants: superoxide dismutase, also called SOD, alpha lipoic acid, or ALA, coenzyme Q10, or CoQ10, catalase and glutathione peroxidase, abbreviated as GPx. Professor Keith says that SOD, catalase, and glutathione peroxidase are the three most important of these because the body can produce more of them when certain free radicals are present(Ipatenco, 2014). However, the role of exogenous antioxidants appears to be on the surface and excessive.

Although,(Kaspar et al., 2009)found that exogenous antioxidants have a priming effect on the antioxidant response system . Exogenous antioxidants carry out their effects through additional mechanisms of action(Figure 1.9).In cases like tocopherols and resveratrol, 2 or 3 different actions can be managed simultaneously to counter the detrimental redox modulation effects. Working together with the endogenous antioxidant response system, exogenous antioxidants stand for a more improved and efficient defense against deleterious redox modulations(Fraunberger et al., 2016).

Melatonin: Is an endogenous antioxidant that plays a vital role in protecting against free radical-induced oxidative damage (Fraunberger et al., 2016). In all organisms,melatonin is generated mainly during the daily period of darkness and only small amounts are being synthesized during the day. Therefore, people should sleep long enough and in complete darkness so that the required amounts of the endogenous powerful antioxidant, melatonin is being achieved and synthesized in addition to its beneficial role in the protection of nuclear and mitochondrial DNA. Melatonin is an antioxidant that is able to cross cell membranes and the blood-brain barrier easily. Melatonin is a direct scavenger of OH, O_2^- and NO. Melatonin is different from other antioxidants as it does not undergo redox cycling, the capability of a molecule to undergo reduction and oxidation repeatedly(Poljsak, 2011).

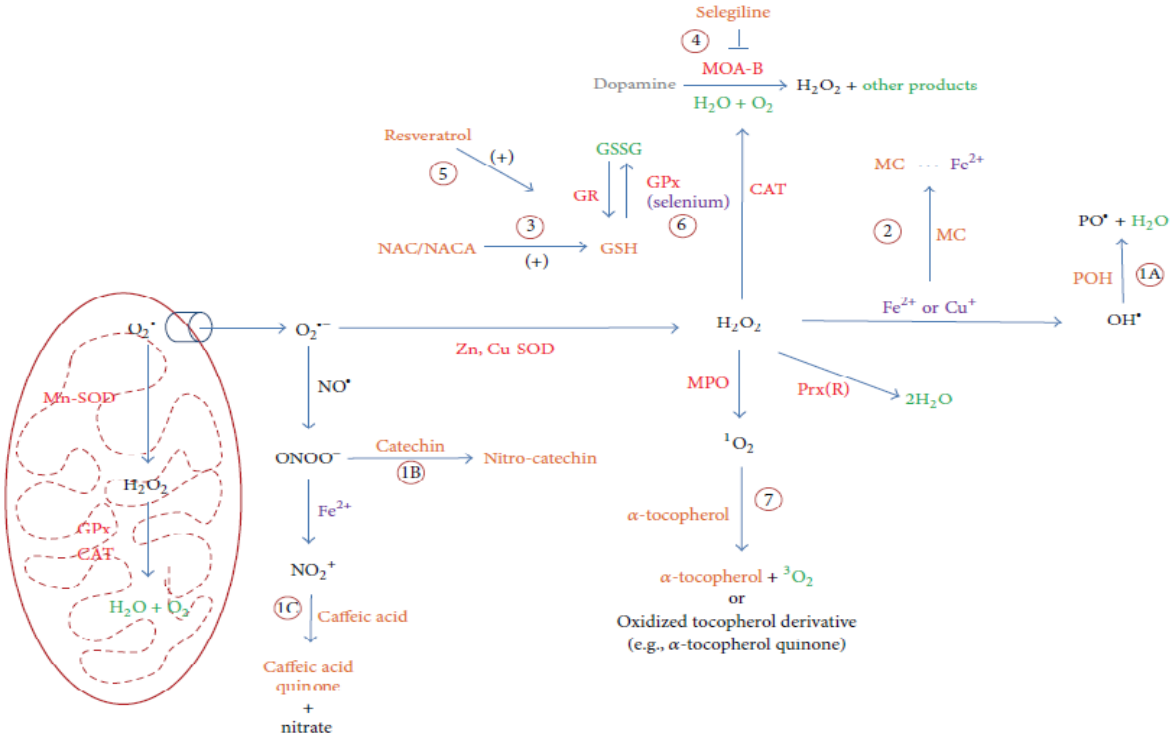


Figure 1.9. Mechanism of action of exogenous antioxidants.

Red: enzymes, green: other products, purple: cofactor/substrate, black: reactive species, CAT, catalase, MC, ametalchelator, 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).

1.2.1.1. Superoxide dismutase and it's biochemical importance

SOD enzymes are categorized into the oxidoreductase enzymes,which, in mammals include either Cu or Mn at the active site and catalyze the alteration of superoxide, the one - electron reduction product of molecular oxygen.

$$O_{2^-} + M^{n+1} \rightarrow O_2 + M^{n+} \qquad (1)$$

$$HO_2 + M^{n+} \rightarrow H_2O_2 + M^{n+1} \qquad (2)$$

Where Mn is the metalloenzyme in the reduced state and M^{n+1} is the enzyme in the oxidized state to oxygen and hydrogen peroxide.Three distinct isoforms of SOD have been identified in mammals (Muscoli et al., 2003). SOD_1 (CuZnSOD) has been found in the cytoplasm, nuclear compartments also have been identified in their intermembrane space of the mitochondria. SOD_2 (MnSOD)is placed in the mitochondrial matrix of the cells and SOD_3 (ECSOD) has been localized in the extracellular portions, where it connects the extracellular matrix within its high – affinity carboxyterminus(Holley et al., 2014; Muscoli et al., 2003; Weydert and Cullen, 2010). All the three enzymes catalyze the dismutation of superoxide radicals into hydrogen peroxide and molecular oxygen(Figure 1.10).Hydrogen peroxide which becomes ROSwhich is decomposed to water by numerous enzyme systems comprising peroxiredoxins, catalase and glutathione peroxidase (GPx) (Holley et al., 2014).

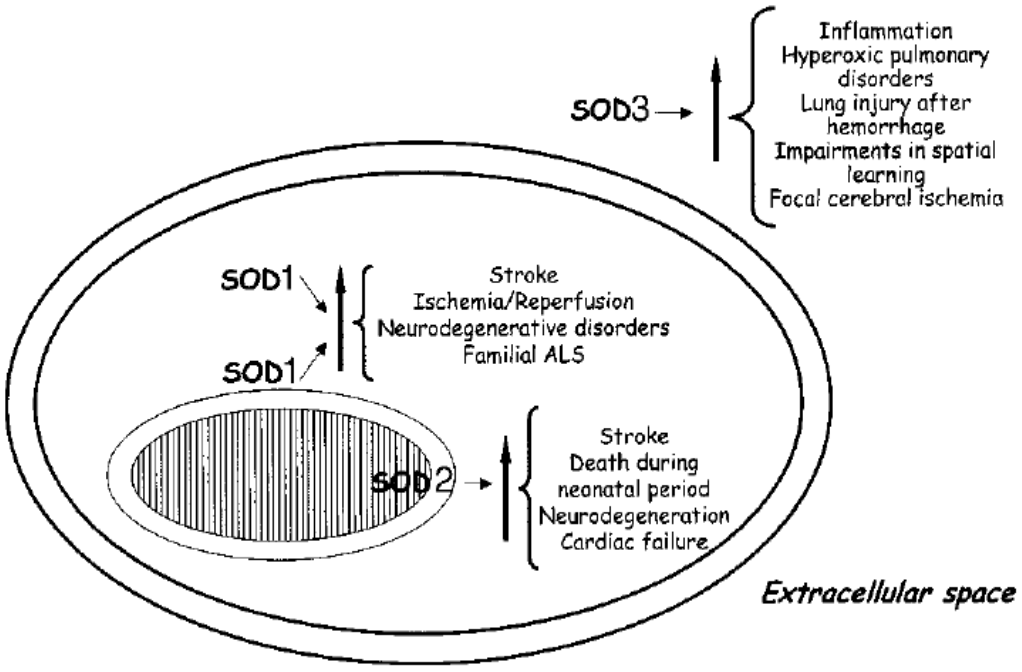


Figure 1.10. Dismutation of superoxide radicals (Muscoli et al., 2003).

Superoxide dismutases (SODs) are the main scavengers. ROS donate to the radio susceptibility of normal and tumor tissues, also the radioresponsiveness of tissues are regulated by SODs, therefore affecting theimpact of radiotherapy (Holley et al., 2014).Superoxide radicals are generated at different sites in the cell, such as the ER, ETC in mitochondria, as well as various enzymatic sources, like NOX and XO, all of which contribute to the generation of ROS after ionizing radiation. Superoxide radicals are detoxified by SODs to form hydrogen peroxide, which is further detoxified by GPx and Prx. GR is used to regenerate GSH. ER: endoplasmic reticulum, ETC: electron transport chain, GPx: glutathione peroxidase, GR: glutathione reductase, GSH: reduced glutathione, NOS, NADPH oxidase,Prx: peroxiredoxin, SOD: superoxide dismutase, XO: xanthine Oxidase (Holley et al., 2014)(Figure 1.11).

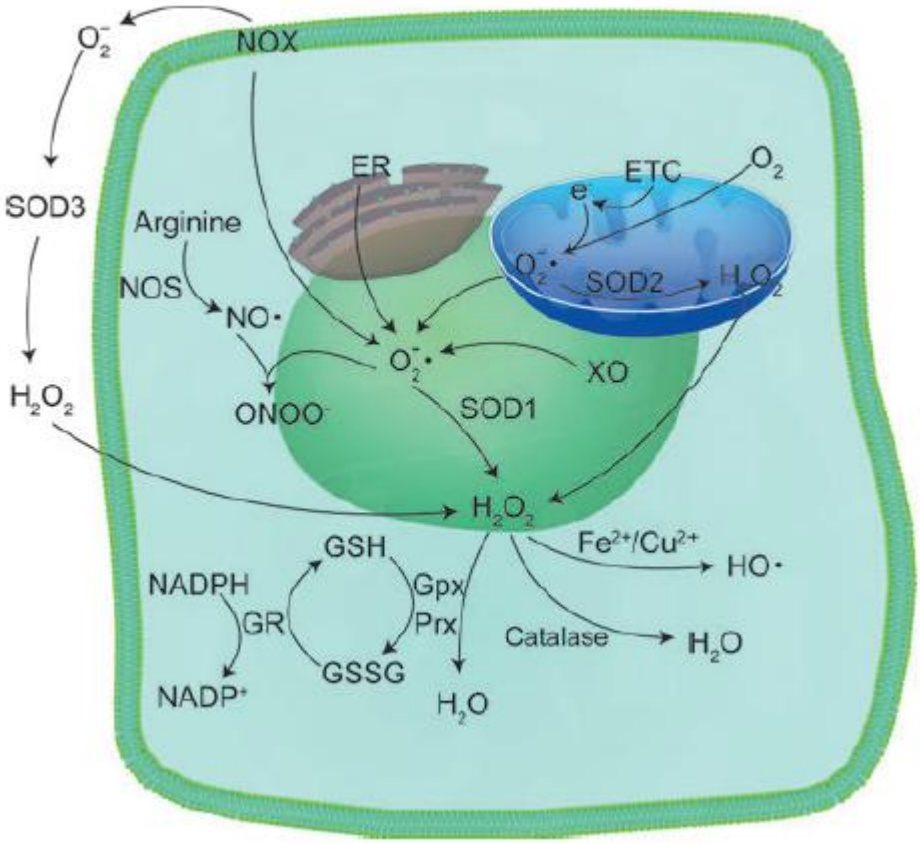


Figure 1.11. Sources and means of detoxification of ROS in the cell.

Superoxide is originated from different sources comprising normal cellular respiration, activated polymorphonuclear leucocytes, endothelial cells, and mitochondrial electron flux. Generation of superoxide by phagocytic NADPH oxidase is familiar to be significant in bacterial destruction. The generation of superoxide is elevated in acute and chronic inflammation at a ratio that defeats capability of the endogenous SOD enzyme defense system to eliminate it. Thus, thesuperoxide-mediated damage results from the consequence of this imbalance meant (Figure 1.12) (Muscoli et al., 2003). Superoxide dismutase (SOD) converts the superoxide anion to hydrogen peroxide, which can then be reduced to water by catalases or peroxidases. SODs are extensively found antioxidants that vary their location and metal cofactor requirements between different organisms. The enzyme activity is dependent on redox cycling of the attached metal cofactor (Morano et al., 2012).

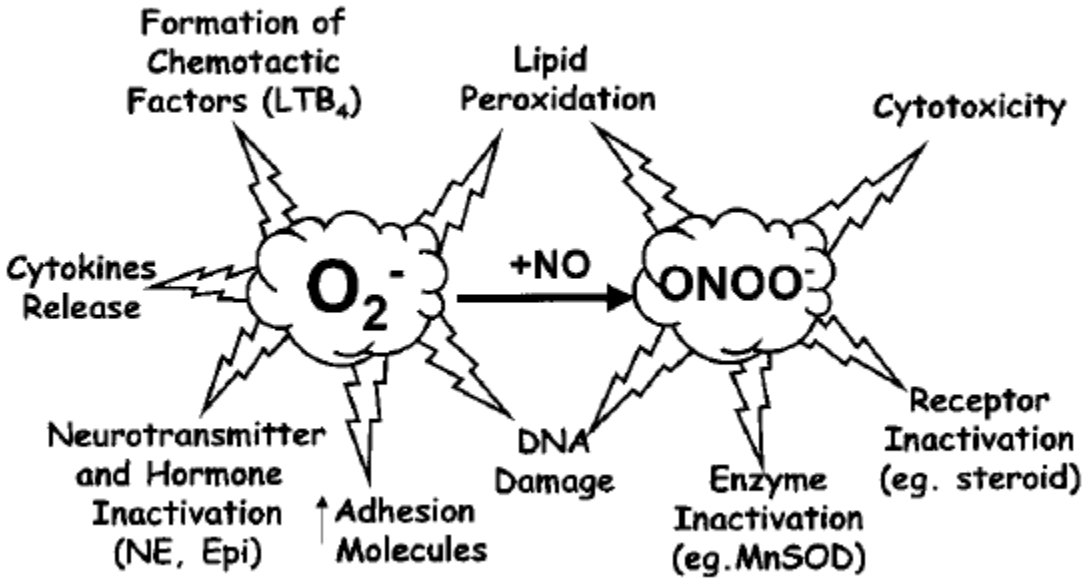


Figure 1.12. The impact of superoxide generation in inflammation.

Excessive production of superoxide can lead to inflammation through various pathways, including the generation of thedestruction of beneficial nitric oxide (NO) and

simultaneous generation of cytotoxic and proinflammatory peroxynitrite ($ONOO^-$)(Muscoli et al., 2003).

1.2.1.2. Glutathione reductase and its biochemical importance

Glutathione reductase (GR) also known as glutathionedisulfide reductase (GSR) is an enzyme that is encoded by the GSR gene in humans. In the glutathione system the oxidation of sulfhydryl groups is one of the initial events observed during ROS- mediated damage, thus the importance of GSH (g-glutamyl cysteinyl glycine) is emphasized that is particularly found as the most plenty low-molecular-weight sulfhydryl compound in almost all of the organisms. The roles of GSH that have been suggested among groups of cellular processes containing amino acid transport, synthesis of nucleic acids and proteins, enzyme activity modulation, and metabolism of carcinogens, xenobiotics and ROS (Morano et al., 2012). Inside the glutathione system, glucose-6-phosphate dehydrogenase (G-6-PD) and glutathione reductase (GR) do not take part in theaction on ROS directly, but GPx is permitted to function (Weydert and Cullen, 2010).The ability of glutathione to regulate and reduce the ROS effect is considered to be a crucial thiol-antioxidant derivative, however, it is dispensable for redox control and cellgrowth beneath specific conditions (Quintana-Cabrera et al., 2012). Glutathione is converted by the oxidative stress to its oxidized disulfide form (GSSG). Although, in yeast and other eukaryotes the glutathione is commonly present in its reduced form due to the fundamental action of glutathione reductase(GR). GR is an NADPH – dependent oxidoreductase that converts GSSG to GSH by using the pentose phosphate pathway.

$$GSSG + NADPH \xrightarrow{GR} 2GSH + NADP$$

GSH is synthesized through two ATP-dependent steps. ɤ-Glutamylcysteine synthetase (Gsh1) catalyzes the first and rate-limiting step where the dipeptide g-Glu-Cys is formed from glutamate and cysteine. The second step is catalyzed by glutathione synthetase (Gsh2) that binds g-Glu-Cys with glycine. GSH is able to be oxidized to GSSG by ROS or in the reactions catalyzed by Grx1–8 and Gto1–3. Reduced GSH is reproduced in an

NADPH-dependent reaction catalyzed by Glr1 (glutathione reductase). GSH can be conjugated to xenobiotics (RX) by GSTs, including Gtt1–2 and Grx1–2. GSH conjugates are carried to the vacuole by the Ycf1 GS-X pump(Figure 1.13)(Morano et al., 2012).

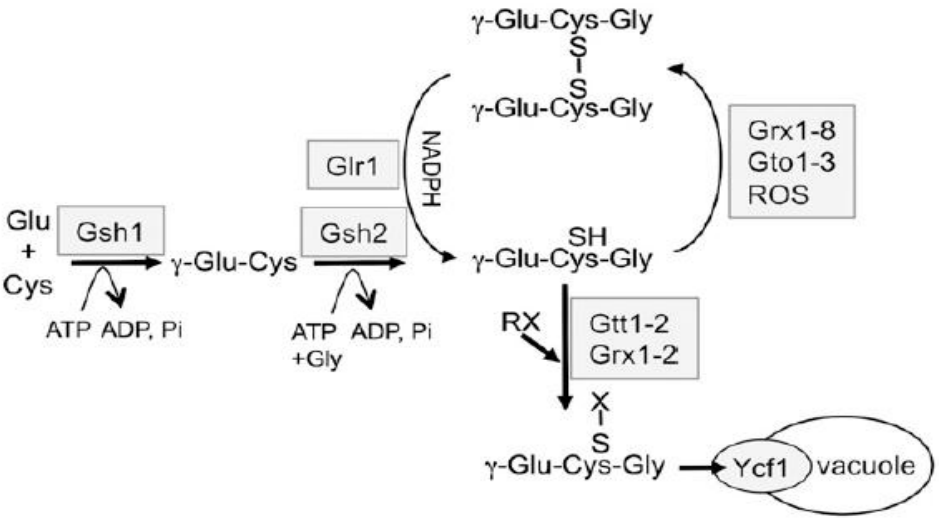


Figure 1.13. The Glutathione system.

In the biosynthesis of ɤ-Glutamylcysteine which is the present glutathione precursor to mitochondria and leads to the detoxification of hydrogen peroxide and superoxide anion despite the cellular concentrations of glutathione.In primary neurons, endogenously synthesized γ-glutamylcysteine completely prevents apoptotic death in many neurotoxic paradigms and, in an in vivo mouse model of neurodegeneration, γ-glutamylcysteine maintains against neuronal loss. Therefore, γ-glutamylcysteine takes over the antioxidant and neuroprotective functions of glutathione by acting as glutathione peroxidase-1 cofactor(Quintana-Cabrera et al., 2012). GSH is important also during the stress circumstances that act as acofactor for stress defense enzymes in addition to glutathione transferase (GST) and glutathione peroxidase (GPx) (Morano et al., 2012).

The most efficient thiol compound in the cells of all organs is the glutathione, which plays a fundamental and protective role in the brain toward the oxidative stress. Glutathione reductase (GR) is the enzyme that is in charge of the recycling of oxidized glutathione to the reduced form, and act as an antioxidant up-regulated in response to the oxidative stress. The oxidative stress,glutathione, and GR model have been involved in the

pathogenesis of both psychiatric and non-psychiatric diseases comprising Type 2 Diabetes mellitus,Leber's Hereditary Optic Neuropathy (LHON), Huntington's disease, schizophrenia and autism. It is observed that GR rises in activity with increasing oxidation in human fibroblasts and keratinocytes as a result of exposition to the elevated concentrations of arsenic (Espinoza et al., 2008).GSH is a beneficial intracellular antioxidant that protects the body systems from the damaging effects of oxidative stress. Commonly, morethan 95% of the total GSH is in the reduced form (GSH) and less than 5% is in the disulfide form (GSSG). Consequently, the decreased ratio of GSH to GSSG within cells indicate an imbalance in redox state and can also be used as a measure of cellular toxicity (Kukongviriyapan et al., 2016).

The pentose phosphate pathway is the origin of the cellular reducing power as the NADPH. In particular, NADPH is crucial when exposed to oxidants, because it maintains the reducing potential for most of theantioxidant and redox controlling enzymes comprising the GSH, glutaredoxin and thioredoxin systems.The 6-phosphogluconate dehydrogenase (6PGDH) and glucose 6-phosphate dehydrogenase (G6PDH) are the source of NADPH and facilitate the first two steps of the pentose phosphate pathway. The NADPH-production key step is catalyzed by G6PDH in addition to its ability in protection against oxidative stress (Morano et al., 2012).

1.2.1.3. Glutathione Peroxidase and its biochemical importance

Glutathione peroxidase (GPx) is the enzyme that is dependent on the micronutrient selenium (Se). It has an efficient role in the lipid and hydrogen peroxide reduction. The hydrogen peroxide presence and its elevation indicate areduction in the GPx activity that leads to the direct tissue damage and activation of nuclear factor factor-κB–related inflammatory pathways. GPx is categorized to four subspecies which facilitate the hydrogen peroxide reduction in certain tissue positions.

GPx1; is widespread and found in the cytosol of most cells, including red blood cells (RBCs).

GPx2; is also cytosolic but is enclosed to the gastrointestinal tract.

GPx3; takes place in the plasma as a glycoprotein and

GPx4; is found in mitochondria and interacts with complex lipids, such as lipoproteins and cholesterol damaged by free radicals (Espinoza et al., 2008).The glutathione peroxidase (hydrogen peroxide:GSHoxidoreductase, EC 1.11.1.9) was first determined by Mills and Randall in erythrocytes andsubsequently in other tissues. A study has maintained an evidence that peroxidase in erythrocytes protects hemoglobin from oxidation to methemoglobin rather than catalase by hydrogen peroxide. Hydrogen peroxide may be the toxic agent in the drug-influenced anemia associated with a genetic deficiency of erythrocyte glucose g-phosphate dehydrogenase. Lately, Hochstein and Utley have indicated that peroxidase in the liver supernatant is also capable of competing effectively with catalase for hydrogen peroxide. The oxidized glutathione formed by the peroxidase may be responsible when peroxide is produced in the erythrocyte for the enhanced glucose shunt. An identical mechanism is supposed for leukocytes during phagocytosis (Little et al., 1970).

$$ROOH + 2GSH \xrightarrow{} ROH + GSSG + H_2O$$

Glutaminolysis is a pathway that occurs inside the mitochondria in which the preliminary deamination of glutamine by the glutaminase takes place, obtaining glutamate and ammonia as a result. Glutamate is afterward converted to alpha- ketoglutarate (α-KG), entering the TCA cycle intermediate and generating both ATP and anabolic carbons subsequently for the synthesis of aminoacids, nucleotides, and lipids. Glutaminolysis also sustains the composition of molecules like glutathione and NADPH, which maintain cells from the oxidative stress. The glutamate conversion to the (α-KG) is catalyzed either by glutamate dehydrogenase1 or other transaminases that convert α-keto acids into their corresponding amino acids in the mitochondria. The key intermediate in the glutamine metabolism is said to be (α-KG) and the product of GDH1, is stabilizing the redox homeostasis in cells. The mitochondrial enzyme glutamate dehydrogenase 1 (GDH1) is generally arranged in cancers found in human body.GDH1 is crucial in cancer cells for redox homeostasis by managing the intracellular levels of its yield alpha-ketoglutarate (α-KG) and the latter metabolite fumarate. Since the ROS homeostasis is vital for the survival and the cell growth,fumarate binds to and maintains the activation of a ROS scavenging

enzyme which is glutathione peroxidase 1 (GPx1). GPx1 isa selenoprotein and GDH1 does not form a protein complex with GPx1, therefore GDH1 may indirectly regulate GPx activity and ROS levels subsequently by controlling the intracellular levels of α-KG(Jin et al., 2016).

1.2.2. Activation of internal antioxidant enzymes

In the course of metabolism ROS such as H_2O_2, $O_2^{-}\bullet$ and •OH and etc, are produced irreversibly. Therefore, methods to decrease the damage generated by oxidative stress are broadly analyzed. Intracellular antioxidant enzymes are an important protective mechanism against ROS. These enzymes are produced in the cell and provide an important defense against free radicals. SOD, CAT, GPX, glutathione reductase (GR), glutathione S-transferase (GST), thioredoxin reductase (TrxR), heme oxygenase and biliverdin reductase are the most important antioxidant enzymes.

The enzyme SOD converts two $O_2^{-}\bullet$ into H_2O_2 and an oxygen molecule (Eqs. 1 and 2).

$$M^{(n+1)+} - SOD + O_2^{-\bullet} - \rightarrow M^{n+} - SOD + O_2 \quad (1)$$

$$M^{n+} - SOD + O_2^{-\bullet} - + 2H^{+} \rightarrow M^{(n+1)+} - SOD + H_2O_2 \quad (2)$$

Where M = Cu (n = 1), Mn (n = 2), Fe (n = 2) and Ni (n = 2).

In this reaction, the oxidation state of the metal ion oscillates between $^{n+}$ and $^{n+1}$. SOD is an important antioxidant defense in nearly all cells exposed to oxygen. Meanwhile, CAT and/or GPX can eliminate H_2O_2 before the Fenton Reaction can create ·OH (Eqs 3 and 4).

$$2\,H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2 \quad (3)\text{and } (4)$$

$$2GSH + H_2O_2 \xrightarrow{GPX} GS{-}SG + 2H_2O$$

Where GSH indicates reduced glutathione and GS–SG stand for glutathione disulfide. GR then reduces the oxidized glutathione (GS-SG) to complete the cycle (Eq. 5)

$$GS\text{–}SG + NADPH + H^{+} \xrightarrow{GRd} 2\,GSH + NADP^{+} \qquad (5)$$

Both GST and GPX are involved in eliminating peroxides that are formed during metabolism. GRd regulates the equivalent of GSH and oxidized glutathione (GSSG), and the ratio of GSH/GSSG is a well-known index of oxidative stress (James et al., 2009). The activation of GRd plays an important role in elevating the concentration of GSH, which maintains the oxide-redox status in the organism (Shih et al., 2007). The brain, which is highly exposed to free radical damage, has seven times more GPX activity than CAT activity (Artur et al., 1991). (Lü et al., 2010; Shih. et al., 2007; Scandalios, 2005).

1.2.3. Redox Modulations

Organisms are constantly challenged by ever-varying variables in their environment, comprising fluctuating nutrient levels, osmotic imbalance, exposure to toxic molecules, and non-optimal temperatures (Morano et al., 2012). Thus, several chemical reactions are frequently occurring inside our cells as a result of our dynamic environment (Fraunberger et al., 2016). ROS give multiples of cell damages because of protein misfolding, changed protein conformation or even fragmentation and lipid peroxidation (Alves et al., 2013). In addition, all organisms are exposed to ROS during the normal aerobic metabolism process or after exposure to radicalformation compounds (Halliwell, 2006). The redox modulation is described as the imbalance in the redox status and if the imbalance occurs as a result of shifting a huge amount towards more oxidized intermediate, characterization of this imbalance occurs by modifications in cellular dynamics and altering degrees of DNA, RNA, protein and lipid damage.In these reactions, one reactant loses electrons (oxidized) and the other gains those same electrons (reduced), in which usually the reaction is provided by an enzyme. An accurate electrical homeostasis between the various macromolecules that contain them is provided by our cells. Thus, the outcome of this balance between oxidized and reduced compounds inside the cell is known as redox status. Under normal conditions, commonly the by-products of metabolism are said to be reactive species. The balance is sustained as a result of the natural endogenous antioxidant

defenses resisting the continuous generation of reactive species in a healthy cell(Fraunberger et al., 2016).

1.3. Oxidative Stress

Oxidative stress is known as a normal fact in the body that the physiologically vital intracellular levels of the reactive oxygen species (ROS) at normal conditions are preserved by different enzyme systems at low levels in which they participate in the in vivo redox homeostasis. Thus, oxidative stress is also observed as an imbalance between the antioxidants and prooxidants (Figure 1.4)within the body (Rahal et al., 2014).The deleterious effect of free ROS and RNS radicals that cause potential biological damage is defined in respective as oxidative stress and nitrosative stress. The complex process of redox stress/oxidative stress has aninfluence on the organism in which it depends on the type of the oxidant, on the site and intensity of its production, on the structure and activities of different antioxidants, and the capability of repair systems.

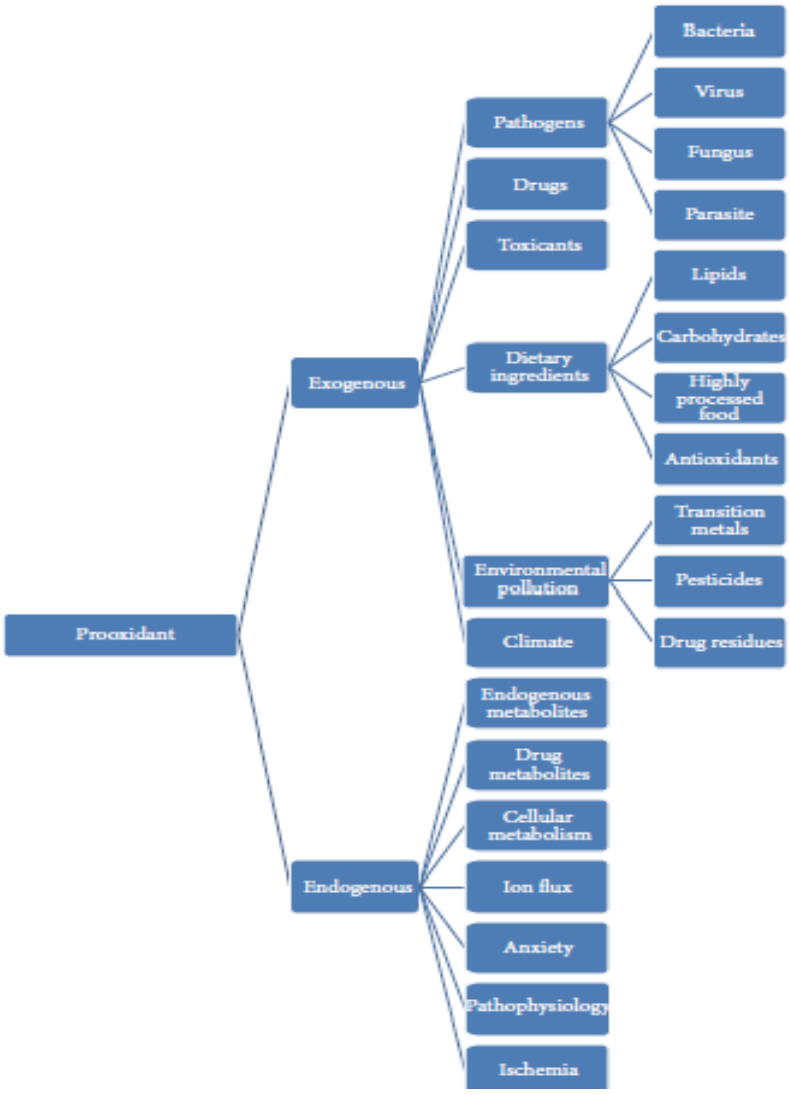


Figure 1.14. General classification of antioxidants (Rahal et al., 2014).

Oxidative stress which is induced by ROS ends with a huge risk for many disorders like inflammatory disease,cancer, cardiovascular disease,cataracts,diabetes, autism and aging (Lü et al., 2010). The imbalance between the generation of reactive oxygen species (ROS), reactive nitrogen species (RNS) and the ability of the biological system to detoxify these reactive mediums cause the oxidative stress. Biochemical modifications are induced by ROS and RNS, which subsequently lipid peroxidation is obtained, proteins are oxidized and DNA damage (Kukongviriyapan et al., 2016). The imbalance in redox signaling often causes the oxidative stress, for example, due to the production of an elevated amount of

oxidants during the normal cellular metabolism (i.e, mitochondrial electron transport or NADPH oxidases) or from environmental stimuli (i.e, cytokines)(Alves et al., 2013).In the oxidative stress theory of aging loss of SOD activity that should result in elevated sensitivity to oxidative stress is predicted, since the organism would be less able to detoxify ROS (Poljsak, 2011)(Figure 1.15).The oxidative stress is described by an increased sensibility of the organism versus the oxidative damage thereafter the meal that is rich in lipids or carbohydrates' consumption. Excess of superoxide generation after the meal due to loads of energy substratemay result in a strong influencing factor that resultsin oxidative stress and reduction in nitric oxide (Poljsak, 2011). Nowadays, the world is facing a huge elevation in diseases that are related to the chronic health disorders like cardiovascular, autoimmune diseases,cancer, diabetes,and so on.

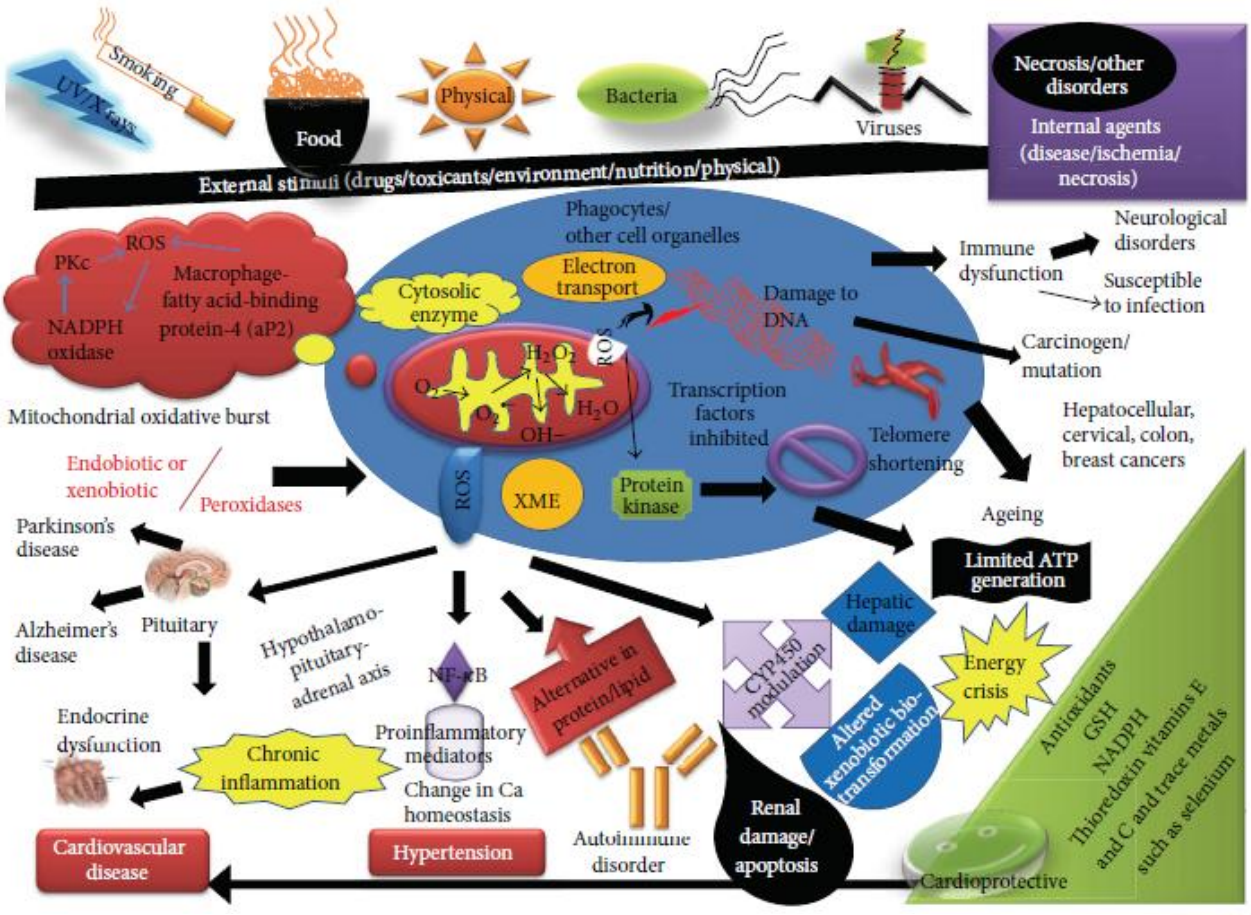


Figure 1.15. Oxidative stress and development of diseases.

As soon as the initiation of lipid peroxidation occurs, a propagation of chain reactions will happen until the products of termination are obtained (Rahal et al., 2014). Hence, lipid peroxidation end products are malondialdehyde (MDA), 4-hydroxy-2-nonenol (4-HNE) and F2-isoprostanes are gathered in the biological systems and these are also indicated as markers of lipid peroxidation, in which all of these are known as neurotoxic agents(Jomova et al., 2011; Rahal et al., 2014). Acute oxidative stress can result in cell death. The experienced degree of oxidative stress by the cell will be a function of the ROS activity that forms reactions and the ROS scavenging system activity. According to a study the common signaling failure with aging might be due to deficient reactive species or production of improper reactive species,however, it's defined that oxidative damage increases in various tissues and animal samples with age.

The reasons that increase ROS production include endogenous causes (elevation in O_2concentration,inflammation, and increased mitochondrial leakage) and exogenous (environmental pollution, active exercise, nutrition, smoking, chronic inflammation, psychological and emotional stress, and others). Causes of decreased antioxidant defenses include reduced activity of endogenous antioxidative enzymes and reduced intake or absorption of antioxidants from food (Poljsak et al., 2013). Totally, the factors responsible for the oxidative stress directly or indirectly take a part in the immune mechanism of thedefensesystem. Any change leading to immune-termination can initiate the disease generation (Table 1.2).The main pathway of cell damage pathogenesis is through lipid peroxidation especially in microsomes, endoplasmic reticulum and mitochondria due to oxidative stress and free radicals (Rahal et al., 2014).

Table 1.2. Deadly diseases that have got positive correlation to oxidative stress.

Sl. number	Disease	Organs involved	Etiology
(1)	Macular degeneration	Eyes	Reactive oxygen intermediates (ROI)
(2)	Diabetes	Multi-organ	Superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase
(3)	Chronic fatigue	Multiorgan	C-reactive protein
(4)	Atherosclerosis	Blood vessels	Reduced NADPH oxidase system
(5)	Autoimmune disorders (systemic lupus erythematosus)	Immune system	R_o ribonucleoprotein
(6)	Neurodegenerative diseases (Alzheimer's and Parkinson's disease)	Brain	Reactive oxygen species (ROS)
(7)	Asthma	Lungs	ROS particularly H_2O_2
(8)	Rheumatoid and osteoarthritis	Joints	Radical oxygen species
(9)	Nephritis	Kidney	Glutathione transferase kappa (GSTK 1-1)
(10)	Melanoma	Skin	Pathophysiological processes including DNA damage and lipid peroxidation (LPO)
(11)	Myocardial infarction	Heart	Reactive oxygen species (ROS)

(Rahal et al., 2014).

Mechanisms that maintain cells from oxidative stress (e.g., DNA repair processes, endogenous antioxidants) are consuming critical amounts of energy whenever they are activated for anextended period in all subdivisions of the cell.Many energy may be required to maintain all the oxidative damage during the life of an organism (Poljsak et al., 2013). Several studies have indicated the interdependency and cooperation of oxidative stress, immune system, and inflammation. The relationship between oxidative stress and immune function of the body is well based. The immune defense mechanism utilizes the fatal causes of oxidants in a manner that is advantageous with ROS and RNS playing the main role in destroying pathogens. Oxidative stress can stimulate the generation of free radicals that can alter proteins.

Modifications in self-antigens (i.e., modified proteins) can induce the process of autoimmune diseases. Underneath the OS, cells may generate an excess of ROS/RNS which react and alter lipids and proteins in the cell.OS produced due to unresolved and permanent inflammation can be the main factor that is concerned with immune responses in the alteration ofdynamics. These alterations can form an immunological chaos that could lead to loss of architectural integrity of cells and tissues finally leading to chronic

conditions or cancers. Oxidative stress is informed to be the cause of induction of allergies, autoimmune or neurodegenerative diseases along with modified cell growth, chronic infections leading to neoplasia, metastatic cancer, and angiogenesis. The oxidative stress theory is the most accepted clarification for the aging nowadays, which states that elevation in ROS lead to physiologicalchanges, pathological situations and other clinically observable signs of aging and eventually death (Rahal et al., 2014). Aging is ordinarily associated with an elevated amount of reactive oxygen species (ROS) that cause severe detriment to cells and tissues. The disequilibrium between the production and elimination of ROS and the oxidative stress development plays a crucial role in age-related diseases, like Alzheimer's, cancer and heart disease (Aliahmat et al., 2012).

Oxidative stress existsat genetic,cellular,molecular, tissue and system levels of all living beings and usually, OS reveals a developing accumulation of various detrimental alterations in the cells and tissues within developing age in addition to increasing the risk of disease and death. Recent studies have demonstrated that ROS levels accumulate within age in the main organ systems as liver,brain, heart and skeletal muscle and are represented either due to their elevated yield or reduced detoxification.Oxidative detriments moderated by free radicals lead to protein modification and then cellular damages and finally disease pathogenesis. There exists equilibrium between the antioxidants level and cellular prooxidants under normal conditions of physiology. But if environmental factors or stressors start to exist, an imbalance occurs in the homeostasis that is in favor of prooxidants, hence resulting in the phenomenon of oxidative stress(Rahal et al., 2014).

1.3.1. Lipid Peroxidation and Malondialdehyde

One of the most prior markers of the oxidative stress is said to be the lipid peroxidation of polyunsaturated lipids(Rahal et al., 2014). Lipid peroxidation is a chain reaction that comprises the oxidation of polyunsaturated fatty acids in the membranes stimulated by free radicals and also it’s an indicator of oxidative cell damage. Measurement of oxidative stress in humans directly is hard since the active oxygen species and free radicals are very short-lived. Instead, oxidative stress products are measured (Güney et al., 2004).

In which the product of this peroxidation is indicated as malondialdehyde, that is detected in bloodplasma easily and is defined and used as a measure of oxidative stress (Güney et al., 2004; Rahal et al., 2014). Oxidative stress correlated with an elevation in MDA and reduction in antioxidant parameters was also indicated in gastric,renal,oropharyngeal, lung and breast cancer and colorectal adenomas.According to studies, it was proven that in the mentioned cancer locations MDA levels are significantly higher and parameters related to antioxidants, such as CATactivity, lower compared to healthy controls (Didziapetriene et al., 2014).“MDA is concluded as a useful biomarker for lipid peroxidation and oxidative stress” according to Singh et al. research. MDAhas produced from peroxidation of polyunsaturated fatty acids the reaction of deoxyribose with a hydroxyl radical, as a by-product of prostaglandin synthesis, and by ɤ–irradiation of carbohydrates. It could be discovered in foods and absorbed from gastrointestinal tract, which changes MDA levels. Cigarette and intake of some drugs will also affect the MDA levels (Jafari et al., 2015).

Elevated lipid peroxidation in abnormally proliferation cells is described as being released into the systemic circulation that results in raised MDA levels in cancer patients as observed in most studies (Güney et al., 2004). Also, the instability of malondialdehyde in the existence of hydrogen peroxide appears to obtain unstable results in studies and this correlates the handling of intermediate hydrogen peroxide levels in order to initiate the lipid peroxidation (Çekiç et al., 2013).

1.4. Familial Mediterranean Fever (FMF)

Familial Mediterranean fever (FMF) is an autosomal recessive disorder represented by recurring attacks of fever; serositis and some patients suffer from episodes of pleuritis, arthritis and erysipelas-like skin disease, orchitis and pericarditis. Especially it’s widespread in Mediterranean population in which it mostly affectsArabs, Non-Ashkenazi Jews, Turks, and Armenians. Additionally, this periodic disorder results in painful attacks cause inflammation comprising the membranes in the abdomen, chest or joints in which the inflammation lasts one to three days (Gershoni-Baruch et al., 2002; Gürbüz, 2016; Mattit et al., 2006).

The mutations that have been identified in MEFV (Mediterranean FeVer) gene are over 50 mutations that are responsible for FMF (Mattit et al., 2006). These mutations encode apyrin-marenostrinprotein that causes FMF disease. The protein encoded by MEFV was called Marenostrin by the French group in reference to the Latin name of the Mediterranean Sea, and Pyrin by the International consortium in reference to the Greek name of fever. Marenostrin/ Pyrin is a 781 amino acid protein of a predicted 86 kDa molecular weight. It has been thought for a while that Marenostrin/Pyrin was a transcription factor, because of the existence of two nuclear localization sites in the gene sequence (Touitou, 2001). This protein is displayed in myeloid/monocytic cells mainly and Pyrin acts as a regulator of the inflammatory response in the hereditary immune system.

A mutated pyrin may result in uncontrolled inflammation due to not precise regulating nature revealed by pyrin. Thus pyrin, through a complex called inflammasome assists as a regulator of the conversion of pro-interleukin (IL)-1β to mature IL-1β (Figure 1.16)and its modulation process leads to activation of NF-κB and apoptosis (Gürbüz, 2016; Onen, 2006).

The FMF gene (MEFV) is positioned on the chromosome 16p13.3, and this was identified in the summer of 1997 by positional cloning and 10 exons are included (Touitou, 2001).The marenostrin-encoding fever gene (MEFV) was increased and four mutations (M694V,M680I,M694I, V726A) have been detected in exon 10 in a huge proportion of influenced patients , and this cloning has clinical importance since the detection of mutations in the MEFV gene maintains the diagnosis of FMF eventually (Brik et al., 1999). In addition, these mutations in the MEFV gene are considered to be involved in non-FMFdisorders, and carrier rates for FMF mutations may be as high as 1:3 in some populations. Classically the four affected ethnic groups according to early studies on MEFV mutations had focused on commonly affected populations, namely: Arabs (North Africans i.e., Maghrebins, and Orientals), Armenians, Non- Ashkenazi Jews (NAJ i.e.,Sephardim and Orientals) and Turks.

The disease perhaps is more recognized in these four ethnic groups not only because the prevalence of MEFV mutations is high, but also because their clinical picture is usually complete. M694V is the most frequent mutation in all four populations, ranging from 20

to 65%. Comparative studies are now available which further address the spectrum of mutations according to the geographic origin of these patients. In North African Jews, M694V is overrepresented (71%), while in East European Jews (Ashkenazim), a moderate mutation V726A is the most frequent (38%).

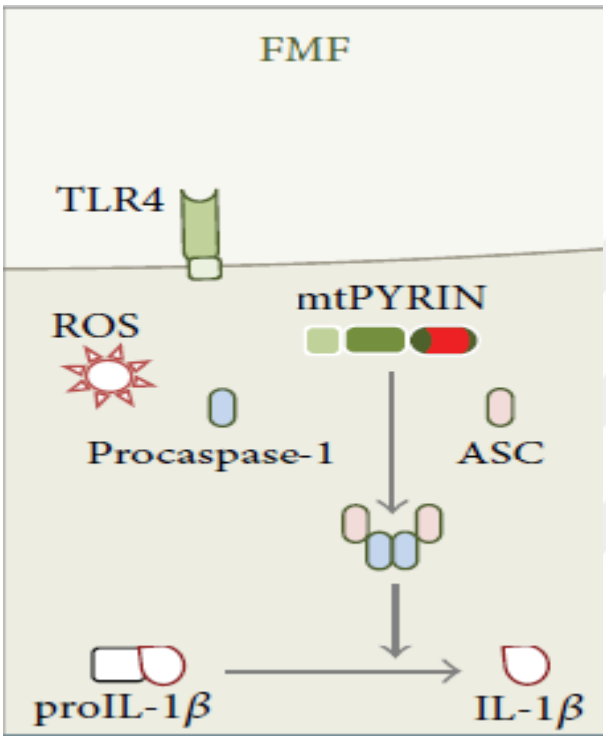


Figure 1.16. Schematic sketch of FMF mechanism.

This sketchshows the main pathophysiologic mechanism of themonogenicautoinflammatory syndrome, Familial Mediterranean fever (FMF)(Caso et al., 2013).

Interestingly, the prevalence of these two mutations in Oriental Jews is in between that of North African Jews and Ashkenazim, suggesting that M694V and V726A likely spread from the Middle East. Arabs have various mutational patternsdepending on their origin. Oriental Arabs have more V726A than Arabs from North Africa, while M694I seems to be relatively specific to Maghrebins. Only 16% of Armenian patients have unidentified MEFV mutations. No differences in the distribution of MEFV mutations has been detected

between Armenian patients from Yerevan and those living in California or France (Touitou, 2001).

More recent studies extended MEFV mutation screening to patients from other origins and from other ethnic groups, mostly Europeans not from the ancestries that mentioned in other studies. Italians, especially those from the South of Italy and Sicily, should no longer be considered as rarely affected populations. A broad range of mutations is found in Italians, the most frequent being E148Q (18%). In Spanish patients, M694V and E148Q are the first (32%) and second (14%) most frequent mutations respectively, as in NAJ. This is not astonishing because most of the NAJ are descendants of Sephardi Jews who were forced to escape to Spain in the 15th century (Sephardi means Spain in Hebrew). A number of Portuguese patients have been referred for genetic testing, but V726A was the only mutation found in 17% of them. The distribution of MEFV mutations in Greeks correlates that of Arabs and Italians. Three British FMF families have been described with probably true dominant FMF. Patients of Indian, Chinese, Afghan and Hungarian origin have also been described (Bernot. et al., 1998; Booth et al., 1998).

The MEFV gene that is responsible for the disease has been informed to be on the short arm (16p13.3) of the 16^{th} chromosome. Determination of mutations in the MEFV gene maintains the diagnosis in FMF patients with clinical findings.Inspite of indefinite and uncertain diagnostic laboratory test for FMF, commonly the elevated acute-phase response observed during these attacks returns to normal in attack-free periods. The disorder is characterized by recurrent attacks of fever, pleuritis, peritonitis, arthritis and skin lesions. Although FMF is a clinical condition that is identified as painful inflammatory attacks,cytokines, high levels of acute-phasereactants, and inflammation-generated proteins that measured during the attack-free phases in most patients still recommend the proceeding of inflammation (Kosan et al., 2013). According to the studies, 12 known FMF mutations have been detected and screened by using a reverse-hybridization test strip-based assay (FMF StripAssay, ViennaLab Labordiagnostika, Vienna, Austria) that permit the detection of most common ones:

p.E148Q (c.442G>C) in exon 2; p.P369S (c.1105C>T) in exon 3; p.F479L (c.1437C>G) in exon 5; and p.M680I (c.2040G>C), p.M680I (c.2040G>A), I692del (c.2076>2078del), p.M694V (c.2080A>G), p.M694I (c.2082G>A), p.K695R (c.2084A>G), p.V726A

(c.2177T>C), p.A744S (c.2230G>T), and p.R761H (c.2282G>A) in exon 10 (Özer et al., 2015). More than 40 MEFV mutations are associated with FMF encode either single amino acid substitutions or deletions. Mutations that cause diseases occur mostly in exon 10 but also occur in exons 1, 2, 3, 5, and 9. Mutations in each of the two MEFV alleles are found in 85% of patients with FMF, while the numerous majority of individuals with a single mutated allele are healthy carriers(Lachmann and Hawkins, 2009).

MEFV mutation was investigated by extraction of the genomic DNA from peripheral leukocytes using standard procedures. The part of exon 10 was analyzed in the MEFV gene where most of the previously reported mutations were located. Polymerase chain reaction (PCR) was performed using 5' GAGCCTGCAAGACATCCATA- 3' and5'-TGACCACCCACTGGACAGAT- 3' as primers (Tomiyama et al., 2008). According to studies demonstrations IL-1b, which is the main determinant of theimmune response in FMF, is an efficient pro-inflammatory and atherogenic cytokine. IL-1b up-regulates the expression of various effectors through the induction of IL-1 receptors and the nuclear factor-kB pathway (Gürbüz, 2016). Additionally, several studies have detected the increased levels of interleukin-6 (IL-6), interleukin-10 (IL-10), serumsoluble interleukin-2 receptor (sIL-2r), and tumor necrosis factor alpha (TNF-α) between or during acute attacks of FMF (Özer et al., 2015). FMF-relatedMEFV mutations, which affect pyrin-mediated regulation of caspase 1 activity in the inflammasomes, are associated with increased IL-1β production in mice and humans. Thus, inhibition of IL-1 activity may decrease both frequency and severity of acute attacks in patients with FMF. Many reports of patients with FMF treated successfully with agents blocking IL-1 activity, generally with daily injections of the recombinant form of IL-1 receptor antagonist (IL-1Ra) of anakinra, have confirmed the critical role of IL-1 in the pathogenesis FMF(Gül et al., 2015). Proteins with pyrin domain (PYD)s play vital roles in the regulation of caspase-1 and therefore regulate the production of interleukin-1 (IL-1) (Lachmann and Hawkins, 2009).

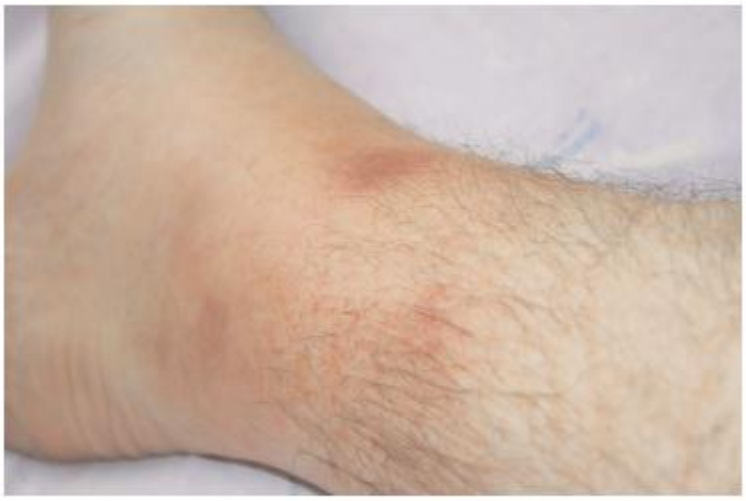
1.4.1. Clinical Features

FMF is widespread in Middle Eastern populations but occurs worldwide. The prevalence of FMF is estimated to be 1/250 to 1/500 among Sephardic Jews and 1/1,000 in the

Turkish population. Carrier frequency exceeds 1 in 4 in some eastern Mediterranean populations, prompting speculation that the FMF trait may have discussedthe existing benefit, possibly through enhanced resistance to microbial infection mediated via an upregulated hereditary immune response.Males and females are affected equally and the disorder usually presents in childhood. Attacks of FMF occur irregularly and apparently spontaneously although some may be precipitated by minor physical or emotional stress, the menstrual cycle, or diet. Attacks develop rapidly and symptoms resolve within 72 hours (Lachmann and Hawkins, 2009). The clinical symptoms can change in several patients, sometimes even in the same family. Similar to amyloidosis is observed in other chronic inflammatory diseases such as rheumatoid arthritis which is the most severe complication of FMF that leads to renal failure. Some individuals develop amyloidosis without having recurrent inflammatory episodes (FMF type 2) and therefore, identification of an amyloidosis associated mutation will allow for directing prophylactic colchicine therapy to pre-symptomatic individuals that can greatly benefit from it (Mimouni et al., 2000).

Feverswith serositis are the cardinal features, and these can changefrom mild to incapacitating. Peritonitis that can mimic an acute surgical abdomen occurs in 85% of cases, and indeed 40% of patients will undergo exploratory surgery before FMF is diagnosed. Pleuritic chest pain occurs in 40% of patients, characteristically unilaterally, either alone or with peritonitis. A headache with features of meningism has been reported in children in particular, but the nervous system is not usually involved. Orchitis occurs in less than 5% of patients, most commonly in early childhood, and can be confused withtesticular torsion. Joint involvement usually affects the lower limbs: arthralgia is common in acute attacks and usually subsides within a couple of days, but a chronic destructive arthritis can rarely occur. A characteristic erysipelas-like rash occurs in 20% of patients, usually around the ankles (Figure 1.17). A degree of myalgia can occur during acute attacks, but up to a fifth of patients complain of persistent muscle pain on exertion, usually affecting the calves. Protracted febrile myalgia is rare and is characterized by severe pain in the lower limbs or abdominal musculature which may persist for weeks and can be accompanied by a vasculitic rash; it usually responds to corticosteroids therapy.Acute attacks are accompanied by a neutrophilic leukocytosis, raised ESR, and a

dramatic acute-phase response. Investigations may be required to exclude other diagnoses but imaging by x-ray, ultrasound, or echocardiography during attacks is usually unrewarding (Lachmann and Hawkins, 2009).



Erysipelas-like erythema around the ankle, the characteristic painful rash seen in attacks of familial Mediterranean fever.

Figure 1.17. Erysipelas.

Pyrin is expected to interact with several proteins through the cytoplasm and to play the main role in the modulation of inflammation and apoptosis. One certain pyrin variant, E148Q encoded in exon 2 has allele frequencies of 10% to 20% in Asian populations and up to 1% to 2% in Caucasians. Since pyrin E148Q can cause FMF when coupled with an exon 10 mutation, homozygosity for E148Q alone is not associated with the disease in the enormous majority of cases. There is some evidencethat FMF carriers, perhaps especially those with pyrin E148Q, may have an augmented response to some types of non-FMF inflammation (Lachmann and Hawkins, 2009).

1.4.2. Diagnosis

The FMF diagnosis is maintained by DNA analysis but essentially remains clinical and centers on the history of recurrent self-limiting idiopathic attacks of fever and serositis that can be prevented by prophylactic colchicine treatment. Genetic results must be interpreted

cautiously given that certain individuals with paired pathogenic MEFV mutations never develop FMF and that others with heterozygous carrier status can do so. Furthermore, most diagnostic laboratories offer only limited analysis of the large 10-exon MEFV gene (Lachmann and Hawkins, 2009).

1.4.3. MEFV mutations and other inflammatory diseases

An increased set of data led to the hypothesis that the MEFV gene may be involved in non-FMF conditions. A high prevalence of MEFV mutations has been demonstrated in patients suffering from Behcet disease, non-FMF secondary amyloidosis (e.g.Rheumatoid Arthritis), recurrent abdominal pain. Additionally, some polyfactorial inflammatory diseases are morefrequent in FMF patients than in the ethnically matchedgeneral population: Inflammatory bowel disease, Behcet disease. It has been suggested that some MEFV mutations may non-specifically upregulate the inflammatory response. This hypothesis is further supported by the fact that acute phase reactants are enhanced in asymptomatic individuals with one or two MEFV mutations (Touitou, 2001).

1.4.4. AA amyloidosis

Reactive systemic (AA) amyloidosis is an often fatal disordermainly affecting the kidneys, which occurs in a small proportion of patients with one of a wide range of chronic inflammatory diseases. AA amyloid fibrils are derived from the circulating acute-phase reactant serum amyloid A protein (SAA), and their accumulation in tissues throughout the body progressively damages the structure and function of vital organs. SAA is synthesized by the liver under thetranscriptional regulation of IL-1, interleukin 6 (IL-6) and TNF-α, and its plasma concentration, which in health is less than 3 mg/l, may rise a thousand-fold in the presence of inflammation.During the lifetime incidence of AA amyloidosis is about 1% to 5% in patients with chronic inflammatory diseases generally, it is much more common among patients with inherited periodic fever syndromes, although the factors that determine susceptibility to its development, other than the presence of an acute-phase response for a long period, are not known. The median duration of inflammatory disease in patients who develop amyloidosis is about 20 years, and the life-long nature of inherited

periodic fever syndromes is probably a factor in the high prevalence of amyloid in these diseases; another factor may be the unusually high plasma concentrations of SAA that typically occur in inherited periodic fever syndromes. Up to 60% of patients with FMF died of renal failure due to AA amyloidosis before prophylactic colchicine was widely prescribed, and even recently it was reported in 13% of a large Turkish series (Lachmann and Hawkins, 2009).

1.4.5. Treatment

The provided measures, including analgesia, are commonly required during acute attacks, but the based point of management is long-term prophylactic treatment with low-dose colchicine. This was discovered in 1972 by Goldfinger and has entirely transformed the outlook of this previously disabling disease. Continuous treatment with colchicine at a dose of 1 to 2 mg daily in adults prevents or practically reduces symptoms of FMF in at least 95% of cases and almost completely eliminates the risk of AA amyloidosis. The mechanism of action of colchicine remains incompletely understood, but colchicine binds to tubulin and evidently modulates neutrophil adhesion, mobility, and cytokine release in a presumably rather specific manner in patients with defective pyrin variants (Lachmann and Hawkins, 2009). Colchicine has extendedlybeen used to prevent inflammatoryattacks and reduce the risk of secondary amyloidosis inpatients with FMF.

Inspite of the efficacy of colchicine, some manifestations, such as arthritis, are less responsive to colchicine. Furthermore, 5−10% of patients are non-responders to colchicine, and the number ofFMF patients who are either intolerant or resistant to colchicine and continue to experience frequent and/or severe inflammatory attacks is increasing (Gül et al., 2015). Since colchicine has been displayed to be effective in preventing the development of amyloidosis,continuouslifelong treatment has been recommended for all FMF patients. Based on investigations, however, it seems that children who are homozygotes for M694V are at the highest risk, and this group should be given colchicine treatment for life. This requires a high level of awareness of all pediatricians working in communities with a high prevalence of FMF. In mildly affected patients (those with infrequent inflammatory attacks) who do not carry this mutation, it

has been recommended that the patients should be either treated or tested every 6 months for the presence of proteinuria. The need for continuous treatment seems to be less indicated in the case of those patients where 1 or both mutations are E148Q, and in this group, colchicine treatment should be given to those patients who develop severe inflammatory episodes and/or proteinuria caused by amyloidosis (Mimouni et al., 2000).

Long-term colchicine is advisable for every patient with FMF and compulsory in those who already have AA amyloidosis. Although colchicine is very toxic in acute overdose, the low daily doses required for treatment of FMF are generally very well tolerated. Diarrhea is the most common side effect and usually can be avoided by thegradual introduction of the drug. Despite theoretical concerns, there is no evidence that colchicine causes infertility or birth defects and it can be taken safely by nursing mothers. Colchicine is a purely prophylactic treatment in FMF, and introduction or dose escalation during an acute FMF attack is not generally effective (Lachmann and Hawkins, 2009).

2. MATERIALS AND METHODS

2.1. Material

In our investigation, we took blood from 35 healthy individuals, and 35 patients with FMF in both male and female children. From each of healthy and patient individuals, we took 4ml of blood from anantecubital venous vein and added 2ml to the biochemistry tube and the other 2ml to the EDTA tube.

2.1.1. Devices and materials

Vortex

Deep Freezing Tubes

Serum Storage Tubes

Spectrophotometer

Adjustable Automotive Pipettes

Thermostated Water Bath

Glass pipette

Cooled centrifuge

Deep freeze

Oven

Stopwatch

Sensitive balance

Spectrophotometer cuvette

Automated Pipette Tip

PH-meter

Magnet

Beaker

Flask

Erlenmeyer flask

2.1.2. Reagents and chemicals

KOH

H_2O_2

Na-azide

GSH

GR

Xanthine

Nitro Blue Tetrazolium

Na_2CO_3

Bovine Serum Albumin

Xanthine oxidase

$NH_4(SO_4)$

CuCI2

Hydrogen peroxide solution

Phosphate buffer

Sodium hydroxide solution

DTNB

Sodium citrate

NADPH

GSSG

Ethylenediamine tetra-acetic acid solution

Butylhydroxytoluene solution

Thiobarbituric acid solution

Trichloroacetic acid solution

Concentrated alcohol

2.2.Method

The total population of the studythat diagnosed and monitored wasranging from 0-18 year(s) of age whichincluded35 patients who had been diagnosed with Familial Mediterranean Fever and 35 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 YüzüncüYıl University Medical Faculty of Educational Research and Training HospitalDepartment of Child Health And Diseases Clinic And Laboratory Research Center. Fromselected healthy and sick individuals 4 mlblood was taken from venousconveniently as the study subject and centrifuged with 5000 r/min for 10 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.

2.3. Analysis Methods

2.3.1. Determination of superoxide dismutase (SOD) activity

SOD 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^{-}•) 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.

$$\% \text{ İnhibition} = [(\text{Blank OD} - \text{Sample OD}) / \text{Blank OD}] \times 100$$

2.3.2. Determination of reduced glutathione (GSH) activity

In the hemolysis of EDTA blood which prepared with distilled water,all the proteins that don't carry sulfhydryl (SH) group are precipitated with precipitation solution. The reduced glutathione (GSH) was measured as the final product of the reaction was achieved.That was theformation 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 theinteraction ofGSH andDTNB.

2.3.3. Determination of glutathione peroxidase (GPx) activity

Beutler's determination method was used to determine glutathione peroxidase activity. The principle of this method is based on the activity of glutathione peroxidasethat catalyzeoxidation of oxidized glutathione(glutathione: H_2O_2 oxidoreductase, EC 1.11.1.9) resulted from the reaction of reduced glutathione with the hydrogen peroxide in which oxidative glutathione (GSSG) by glutathione reductase (GSH-R) enzyme is reduced to GSHin the presence of NADPH, and the observed reduction of NADPH is monitored at 340 nm .

$$\text{Activity (U/ml)} = (\Delta OD/t) * [(Vt) /6.22*Vs)]$$

ΔOD = Absorbance change according to time.

t = time

Vt= Total volume of the reaction(ml).

Vs= Volume of the sample(ml).

6.22 is the optical density of 1 mmol NADPH in the 1 cm light path.

2.3.4. 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 (Gutteridge, 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.

2.4. Statistical 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.

3. RESULTS

When SOD (superoxide dismutase) enzyme activity was inspected (Table 3.1), the relationship between control group levels (5.028 ± 2.198U/L) and patient group levels (1.876 ±1.400 U/L) was found to have a statistically significant relationship ($p<0.05$).

When GSH (reduced glutathione) enzyme activity was inspected (Table 3.1), the relationship between control group levels (0.018 ± 0.011 U/L) and patient group levels (0.019 ± 0.009 U/L) was found to have a statistically insignificant relationship ($p<0.001$).

When GPx (glutathione peroxidase) enzyme activity was inspected (Table 3.1), the relationship between control group levels (0.038 ± 0.040 U/L) and patient group levels (0.047 ± 0.044 U/L) was found to have a statistically insignificant relationship ($p>0.001$).

When MDA (malondialdehyde) level was determined, as shown in (Table 3.1), the relationship between control group levels (1.214 ± 0.214 U/L) and patient group levels (1.521 ± 0.200 U/L) was found to have a statistically significant relationship ($p<0.05$).

Table 3.1. Comparison According to the Control group and patients with FMF.

Parameter	Control	Patient
	$\bar{x}$ **±SEM (n= 35)**	$\bar{x}$ **±SEM (n=35)**
SOD (U/L)	5.028 ± 2.198*	1.876 ± 1.400 *
GSH(U/L)	0.018 ± 0.011 ^	0.019 ± 0.009 ^
GPx (U/L)	0.038 ± 0.040^	0.047 ± 0.044 ^
MDA (µmol/L)	1.214 ± 0.214 *	1.521 ± 0.200*

*: $p<0.05$^ : $p>0.001$

The (SOD, GSH, and GPx) activity of serums and serum (MDA) level of the studypopulation.

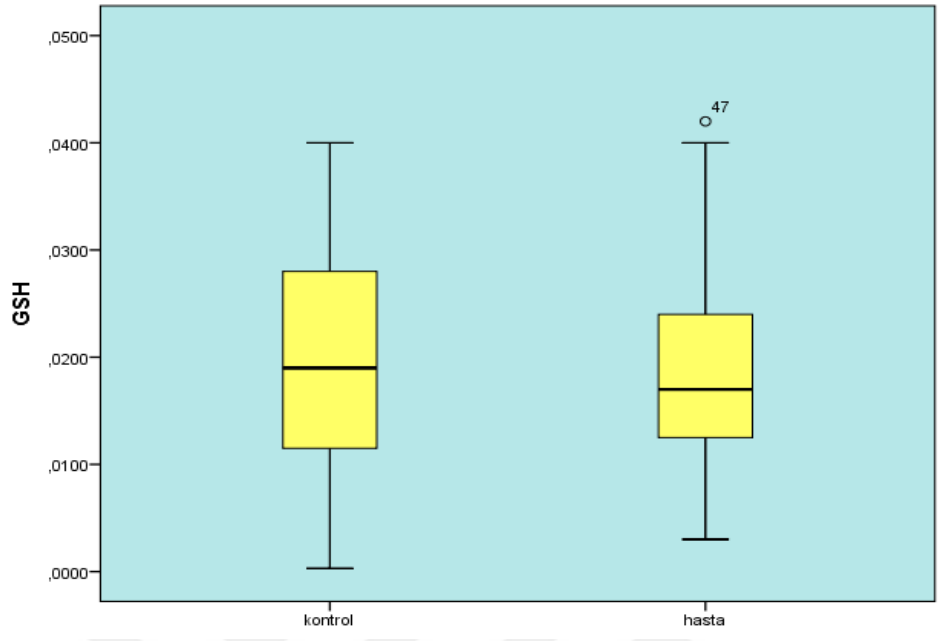


Figure 3.1. GSH enzyme activity level in both control participantsand patients with FMF.

From the obtained results while we examined GSH (reduced glutathione) enzyme activity as in (Table 3.1).As control group levels (0.018 ± 0.011U/L) and patient group levels (0.019 ± 0.009 U/L) were compared (p>0.001) statistically insignificant relationship was found.

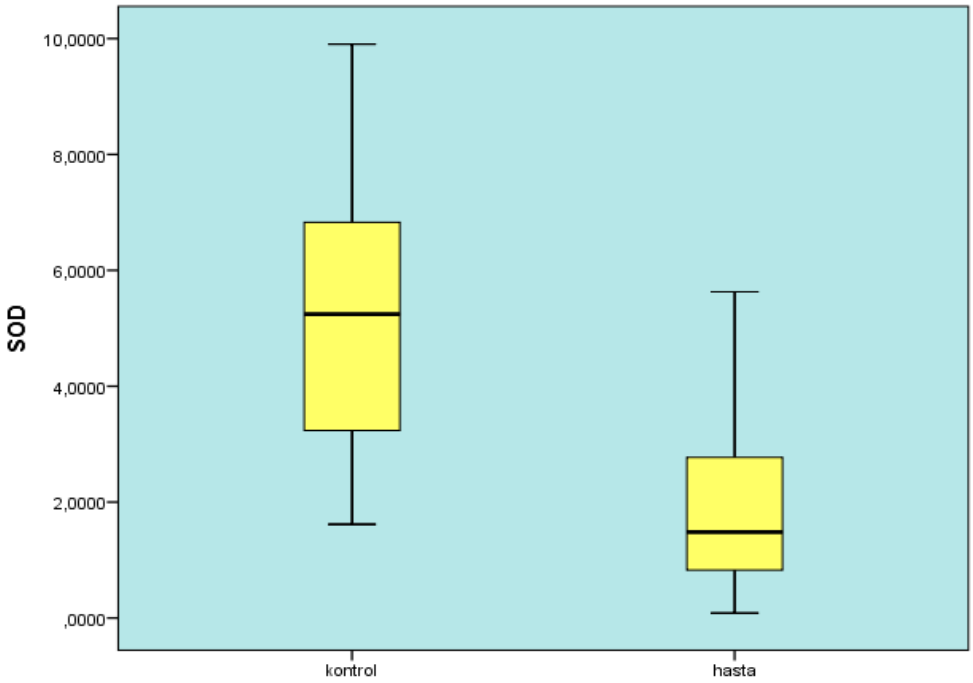


Figure 3.2. SOD enzyme activity level in both control participants and patients with FMF.

When SOD (superoxide dismutase) enzyme activity was inspected (Table 3.1), the relationship between the control group levels (5.028 ± 2.198U/L) and patient group levels (1.876 ± 1.400 U/L) was found to have a statistically significant relationship ($p<0.05$). As can be seen, patient groups' SOD enzyme activity was found to be relatively low as compared to control groups.

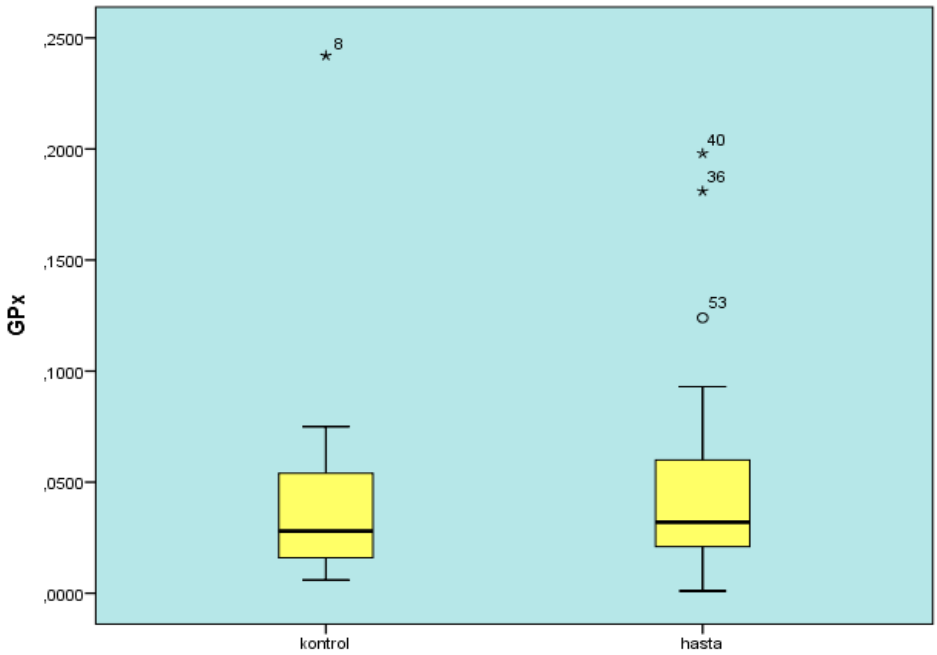


Figure 3.3. GPx enzyme activity level in both control participants and patients with FMF.

While we examined GPx (glutathione peroxidase) enzyme activity as in (Table 3.1). As control group levels (0.038 ±0.040U/L) and patient group levels (0.047 ± 0.044 U/L) were compared ($p>0.001$) statistically insignificant relationship was found.

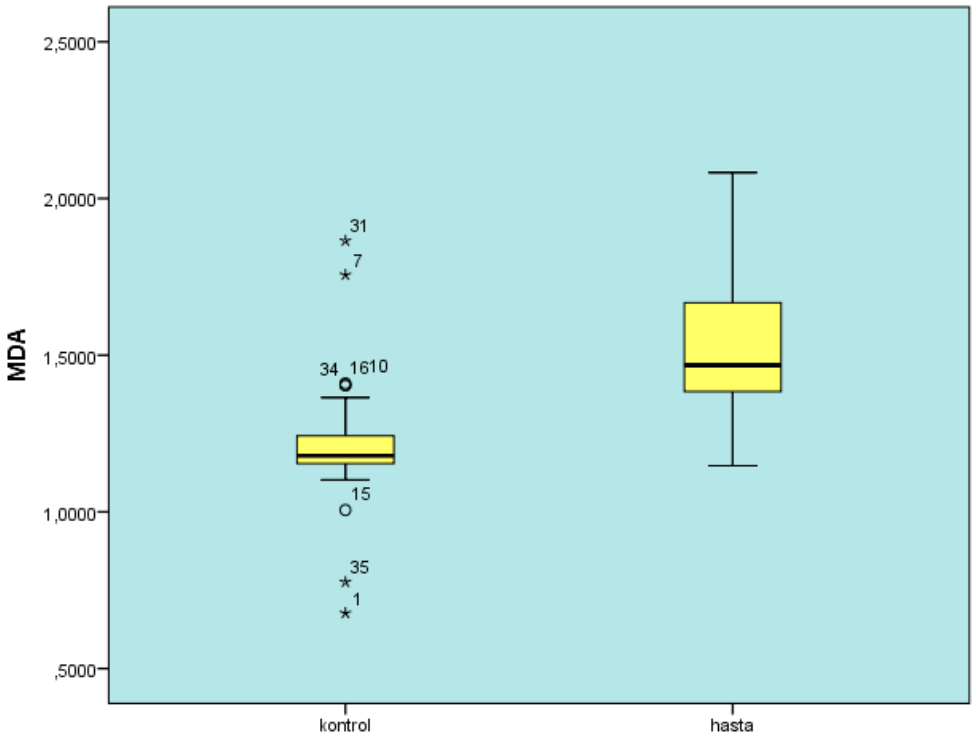


Figure 3.4.MDA level in both control participants and patients with FMF.

When MDA (malondialdehyde) level was inspected as shown in (Table 3.1), a statistically significant relationship ($p<0.05$) between control group levels (1.214 ± 0.214 U/L) and patient group levels (1.521 ± 0.200 U/L) was obtained. As we observed from the (Table 3.1), patient groups' MDA level was found to be relatively high as compared to control groups.

4. DISCUSSION AND CONCLUSION

In this investigation, the malondialdehyde level, which is revealed as the marker of the oxidative stress in children with familial Mediterranean fever and other antioxidants such as reduced glutathione, glutathione peroxidaseand superoxide dismutase have been analyzed and compared.

In the biochemistry field, advanced techniques involving enzymology have a crucial role for inhibiting and eliminating the harmful impact of oxidants, such as the utility of several enzymes including endogenous and exogenous antioxidants which possess low molecular weight.Ansari and Scheff introduced an intense correlation between variousdegenerative disorders and the oxidative damage levels such as glutathione,catalase ,total SOD, thiobarbituric acid reactive substances ,acrolein ,protein carbonyl, 3-nitrotyrosine, and 4-hydroxynonenal(Ansari and Scheff, 2010).The obtained results from the studies yet are not convincing to realize the antioxidant status of each organ whenever they are accessible for the oxidative stress attack that may cause damage and induce involvement and interaction of genetic codes and protein genes. Molecular mechanismsand genetic alterations particularly provide us to figure out and indicate the interaction of free radicals andfurthermoretheessential role of proteomics,genomics, and process of the disease development. Besides , further environmental factors such as oxygen tension, concentration of transition metals accompany with their redox status will also be a resolving factor (Rahal et al., 2014).

Eukaryotic glutathione peroxidases (GPx)s are considered to maintain the main enzymatic defense against oxidative stress caused by hydro-peroxides. By using the reducing power provided by GSH, hydrogen peroxide and other organic hydro-peroxides, such as fatty acid hydro-peroxides, are being reduced to the corresponding alcohol(Carine et al., 1994; Morano et al., 2012). In some studiesglucose intolerance and diabeteshave been related with increased GPx activity and decreased activity in others. In a study, low GPx activity was obtained in individuals that were suspected from coronary disease and cardiovascular disorders were expected from them. However, the relationship between (GPx) activity and cardiovascular disease was insignificant in their study. But logically the associationdirection was agreeable with former studies. The reason of (GPx)

activityreduction in cardiovascular disorder and elevation in diabetes, which are commonly interrelated diseases, is indefinite and unclear. Studies upon animals, demonstrate the initiation of oxidative stress throughhydrogen peroxide addition leads to up-regulation of (GPx) statement. In diabetes accompanied by chronic oxidative stress we may predictelevated GPx activity while determination due to up-regulation of the enzyme.Whilstin myocardial infarctionfeaturing acute oxidative stress, overproductionof free radicals may be observed. From the achieved results we conclude that (GPx) activity changes with age and may be changed by particulardiseases in addition to body constitution(Espinoza et al., 2008).

FMF is an auto-inflammatory disease with a progressing inflammatory activity and inflammation takes the first place in the improvement and development of atherosclerosis in some of rheumatic disease. During the investigation, early markers of atherosclerosis were intended in patients with FMF by various measurements which also include MDA level. They achieved that MDA levels were the same in FMF patients with acute& free-period attack. In patients with FMF, independent of OS status, results leaded to increase the tendency to atherosclerosis rather FMF .But during our investigation we concluded a statistically significant relationship ($p<0.05$) among FMF patients and healthy controls duringthe inspection of MDA level. Therefore, presence of further inflammatory diseases within FMF patients may alter oxidative stress status(Arıtürk et al., 2013).Our findings are supportive of the recent literature. As a result, high MDA levels in blood can be considered as reducing factor for the risk of cancer is in the studies.

Several studies have revealed that atherogenesis is highly accelerated by up-regulated autoimmune attacks and inflammationin inflammatory disorders with highlevelssuch as rheumatoidarthritis andSystemic lupus erythematosus (SLE). Relationship betweenatherogenesis and low-grade inflammatory conditions such as ankylosing spondylitis and FMF has been relatively recently suggested and studies about this relationship build up over time. However, studies investigating carotid atherosclerosis in FMF found less aggressive course of atherogenesis than atherogenesis in highgrade inflammatory disorders. Ediz et al. have measured the levels of some antioxidant markers in serum and entire blood in FMF-attack period(AP) and FMF-attack-free period(AFP)and

suggested that FMF patients during the attack period has increased level of oxidative stress.Due to the lipid lowering effect of colchicine, further investigations are needed to explain impact of LDL and HDL levels on atherogenesis in FMF patients(Ediz et al., 2011). Erden et al. showed that colchicine which is used in treatment of knee osteoarthritis increases the total antioxidant capacity and decreases malondialdehyde levels. In in vitro studies, especially those performed with the multidrug resistant cell lines, it has been demonstrated that colchicine increases the glutathione level and GST activity(Erden et al., 2012). Therefore, colchicine therapy may be responsible for less aggressive course of atherogenesis in most studies on FMF. In this study; LDL, ESR, and fibrinogen levels were statistically significantly elevated in patient groups. The difference between groups was not significant in terms of other biochemical parameters. There are different results about LDL and HDL levels of FMF patients in the literature(Gürbüz, 2016).

According to another investigation which they studied the oxidant status and antioxidants' status throughout the attack period(AP) and attack free periods(AFP) of patients suffering familial Mediterranean fever (FMF). Levels of GSH, MDA, protein carbonyl (PC) , and antioxidant vitamins (A,C and E) were measured in addition to GPx andCAT activity in both serum and total blood of FMF patients. PC and MDA levels were observed significantly ($P<0.05$) increased of both serum and entire blood of FMF during attack period as compared to attack free period. GPx and CAT activities in the attack period of FMF patients showobvious lowered result ($p<0.05$) when compared to healthy controls. Besides , no significant statistical results or differences in the antioxidant vitamin levels were observed between both healthy and patient groups.Conclusive results of this study indicated elevations in OS in patients with FMF during attack period (Ediz et al., 2011).

In a study referring toarthritis and rheumatoid arthritis, which are classified to inflammatory disorders. This investigation clarified that patients of arthritis and rheumatoid arthritis are subjected to OS since MDA levels were significantly higher ($p<0.01$) as compared to healthy controls. Besides, other studies demonstrated the low levels of ascorbate and α-tocopherol in patients with arthritis and Rheumatoid arthritis ,and this show inverse association between serum antioxidant levels and inflammation present in these patients (Sharma et al., 2013).

Deficiency of vitamin-D is common with a great extend in children with FMF than healthy ones. 25.OH-D vitamin levels change according to localization, skin type, sun exposure and season. Some researchers demonstrated that small levels of Vit-D is a consequence of inflammatory process and yet not mainly conclusive.But this study clarified this.And with increasing age, negative correlation was observed in which Vit-D levels were decreased. Significantly lower 25-hydroxy vitamin D levels between patients with FMF and healthy controls ($p<0.01$) and mostly females were affected (Onur et al., 2016).

Difference in mutations also alter the diagnoses that are made for FMF patients. In almost all studies M694V was seen as the most common mutation and serum amyloid A (SAA) on admission with homozygous M694V mutation were significantly higher according to other mutations like heterozygous mutations and compounds. In addition , (97.3 %) is indicated to be the amount that represent the most common symptom of FMF which is fever (Kilic et al., 2015).

Many studies have recognized the MDA concentration which indicate lipid peroxidation. Elevated MDA levels have been observed in patients with gastric cancer as compared to healthy groups.According to a study which analyzed IL-18 and MDA levels in patients with renal cell carcinoma before and after surgical treatment. It was determined that IL-18 (an inflammatory mediator) was significantly higher after surgery while MDA was higher. Furthermore, analysis according to gender showed that in the male group IL-18 concentration was higher and MDA levels were lower after surgery. Meanwhile in female group although IL-18 levels were also higher, MDA levels were not significantly different and CAT activity was higher. Finally SOD activity was found to be higher after surgery in both groups.Therefore it has been proven that oxidative stress is present not only in cancerous cells, but in the whole organism affected by the tumor(Didziapetriene et al., 2014).

Before the characteristic features and eliminating other reasons of periodic fever,the diagnosis of FMF has been set. Thereforediagnosis in patients becomes difficult to obtain a correct result with milder or atypical report of the disease. In the FMF clinical status of, the existenceof two mutations on dissimilar alleles being homozygous or combined heterozygous possiblymakethe diagnosis of FMFapproved.But when only one mutation is

present, the diagnosis is indefinite.According to a study thattargeted to detect the basicgenotypic distribution and variable clinical presentations for patientswith FMF and colchicine therapy impact on treatment of mentionedFMFgroups after a year subsequently. Thediseaseintense pointwas calculated and based on the Tel Hashomer Severity Score. Although, nearly1/3 of FMF patients carry only one mutation on an allele. On the other hand an additional genethat is not completely identifiedmight be responsible for the disease with single allele mutation alternatively in these cases. The reasons that cause lacking in response to colchicine could be clarified by the realization that colchicine must pass throughout various stages to regulateinflammation on its path, thus its impact may be affected at many points.Transition of colchicine metabolism at cytochrome 3A4levelby severalfactors (erythromycin, grapefruit juice, clarithromycyn, lovastatin, simvastatin, cyclosporin, etc.) can also influence the efficacy of colchicine.

Recently, 70 patients with FMF mostly suffering from(fever, abdominal pain and arthritis)approved the most common presenting features for homozygous, heterozygous and uncharacterized patients.M680I, V726A and E148Q and were recommended as the most frequent mutations thatwere indicated in the heterozygous group. The average colchicine dose required to control the attacks was concluded assignificantly lower and response of patients to colchicine therapy was significantly more effective in the heterozygous group as compared to homozygous group(Talaat et al., 2012). A Turkish group (Yalçınkaya-Özen) recently suggested new criteria for FMF diagnosis in children. This study group concluded that the criteria of pediatric had accomplished better in identifying symptoms of patients with FMF in early years with respect to and Livneh and Tel Hashomer criteria, since pediatric criteria gave more precious and more sensitive results for children with FMF (Demirkaya et al., 2013).

And if we come to the metabolic effects of colchicine, inspite of its ancient origin and usage in gout. Researchers mention that it was tried in various acute inflammatory disorders like rheumatoid arthritis and during acute attacks of FMF despite of its little influence. Colchicine, which is an extract from the colchium autumnale plant established for treatment of acute gout in the sixth century, and continuouslyprescribed. However, its effect is not strong in little doses, but its efficacy depends on interaction with various steps in the inflammatory reaction. And finally the Tel Hashomer group in Israel established

and setcolchicine as the drug choice in dealing and controlling FMF after their research on 350 patients (Schwabe et al., 1977). Additionally , high dosages of colchicine usage lead to significant side effects, taking more than 1.8 mg of colchicine a day may cause mal-absorption of D-xylose, fat and Vitamin B_{12} as well as inhibition of intestinal enzymes, In even higher doses; its utility may be toxic to gastrointestinal mucosa , kidneys , bone marrow , lungs and cardiovascular system. The recommended safe daily dosage is said to be (1-1.8 mg) as underlined in almost all researches (Peters et al., 1983).

In a study that was done on 20 patients during the acute phase of FMF and compared to 15 healthy controls. Markers of acute phase response were indicated as; C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), fibrinogen and leukocyte levels. Markers of lipid peroxidation were evaluated as thiobarbituric acid reactive substances TBRS, conjugated diene, and lipid hydroperoxide levels. In order to express DNA oxidation, 8-hydroxy-2-deoxyguanosine (8-OHdG) was evaluated. Carbonyl group and thiol (T-SH) levels were inspected to indicate the proteinoxidative damage. On the other hand reduced glutathione level, glutathione peroxidase,CuZn superoxide dismutase andcatalase activities were revealed and measured as antioxidant status marker. In FMF patients,significantly higher levels ($p<0.001$) of conjugated diene and carbonyl group ($p<0.05$) were observed respectively and GPx activity was significantly lower ($p<0.01$) while compared to healthy controls. During the attack period significantly higher($p < 0.001$) CRP,ESR, leukocyte levelsand fibrinogenwere obtained in FMF patients as compared to patients during the attack-free period. The CuZn SOD activity was significantly lower ($p < 0.05$) and T-SH level was significantly higher($p < 0.05$) in FMF patients during the attack period. Thus, results displayed adjustment and up-rising the acute phase response (APR) during the attack period and raised oxidative stress levels in FMF patients when matched to healthy controls(Guzel et al., 2012). Besides, this research demonstrates that SOD and CuZn SOD may not act similarly as in many studies SOD levels will be higher than controls or the measurements done in attack period and attack-free periods reveal different results. Thus, further detailed studies will clarify the absolute results.

There is alsoobvious evidence that during the pain crises of the disorder the associated oxidative stress elevation is more.The stress is eitherdue to the symptoms related to FMF or the consequence of the condition is not identified completely, but the possibility of strengthening antioxidant defensesto reduce the symptoms is more. Besides, some evidences that are present providethe ideaof elevating the tissue antioxidant levels by dietary supplementation with α-tocopherol which prevents lipid peroxidation.Since dietary supplementation with α-tocopherol is an effective treatment of FMF thus investigations can be progressed(Karaguezyan et al., 1996).

In addition, alterations in genes may influence the inherited immunity and regulation of oxidative stress. In populations of different ethnicity, the incomplete penetration or variable expressivity of R202Q could influence the autoinflammatory disease expression of phenotypes (Milenkovic et al., 2016). When M694V homozygous patients were compared with any other homozygote or combinedheterozygote combination of the other three mutations, similar results were obtained. Patients,whom were homozygous with M694V had more attacks per a month ($P<0.001$) and significantly greaterarthritis incidence($P <0.015$)is observed (Brik et al., 1999). A study that made in Iran and reported from FMF registration center indicated that M694V-M694V mutations are the most commonly found in patients and presented in a period of 10 years life-time. Besides, in those that disease presents during the latter10 years V726A-M694V was common. Younger patients are identified with homozygous mutation M694V and the main symptom is the abdominal pain and involves joints and the genotype has similarity to those of Arabs and somehow Armenians (Salehzadeh, 2015).

FMF is indicated as the most common cause of amyloidosis in Turkish patients according to a study recently and it is reported that the highest frequency of FMF (1/123) are from Tokat region in Turkey. Abdominal pain can be very dull and yet is sometimes very serious and seems to be an acute abdomen. Besides, it is easily misdiagnosed as appendicitis which leads to an unnecessary appendectomy. Patients with typical phenotype and genetically confirmed mutations of FMF are defined as phenotype Ibut patients withphenotype II develop amyloidosis without any prior attacks of FMF distinctive(Yilmaz and Ozer, 2010). Investigations expect that causes which contribute to the recurrent disease attacks remain unreliable and may be due to physiological,emotional,

or physical stress, menstrual periods,change of diet, and other uncertain factors. In a recent study which they utilized the case-crossover design, the investigation reporters found the presence ofa positive and statistically significant association among the stressful events and FMF attacks(Ktsoyan et al., 2013).

Studies support that the risk of cardiovascular disease is increased in FMF patients as compared to the population generally. This study is targeted on the importance of inflammation after prescribing FMF patients with regular treatment and then compared them to dialysis patients, whom are considered to face more risk from development of cardiovascular disorder. Furthermore,they clarified the correlation between pathophysiological effects of acute phase proteins and inflammation. And this correlation is crucial for improving FMF disease complications (Akalin et al., 2015).

Due to the risk of amyloidosis with not severe inflammation, various studies have focused their targetto determine subclinical inflammation in FMF patients to discover new markers. In FMF patients subclinical inflammation elevates the risk of developing problems and disturbances such as anemia, heart disease, decreasedbone mineral density, splenomegaly, and especially amyloidosis that may be lethal.Usually inflammation occurs through the secretion of inflammatory cytokines by macrophages and monocytes. IL-1, sIL-2r, α-TNFand IL-6 play a major role during acute attacks in FMF patients. In contrast, IL-1b levels are increased in FMF patients during the attack-free period, and are correlated with CRP levels. IL-1b levels may be important to display subclinical inflammation during the attack-free period in FMF patients. Also, IL-6, IL-8, and α-TNF levels were observed in attack-free FMF patients. In this study, neutrophil/lymphocyte ratio(NLR) was found to be a reliable inflammatory marker in FMF. Besides, NLR was statistically significantly higher in patients with chronic renal failure than in healthy individuals and levels were increased in proportion to the degree of chronic renal failure. As in final-stage renal disease, NLR can be utilized to detect inflammation in patients who receive hemodialysis or peritoneal dialysis, have cardiac disorders (especially myocardial infarction), endometriosis, and type 2 diabetes mellitus. NLR is a simple marker to determine subclinical inflammation because it can be achieved from CBC easily and does not require additional cost(Özer et al., 2015).

During the seeking for information and literature review, I didn’t face exact research as mine. Besides, near investigation was found. Their ages were ranged between 20-40 years old, in which the aim behind that study was to indicate the oxidant &antioxidant status in FMF patients within the attack period (AP) and attack free periods (AFP).Level of MDA activitywas found significantly higher ($p < 0.05$) both in serum and total blood of attack period group of FMF as compared to other groups. The activities of CAT and GPx of attack-free period of FMF group were found obviously lower ($p<0.05$) as compared to healthy control groups. However, statistically insignificant variationswere found in the antioxidant statement vitamin levels between the groups. Therefore, we can conclude that antioxidant levels and their impact is much more in children as compared to adults(Ediz et al., 2011).

As a result, MDA levels increased in patients that suffer familial Mediterranean fever disorder. Whereas, firstly antioxidant enzymes activity of SOD and GSH have been decreased. Due to these reasons, new approaches should be exhibited clinically for FMF patients and antioxidant enzymes can affect the etiopathogenesis of the disease. In addition to this, the increase of MDA level which is the product of lipid peroxidation or oxidative stress levels should assess clinically and studies for treatment should be performed. We believe thatthis investigationwill enlighten later studies.

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EXTENDED TURKISH SUMMARY (GENİŞLETİLMİŞ TÜRKÇE ÖZET)

AileviAkdenizateşiçocuklardaoksidatifstresdüzeyi ve bazıantioksidantenzimaktivitelerinbelirlenmesi

Giriş

Bu tezçalışmasında, Yüzüncü Yıl Üniversitesi Tıp Fakültesi Çocuk sağlığı ve HastalıklarıAnabilimDalındaAileviAkdenizateşintanısıkonulan ve alınan kan serum örneklerindenantioksidanenzimaktiviteleriolan SOD, GSH ve GPx ve lipid peroksidasyonun son ürünüolanmalondialdehit (MDA) seviyeleriölçülmüştür.

Materyal ve metot

Materyal

Bu çalışmada, 35 AAA hasta kız ve erkek çocuk 35 sağlıklı çocuklardan oluşan bireylerden 4 ml kan örneklerini aldık. 2 tüp ETDA ve 2 ml ise biyokimya tüplerine alındı.

Alet ve malzemeler

Vorteks
DerinDondurucuTüpleri
Serum SaklamaTüpleri
Spektrofotometre
AyarlanabilirOtomotikPipetler
Termostatlı Su Banyosu
Cam Pipet
Soğutmalı Santrifüj
Derin Dondurucu
Etüv
Kronometre
Hassas Terazi

SpektrofotometreKüveti

Otomatik Pipet Ucu

PhMetre

Magnet

Beher

Balonjoje

Erlen

Reaktantlar ve kimyasallar

KOH

H2O2

Na-azid

GSH

GR

Ksantin

Nitro Blue Tetrazolium

Na2CO3

Bovin Serum Albumin

KsantinOksidaz

NH4(SO4)

CuCI2

Hidrojenperoksitçözeltisi

Fosfattamponu

Sodyumhidroksitçözeltisi

DTNB

Sodyumsitrat

NADPH

GSSG

Ethylenediamine tetra-asetikasitçözeltisi

Butylhydroxytoluene çözeltisi

Thiobarbituric acid çözeltisi

Trichloroacetic acid çözeltisi

Konsantrealkol

Metot

AileviAkdenizAteşitanısıkonmuş 35 hasta ve 35 sağlıklı bireyolan ve yaşaralığı 0-18 idi. Biyokimyasalparametreler serum örnekleriilebelirlendi. Bu çalışmada kan örneklerinintoplanmasındanönce, Yüzüncü Yıl Üniversitesi Tıp FakültesiEğitim ve AraştırmaHastanesideyereletikonayı alındı. Seçilen sağlıklı ve hasta bireylerden, usuluneuygunolarakolarakvenözden 4 ml kan alındı ve 5000 rpm / dkile 10 dakikasantrifüjedildi ve dahasonraserumlarplazmadanayrıldı. Ayrılan serum örneklerindensüperoksitdismutaz (SOD), indirgenmişglutatyon (GSH), glutatyonperoksidaz (GPx) ve malondialdehit (MDA) düzeylerinibelirlemekiçinkullanıldı.

AnalizMetotları

Süperoksitdismutaz (SOD) aktivitesininbelirlenmesi

SOD aktivitesiönerilenyöntem (Popov ve ark., 2004) kullanılarakbelirlendi. SOD, oksidatifenerjiüretimisırasında oluşan toksiksüperoksitradikallerinin($O_2^{\cdot-}$) hidrojenperoksit ve moleküleroksijenedismutasyonunuhızlandırır. Bu yöntem, ksantin ve ksantinoksidaz (XOD) kullanılarakoluşturulansüperoksitradikallerinin, nitro blue tetrazolium (N.B.T) ilemeydanagetirdiğimavirenkli formazan boyasının 560 nm dalgaboyundaverdiğioptikdansititenin (OD) okunmasıesasınadayanmaktadır. Örnektebulunan SOD, süperoksitradikalleriniortamdanuzaklaştırarak formazan reaksiyonunuinhibeeder. Deneyselkoşullaraltında, 1 birim SOD unitesi , N.B.T indirgemeoranının % 50 inhibisyonudur.

$$\% \text{ İnhibisyon} = [(\text{Blank OD} - \text{Sample OD}) / \text{Blank OD}] \times 100$$

İndirgenmişglutatyon (GSH) aktivitesininbelirlenmesi

Destilenmişsuilehazırlanan EDTA kanınınhemolizinde, sülfhidril (SH) grubunutaşımayantümproteinlerçökeltmeçözeltisiileçökeltilir. indirgenmişglutatyon (GSH) reaksiyonun son ürünüolarakölçülmüştür. Eldeedilenberraksülfhidrilgruplarının ve DTNB'nin (5 ', 5' - (ditiyobis 2-nitrobenzoik asit) sarı renklioluşumudur. EDTA kandakiindirgenmişglutatyonseviyesininölçümü 412 nm dalgaboyunda 24 saatiçindespektrofotometre (Beutler ve diğ., 1963) ölçüldü.

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

OD1 = DTNB 412 nm de İlk absorbanseklenmeden once

OD2 = DTNB 412 nm de İlk absorbanseklenmedensonra

E1 = 1 hesaplanan.

13600, GSH ve DTNB'ninetkileşimisırasında oluşan sarı rengin molar eksiksiyonkatsayısıdır.

Glutatyonperoksidaz (GPx) aktivitesininbelirlenmesi

Beutler'inbelirlemeyöntemiglutatyonperoksidazaktivitesinibelirlemekiçinkullanılmıştır. Bu metodunprensibiredükteglutatyonunhidrojenperoksitlereaksiyonusonucuoksideglutatyonay ükseltgenmesinikatalizleyenglutatyonperoksidazın (glutatyon: H2O2oksidoredüktaz, EC 1.11.1.9) aktivitesininoksideglutatyonun (GSSG), NADPH varlığındaglutatyonredüktaz (GSH-R) enzimitarafındanGSH‘aindirgenmesi, NADPH ‘dakiazalmasının 340 nm‘ de takipedilmesiesasınadayanır.

Malondialdehit (MDA) seviyesinintayini

Yağasitlerininserbestradikallerlereaksiyonu, lipid peroksidasyonununsonürünüolanmalondialdehidilesonuçlanır ve renkformunuverentiyobarbitürikasitileölçülür (Gutteridge, 1995). Birtüpeserumdan 200 ml alındı. Üzerine 800 ml fosfattamponu ve 25 µl BHT çözeltisi ve 500 ml % 30’ luk TCA eklendi. Tüplervortekstekarıştırıldı, kapaklarıkapatıldıktansonra 2

saatbuzbanyosundatutuldu. Tüplerodasıcaklığınagetirildi. Dahasonra, tüplerinkapaklarıçıkartıldıktansonra, 15 dk 2000 rpm'desantrifüjedildi. Santrifüjdeneldeedilensüpernatantın (süzüntünün) 1 ml'sialınarakbaşkatüplereaktarıldı. 1 ml'sialınansüzüntülerinüzerine 75 ml EDTA, 250 ml TBA eklendi. Tüplervortekstekarıştırıldı ve 15 dk (70˚C) sıcaksubanyosundatutuldu. Sonraodaısısınagetirilerek 532 nm'de UV/Vis spektrofotometredeabsorbanslarıokundu.

Malondialdehitdüzeyihesaplaması:

C= konsantrasyon

F=Seyreltmefaktörü

A=Absorbans

C= F x 6.41 x A

Düzeyhesabı; µmol/L olarakhesaplandı.

İstatistikselanaliz

Çalışılanparametreleriçintanımlananistatistiklerstandartsapmadaifadeedildi.
Eşleştirilmişgrupkarşılaştırmalarında, normal sapmanıneldeedildiğiyerde T-testikullanıldı ve normal sapmanınolmadığıyerlerde ise Mann-Whitney U testikullanıldı. Anlamlılıkdüzeyi % 5 olarakkabuledildi ve tümhesaplamalar SPSS istatistikpaketyazılımıileyapıldı.

Bulgular

Çizelge3.1. AAAilekontrolgrubukarşılaştırılması.

ParametrelerKontrol Hasta		
	$\bar{x}$ **±SEM (n= 35)**	$\bar{x}$ **±SEM (n=35)**
SOD (U/L)	5.028 ± 2.198 *	1.876 ± 1.400 *
GSH (U/L)	0.018 ± 0.011 ^	0.019 ± 0.009 ^
GPx (U/L)	0.038 ± 0.040 ^	0.047 ± 0.044 ^
MDA (µmol/L)	1.214 ± 0.214 *	1.521 ± 0.200 *

*: $p<0.05$ ^ : $p>0.001$

Sonuç ve Tartışma

Bu tezçalışmasında, Yüzüncü Yıl Üniversitesi Tıp Fakültesi Çocuk sağlığı ve HastalıklarıAnabilimDalındaAileviAkdenizateşintanısıkonulan ve alınan kan serum örneklerindenantioksidanenzimaktiviteleriolan SOD, GSH ve GPx ve lipid peroksidasyonun son ürünüolanmalondialdehit (MDA) seviyeleriölçülmüştür. AileviAkdenizateşiolanhastalarda, hasta gruplarının SOD aktivitesi sağlıklı kontrolgrubunagöreanlamlıolarakdüşükbulundu ($p<0.001$) (Çizelge 3.1.). Hasta gruplarınserumlarında GSH aktivitesinindeğerlendirdiğinde, sağlıklı kontrolgruplaragöreanlamlıbirfarkgözlenmedi. Ayrıca, serum GPx aktivitesiiçin hasta grubukontrolgrubuilekıyaslandırıldığında ise anlamlıbirfarkbulunamadı. Serum MDA düzeyi hasta grubu sağlıklı kontrolgrubunakarşılaştırıldığında ise anlamlıderecedeyüksekbulunmuştur. GSH ve GPx ortalamalarıarasındakifarkönemsizbulunurken, hasta ve kontrolgruplarında ise SOD ve MDA ortalamalarıarasındakifarkınanlamlıolduğubulunmuştur ($p<0.005$). Sonuçolarak, AileviAkdenizateşiolanhastalarda MDA düzeyleriartmıştır. Bununlabirlikte, öncelikle SOD ve GSH antioksidanenzimlerinaktivitesidüşmüştür. Bu nedenlerdendolayı,

AAA'iolanhastalardaklinikolarakyeniyaklaşımlarsergilenmeli ve antioksidanenzimlerhastalığınetyopatogenezinietkileyebilir. Böylece, AileviAkdenizateşihastalığıgelişiminnedeniantioksidanlar ve oksidatifstresarasındakidengesizliğinsonucuolabileceğisonucunavarılabilir.

CURRICULUM VITAE

Amani Tahsin YASIN was born in 1991- Erbil / Iraq,finished her secondary and high school education from Private Nilufer Girls' College in 2009. The same year got accepted in Chemistry Department of Science College at Salahaddin University – Erbil. In 2014, got graduated from Chemistry Department. In February2015, started her postgraduate study in the department of Chemistry (Biochemistry), Institute of Natural and Applied Sciences at Yüzüncü Yıl University – VAN. Finished her studying at Biochemistry Department inFebruary, 2017.

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THE ISTITUTE OF NATURAL AND APPLIED SCIENCES
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Program: Master

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