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

DETERMINATION OF SOME ANTIOXIDANT ACTIVITY AND OXIDATIVE STRESS LEVELS IN PATIENTS WITH OSTEOPOROSIS

M.Sc THESIS

PREPARED BY: Abdulrahman Abdulrazzaq ISMAEL
SUPERVISOR: Prof. Dr. Halit DEMİR

VAN-2020

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ACCEPTANCE AND APPROVAL PAGE

This thesis entitled **"DETERMINATION OF SOME ANTIOXIDANT ACTIVITY AND OXIDATIVE STRESS LEVELS IN PATIENTS WITH OSTEOPOROSIS"** and prepared by Abdulrahman Abdulrazzaq ISMAEL under consultation of Prof. Dr. Halit DEMIR in Department of Chemistry, on date of 17/01/2020 it has been successful with a unanimous vote by the following jury and it has been recognized as a Master's Thesis, in accordance with Postgraduate Education and training regulation with the relevant provisions

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The Board of Directors of the Institute of Science: 24../.01../...2020.. date and is approved by decision No..2020./ 6-7

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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.

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Abdulrahman Abdulrazzaq ISMAEL

ABSTRACT

DETERMINATION OF SOME ANTIOXIDANT ACTIVITY AND OXIDATIVE STRESS LEVELS IN PATIENTS WITH OSTEOPOROSIS

Abdulrahman Abdulrazzaq ISMAEL
M. Sc. Thesis, Department of Chemistry
Thesis Advisor: Prof. Dr. Halit DEMİR
January 2020, 82 Pages

Osteoporosis is a situation that bones lose their power, density and quality also bone become more porous and fragile. The most effectible risk factors for osteoporosis are calcium and vitamin D deficiency, smoking, high amount alcohol intake, nutrients deficiency and insufficient exercise. In this study, blood serum samples were collected from patients with osteoporosis in the Department of Orthopedics and Traumatology, Faculty of Medicine, Van Yüzüncü Yıl University. The aim of this study was to determine the level of malondialdehyde (MDA), which is the end product of Lipid peroxidation by oxidative stress and some antioxidant activities such as reduced glutathione (GSH), catalase (CAT) and glutathione S-transferase (GST) were measured in the blood serum of osteoporosis patients. CAT, GSH and GST activities decreased significantly in patient groups compared to healthy control group ($p < 0.05$), but MDA levels were significantly higher than healthy control group ($p < 0.05$). In conclusion, any imbalance between antioxidants and oxidative stress may be the cause of the development of osteoporosis.

Keywords: CAT, GSH, GST, MDA, Osteoporosis.

ÖZET

OSTEOPOROZLU HASTALARDA BAZI ANTİOKSİDANT AKTİVİTESİNİN VE OKSİDATİF STRES DÜZEYLERİNİN BELİRLENMESİ

Abdulrahman Abdulrazzaq ISMAEL
Yüksek Lisans Tezi, Kimya Bölümü
Tez Danışmanı: Prof Dr Halit DEMİR
Ocak 2020, 82 Sayfa

Osteoporoz, kemiklerin güç, yoğunluk ve kalitelerini kaybettiği, ayrıca kemiğin daha gözenekli ve kırılgan hale geldiği bir durumdur. Osteoporoz için en etkili risk faktörleri kalsiyum ve D vitamini eksikliği, sigara, yüksek miktarda alkol alımı, besin eksikliği ve yetersiz egzersizdir. Bu çalışmada Van Yüzüncü Yıl Üniversitesi Tıp Fakültesi Ortopedi ve Travmatoloji anabilim dalı osteoporozlu hastalarından kan serumu örnekleri toplandı. Bu çalışmanın amacı oksidatif stress göstergesi olan malondialdehit (MDA) düzeyi ve bazı antioksidan aktiviteleri belirlemektir. Osteoporoz hastalarının kan serumunda lipid peroksidasyonu düzeyi olan malondialdehit asit (MDA) ve redükte glutatyon (GSH), katalaz (CAT) ve Glutatyon S-transferaz (GST) gibi antioksidanlar ölçüldü. CAT, GSH ve GST aktiviteleri hasta gruplarında sağlıklı kontrol grubuna göre anlamlı olarak azaldı ($p < 0.05$) ancak MDA düzeyleri sağlıklı kontrol grubuna göre anlamlı olarak daha yüksek bulundu ($p < 0.05$). Sonuç olarak, antioksidanlar ve oksidatif stres arasındaki herhangi bir dengesizliğin osteoporoz hastalığı gelişmesinin nedeni olabilir.

Anahtar kelimeler: CAT, GSH, GST, MDA, Osteoporoz.

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2020

Abdulrahman ESMAEL

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

Symbols	Description
Cu	Cupper
Fe	Iron
Zn	Zinc
O_2	Oxygen
O_3	Ozone
O_2^-	Superoxide
OH^-	Hydroxyl
^oC	Centigrade temperature
Min	Minute
G	Gram
H	Hour
L	Liter
Ml	Milliliter
µl	Microliter
Mg	Milligram
Mm	Mill molar
M	Molar
N	Normal
rpm/min	Round/Minute
µg	Microgram
µm	Micrometer
B	Beta
U/L	Unit/liter

Abbreviations	Explanation
CAT	Catalase
GST	Glutathione-s-transferase
DNA	Deoxyribo nucleic acid
EDTA	Ethylenediamine tetra acetic acid
GPx	Glutathione peroxidase
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
ROS	Reactive oxygen species
GSSG	Oxidized glutathione
GSH	Reduced glutathione
G6PD	Glucose-6-phosphate dehydrogenase
WHO	World Health Organization
ALP	Alkaline Phosphatase
O_2^-	Superoxide radicals
SOD	Superoxide dismutase
H_2O_2	Hydrogen peroxide
Mn	Manganese
Ni	Nickel
HO•	Hydroxyl radical
OH–	Hydroxyl
RSS	Reactive sulphur species
MPO	Myeloperoxidase
COXs	Cyclooxygenases
LOXs	Lipoxygenases
HRT	Hormone Replacement Therapy
BMD	Bone mineral density
HNE	4-hydroxynonenal
LOOH	Lipid hydroperoxides

Abbreviations	Explanation
MS	Mass spectrometry
GC	Gas chromatography
LC	Liquid chromatography
BMI	Body Mass Index
OSCN	Hypothiocyanite
PTH	Parathyroid hormone
WHI	Women health Initiative study
DRI	Dietary Reference Intakes
FNB	Food and Nutrition Board
LOO•	Lipoperoxyl radical
SCN	Thiocyanate
LT	Leukotrienes
XO	Xanthine oxidase
NAS	National Academy of Sciences
IOM	Institutes of medicine
ETC	Electron-transport chain
ER	Endoplasmic reticulum

1. INTRODUCTION

Osteoporosis is a situation in which bones lose their power and are much more likely to break, generally following a minor bump or fall. Fractures that occur due to decreased bone strength are defined as 'fragility fractures' and lots of these might be because of osteoporosis. even though fragility fractures as a result of osteoporosis can occur in differing components of the body, the wrists, hips and spine are the maximum usually affected sites (James, 2016). Osteoporosis is a disorder characterized via abnormalities in the amount and structural arrangement of bone tissue which results in impaired skeletal power and an undue susceptibility to fractures. These characteristics are difficult to measure directly but are meditated in bone mineral density, which can be measured correctly and non-invasively by means of various radiological strategies. Bone mineral density (BMD) can be used as a surrogate marker of osteoporosis (Gupta, 1996).

Osteoporosis is nearly has effect on over 2 hundred million human beings worldwide and seventy five million humans in Europe, United States, and Japan. In the age older than 50 years nearly 1 in 2 women and 1 in 5 men will eventually experience osteoporotic fractures and the hip fracture increase in the worldwide by 240 % in women and 310 % in men is projected by the year 2050. Osteoporosis is projected to results to 8.1 million fractures (78 % women, 22 % men) during the period between 2010 and 2050. The situation costs our healthcare system $18 billion in step with year. Evidences show that Osteoporosis has been recognized to exist since Egyptian mummies have been located with suspected dowager's hump. More modern findings on all factors of osteoporosis have improved exponentially. The more importantly ones are the creation and improvement in more sensitive diagnostic devices, discovering an ever growing quantity of risk factors which includes oxidative stress, beginning up new information at the involvement of the bone forming cells osteoblasts and the bone resorbing cells osteoclasts inside the improvement of osteoporosis and finding new capsules and the nutritional alternatives for the prevention and treatment of osteoporosis. Treatment of osteoporosis with some drugs like bisphosphonates, although effective in stopping the reception of bone and preventing osteoporosis in

females has been discontinued because of side effects also Hormone Replacement Therapy (HRT), once a primary line of treatment for osteoporosis is also associated with a number of side effects. These side effects have been alarming a large number of females with osteoporosis in such a way that they are now resorting to different mode of treatment such as that from natural food components (Rao et al., 2013).

The etiopathogenesis of osteoporosis is related to the number of free radicals formation, free radicals are endlessly produced in the human bodies which occur as part of normal cell metabolism especially by biochemical redox reactions involving oxygen.

Free radicals are efficiently scavenged, when there is an imbalance between production and scavenging of the same free radical, oxidative stress will occurs and leads to oxidative deterioration of lipids or polyunsaturated fatty acids, thus compromising cellular function and antioxidant status of the body. When the number of free radicals increase also the osteoclastic activity and lipid peroxidation increases in which indicated by increase in the levels of Malondialdehyde (MDA) , in another way there is a decrease of the antioxidant status of the body, reflected by low levels of glutathione peroxidase (GHPx), glutathione reductase (GHR) and superoxide dismutase (SOD). And also Alkaline Phosphatase (ALP), Inorganic Phosphorus (Pi) and Free or ionic calcium (Ca^{++}) also undergo alteration in the osteoporosis scenario which are The Osteoblastic markers, Vitamins E and C are antioxidant vitamins are exert their osteoprotective effect by curbing the excessive free radicals formation and thereby controlling the MDA levels, which indicate the lipid peroxidation in the body (Chavan et al., 2007).

In addition, smoking, high amount alcohol intake, low antioxidant reputation, nutrients deficiency, excessive sports activities interest and immoderate caffeine intake are the major risk factors for osteoporosis (Rao et al., 2013). Risk factors, such as vitamin D deficiency, are the worldwide problem reported from 4 to 80% in different parts of the world. But other risk factors like thalassemia are more essential in some countries and less important in others, also other factors such as diastolic blood pressure, tea consumption, and soy intake and zinc (Zn) level with bone markers are investigated by Some studies and found some relations with osteoporosis (Abdollahi et al., 2005).

2. LITERATURE REVIEW

2.1. Osteoporosis

Osteoporosis is a disorder characterized by means of low bone mass and microarchitectural deterioration of bone tissue and its effects many millions of human beings around the world. And caused to greater bone fragility and consequent increase in fracture risk. Fragility fractures are most commonplace at the wrist, spinal vertebrae and hip, even though they can occur at some point of the skeleton. Osteoporotic fractures are a main reason of morbidity and incapacity inside the aged and, within the case of hip fractures, can cause untimely loss of life. similarly, they impose a large economic burden on health services, costing many billions of dollars every year (Prentice, 2004).

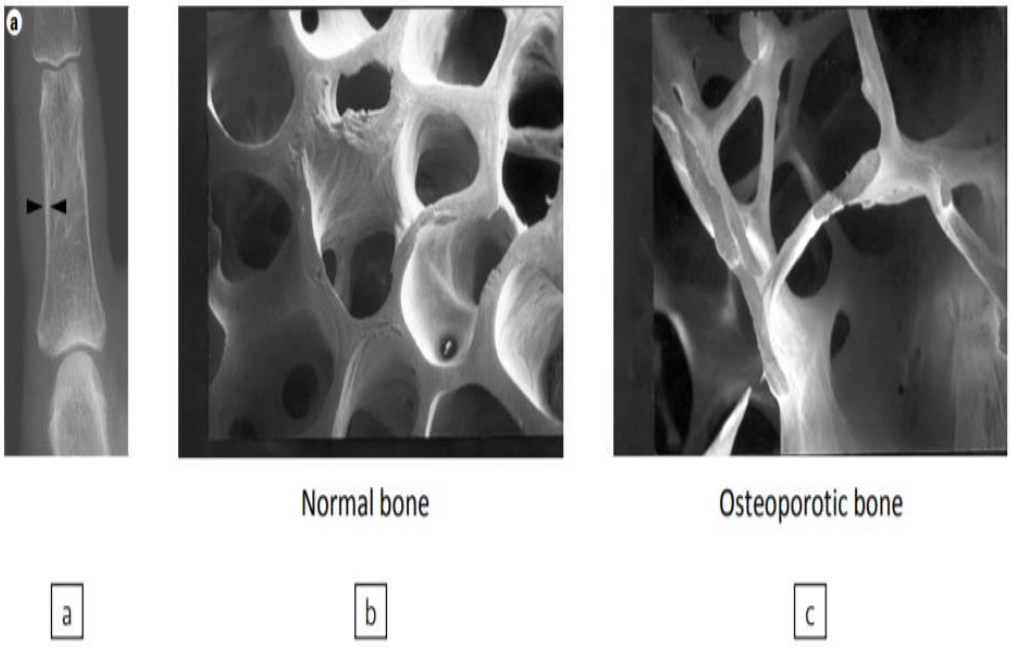


Figure 2.1. Imaging of osteoporosis (Version & Klerk, 2017).

a. Radiograph of the proximal phalanx of the second finger shows thinned bone cortex (arrowheads)

b. normal number of trabecular

c. reduction of trabecular as can be seen in osteoporotic bone

2.1.1. Pathophysiology of osteoporosis

The imbalance between bone resorption and bone formation detects the pathophysiology of osteoporosis , In osteoporosis, bone resorption occur in high extent than bone formation and this makes a negative balance with the loss of bone and also increasing risk of fractures, in which results to deformity and chronic pain when it has been present for at least 3 months , increased bone resorption in the remodeling unit or decreased bone formation within a remodeling unit (incomplete coupling) are two factors that may be combine and make The imbalance between bone formation and resorption (Bruce, 2011).

The process of bone remodeling that maintains a healthy skeleton in which considered a kipping prevention program, continually removing older bone and replacing it with new bone. And when this balance between removing older bone and replacing it with new bone changed then bone loss occur. Figure 2.2 shows the factors associated with an increased risk of osteoporosis-related fractures. Includes general factors that relate to aging and sex steroid deficiency, and also specific risk factors, such as use of glucocorticoids, which cause decreased bone formation and bone loss, reduced bone quality and disruption of microarchitectural integrity. Fractures result when weakened bone is overloaded, often by falls or certain activities of daily living.

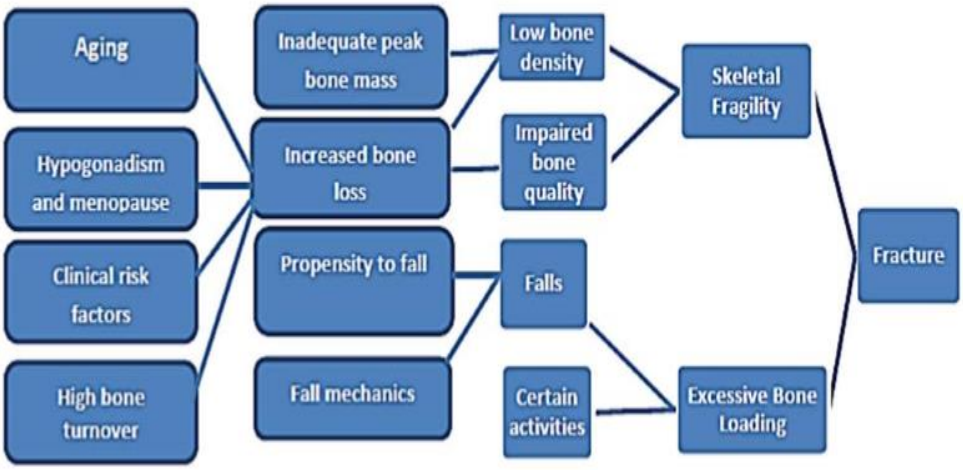


Figure 2.2. Pathogenesis of Osteoporosis-Related Fractures (Cosman el at., 2014).

2.1.2. Classification of osteoporosis

There are two categories of osteoporosis that have been identified. Primary osteoporosis and Secondary osteoporosis:

Primary osteoporosis is the most common form of osteoporosis disease and includes postmenopausal osteoporosis (type I) and senile osteoporosis (type II). Type I is related to a loss of estrogen and androgen resulting in increased bone turnover, bone resorption exceeding bone formation, and a predominant loss of trabecular bone compared with the cortical bone. Type II, that represents the gradual age-related bone loss found in each sexes caused by general senescence, is evoked by the loss of stem-cell precursors, with a predominant loss of plant tissue bone (Dobbs el at., 1999).

Secondary osteoporosis is happens because of the side-effects of different health conditions. As showen in table 2.1 , Like high production of cortisone, as in Harvey Williams Cushing syndrome will cause osteoporosis. Abnormally low production of sex hormones, as in hypogonadism, castration or "total hysterectomy" will cause bone loss

certain malignancies, significantly metastatic tumor (a bone marrow cancer), thyrotoxicosis and glandular digestive, kidney, or liver disorders could cause bone loss (Heinz, 1992).

Table 2.1. Common diseases associated with osteoporosis (Mirza, 2015)

Endocrine Disorders	Gastrointestinal, Hepatic and Nutritional Disorders
Glucocorticoid-induced osteoporosis Hyperthyroidism Hypogonadism Diabetes mellitus Growth hormone deficiency and acromegaly	Celiac disease Inflammatory bowel disease Gastric bypass surgery Anorexia nervosa Hemochromatosis and chronic liver diseases
Hematological disorders	Renal and Autoimmune Disorders
Monoclonal gammopathy of uncertain significance Multiple myeloma Systemic mastocytosis Beta thalassemia major sclerosis	Idiopathic hypercalciuria Renal tubular acidosis Chronic kidney disease Rheumatoid arthritis Systemic lupus erythematosus Multiple

2.1.3. Risk factors of osteoporosis

There are three groups of risk factors on osteoporosis, modifiable, non-modifiable risk factors and secondary causes of osteoporosis.

2.1.3.1 Modifiable Risk factors of osteoporosis

Alcohol

Individuals with excessive alcohol consumption (> 2 units each day) have an increase in the risk of developing any osteoporotic fracture by 40 % compared to people with small or no alcohol intake, High alcohol levels eventually cause secondary osteoporosis on the hormone which controls calcium metabolism and poor nutritional health (calcium, protein and vitamin D deficiency) due to specific adverse effects on bone-forming cells (Kanis et al., 2005).

Smoking

Smoking has been known to be one in all the danger factors for osteoporosis for twenty years past. Smoking is additionally a long time risk factor for osteoporotic fracture. Current and former smokers have a bigger risk of low BMD, thus, smoking has been recognized to cause poor bone health. Female smokers lost around five to ten you look after bone tissue over female non-smokers once they reach change of life (Ahmad et al., 2015). Low (BMD) has been known as one of the main causes of the raised risk of osteoporosis and hip fracture, while the BMI of people is related to the BMD. Smoker peoples often have thinner and lower BMI. One of the mechanisms in which cigarette smoking cause bone loss through its effect on changing body weight by suppressing the appetite of smokers, the article from Klesges et al study found that the difference in weight of smokers than non-smokers is approximately 7–8 pounds in middle age, and this is the evidence that cigarette smoking raises the risk of bone loss (Liu et al.,2016)

Low body mass index

The World Health Organization WHO classifies the value of BMI as (BMI) ≥ 30kg / m2 for obesity, overweight as a BMI=25 to 29.9kg / m2, and underweight as a BMI<18.5kg/m2. Osteoarthritis, diabetes, coronary heart disease, and some forms of cancer are some associated diseases that are found related to obesity (Guh et al., 2009). fracture risk will increase with low BMI probably because low BIM related to low BMD, less soft tissue, and fracture risk muscle weakness (Xiang et al., 2017). hip fracture risk in women was increased by 7.4% for each unit decrease in body mass index (BMI), many earlier studies support that (Matijevic et al., 2016).

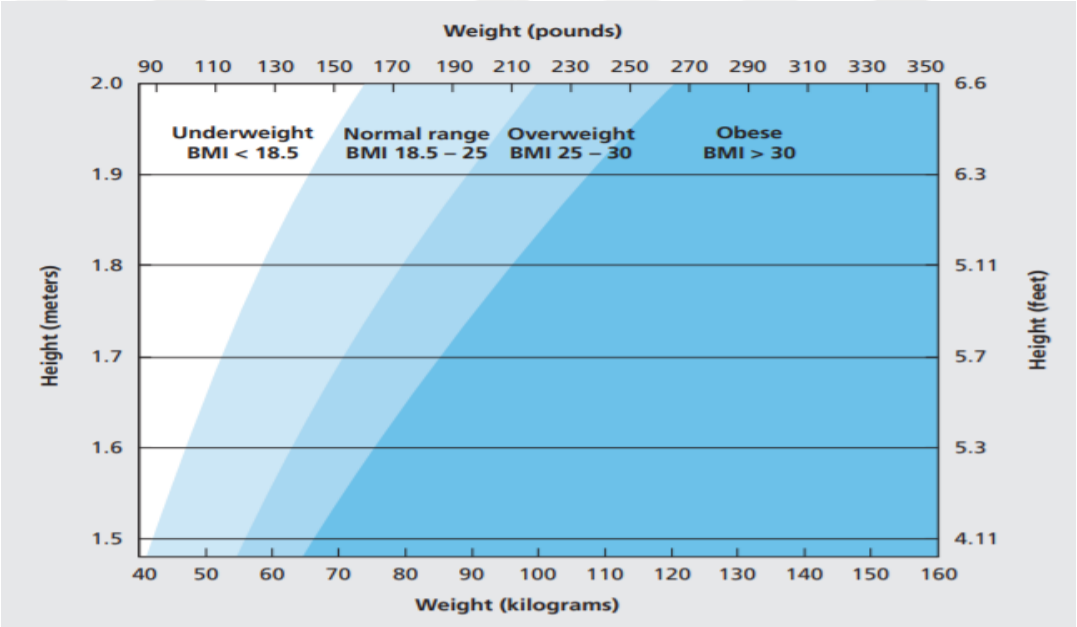


Figure 2.3. Classification or value of BMI ((IOF), 2007).

Diet

Synthesis and secretion of the Parathyroid hormone PTH might be increased with the low-calcium diet which increases osteoclast activity. This could lead to increased bone resorption to normalize serum calcium, which could otherwise be reduced due to nutritional deficiencies (Reginster, 2005).

Vitamin D is another essential dietary factor for bone health and is closely linked to calcium metabolism, In addition to sufficient calcium intake, it is necessary

to maintain an optimal level of vitamin D to avoid bone loss, Vitamin D has been identified as an important factor in the relationship between calcium and Parathyroid hormone PTH and may change the compensatory reaction of PTH to hypocalcemia (Dayem et al., 2017).

An intake diet with a high level of protein may increase urinary calcium secretion by causing urine to be acidic. Also, a diet with a high level of phosphate reduces calcium excretion. Thus diets high in protein and phosphate such as meat, fish and dairy products result in very little increase in calcium excretion. And also insufficient diet of protein is unfavorable due to the fact that some protein is required for calcium absorption. Protein is likewise essential for bone production. Reducing calcium absorption or excess excretion occurs because of excess caffeine intake, but moderate caffeine intake not considered endangering calcium balance in females who eat sufficient calcium. Additionally inside the same way excess sodium may also decrease absorption of calcium to a few degrees (Heinz, 1992).

Insufficient exercise

Osteoporosis is more common in developed countries than in developing countries. This will be because of the fact that less physical activity is necessary for survival. exercise promotes more bone density because of increasing the load on the skeleton and also exercise have an effect on hormone concentrations that prefer bone strength .in postmenopausal women's initial researches have shown that physical hobby can also reduce bone loss, evidences additionally have shown that exercise during growth and development optimizes bone density and delays bone loss later in life. The kind of exercising is important. Weight-bearing exercising which includes tennis is more effective than swimming. But in humans with advanced osteoporosis, the type of workout needs to be carefully selected due to the fact exercising that is too intense, such as tennis can overly pressure already weak bones. Taking walks is a great choice. A body with high muscle is also helps to increase bone density , exercise is most effective with calcium intake (Heinz, 1992).

In addition physical activity is important to the skeleton because the associated weight bearing and muscular activity stimulate bone formation and increase bone

mass, at the same time as immobilization results in rapid bone loss The advantageous responses of the skeleton are site specific to the loading pattern and the type of activity additionally impacts the degree of response of the bone loading. The starting age of activity is important with the gain to bone being doubled if the activity is began before or at puberty , In adulthood, exercising seems to largely maintain bone rather than add new bone and within the immediately put up-menopausal years it's far unlikely that exercising will balance the effect of estrogen deficiency.in my opinion targeted exercises focusing on muscle strength and balance can improve gait, co-ordination, proprioception and reaction time in older people, lowering the risk of falls (Kamau, 2011).

2.1.3.2 Non-modifiable Risk factors of osteoporosis

Age

Ninety percent of hip fractures occur in people aged 50 and older, and that is because of the fact that the BMD decreased as the age increased, however age also a one of the risk issue independent of BMD due to the fact even older adults with normal BMD are more likely to go through a fracture than younger people (Kanis, 2001). A bone loss result of growing age begins in forties or fifties of life (Figure 2.4).

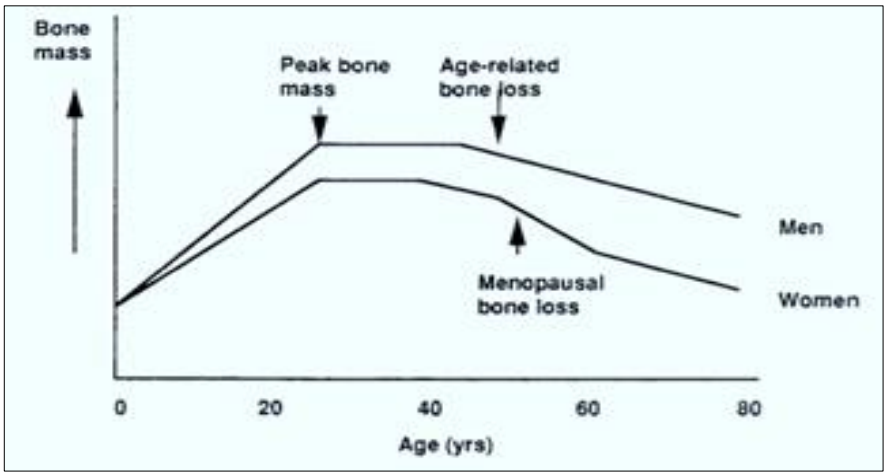


Figure 2.4. Lifetime changes in bone mass (Balasch, 2003).

And this occurs because of the increased bone breakdown through osteoclasts (Figure 2.5) and decreased bone formation via osteoblasts. There are some factors that lead to bone loss in elderly man and women one of this factors that well documented is the role of estrogen deficiency which positively related to bone mass. Also insufficient Vitamin D and secondary hyperparathyroidism are common in aged people and may contribute. Other factors are decreased physical exercises with ageing and decreased production of growth factors insulin (Kenneth & Poole, 1999).

when the age growth naturally the bone will become thinner after the age of 30 this is due to the slowly increasing the dissolving bone tissues and absorbed through the body and reducing the rate of bone-building process of, for this reason typically everybody loses a small amount of bone every year after the age of 30. In man the possess of thinning the bone begins at approximately the age of 45 – 50 when the production of the hormone testosterone decrease but in women this process thinning the bone is more rapid and usually begins between the ages of 45 - 55 after monthly menstrual periods stopped while the process of the hormone estrogen will slow down . for this reason, osteoporosis develops in women more regularly than in men and generally, osteoporosis has not a significant effect on humans till they have become 60 or older (Bowyer, 1996).

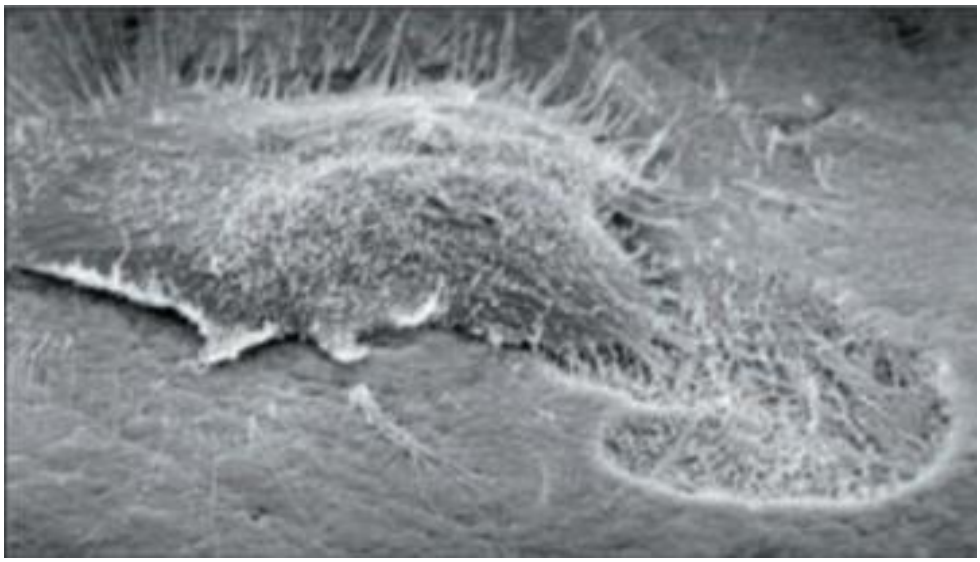


Figure 2.5. Scanning electron micrograph of an osteoclast resorbing bone (Kenneth & Poole, 1999).

Race/ethnicity (more common in Caucasians and Asians)

One of the non-modifiable risk factors of osteoporosis is the race and ethnicity and the evidences stated that Asians and Caucasians are at higher risk of getting osteoporosis in comparison to Africans and Hispanics (Ahmad et al., 2015). In different Race and ethnicity osteoporosis takes place to various degrees because data from the Baltimore men's Osteoporosis study and the study of Osteoporotic Fractures show that, whites and Asians have less BMD than blacks in both sexes, there is many factors that contribute these ethnic/racial differences in bone strength like genetic, nutritional, lifestyle and hormonal factors (Hochberg, 2007). Ethnicity and race are the most important factors that lead to osteoporosis occurrence, based on ethnicity and race there are variations in risk factors and treatment outcomes for osteoporosis. By race and ethnicity the frequency of hip fractures varies greatly inside the worldwide, (Figure 2.6).

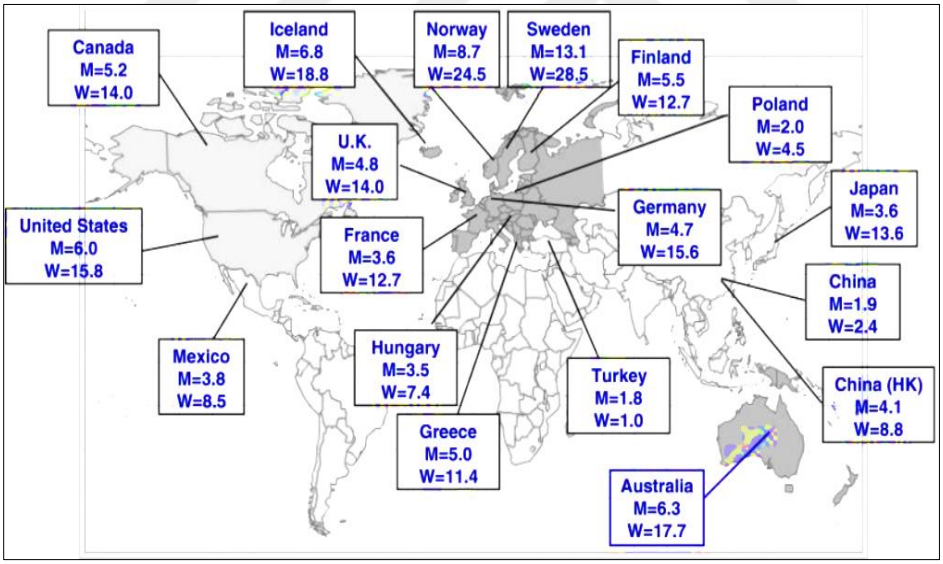


Figure 2.6. Lifetime risk (%) of hip fracture at age 50 years in men (M) and women (W) is shown according to country (Cauley, 2011).

The comparison of lifetime risk of hip fracture at age 50 years inside the united states is 15.8% in women and 6.0% in men, in Chinese is 2.4% in women and 1.9% in men, and 8.5% and 3.8% in Hispanic women and men, respectively.in which the ten-year relative possibility of hip fractures , rates of hip fracture are highest in Northern

European countries and averaged for age and sex and altered to the possibility of Sweden, is 1.24 % in Norway as compared to 0.62 % in Singapore and 0.08 % in Chile (Cauley, 2011).

Female gender

Many females than males were impacted by osteoporosis, About 8 million (or 80 present) of the approximate 10 million Americans with osteoporosis are female (Hyochol, 2017). The cause that females are more likely have smaller, thinner, less dense bone than males and females live for many years longer than males and there is a direct and natural relation between increasing age and loss of bone dense, and also females lose more bone mass after menopause with very low levels of the hormone estrogen. Higher estrogen levels before menopause help protect bone density, due to these factors females are more likely to get osteoporosis (Cawthon, 2011).

Fall facts

most homes contain many fall risks including slippery flooring, insufficient lighting fixtures, loose rugs, unstable fixtures and obstructed walkways and this risks many time cause older peoples characteristic their falls to trips or slips within the home or immediate home environment In response to those observations, home safety evaluation and household modifications have been suggested as essential components of falls-prevention applications. However, the role of environment hazards in increasing falls risk is by no means trustworthy, and neither is the amelioration of this risk by household modification (Lord, 2006). Nearly about 90-95% of hip fractures are because of falls but, not all falls are caused a hip fracture. Only about 1% of falls in aged women result in a hip fracture. the strength of the fall and the point of effect being on or near the hip apart from bone quality and quantity The fracture is affected, Falls in elderly people usually occur with small pace, landing on their hip, with increased hazard of hip fracture however middle-aged people, who move around with higher speed, frequently fall on their arms, having increased risk of fracture, nearly 75% of proximal humerus fractures and 95% of distal forearm fractures are the result of a fall (Dontas et al., 2007)

Family history

International researches classified Osteoporosis as a genetic disorder, for these reason genetic or family history can be one of the non- modifiable risk factors for osteoporosis and osteoporotic fracture. this is because BMD is highly correlated with heredity Genetic factors accounted for 50% of the variance in BMD across the populations based on the study on factors influencing BMD in postmenopausal Malaysian women, it turned into showed that women with family history of osteoporosis are at higher risk of growing osteoporosis in comparison to women without own family records of osteoporosis (Ahmad et al., 2015). researches in twins and families indicate that genetic factors play a major role within the regulation of BMD and other determinants of osteoporotic fracture risk. The heritability of BMD has been estimated to lie between 50% and 85% in twin research, with the most powerful outcomes in the axial skeleton family-based, researches are also yielded strong heritability estimates for BMD with effects which might be maximal in young adults and persist even after adjusting for lifestyle factors which can be known to adjust BMD (Vieira et al., 2014).

2.1.4. Prevention and Treatment of osteoporosis

Hormone replacement therapy HRT

After the menopause estrogen levels decrease sharply at this time Osteoporosis and fractures are more commonplace and by restoring estrogen levels, HRT helps to decrease the rate of bone loss, additionally relieving the signs of menopause based on the research HRT even at low doses, can appreciably increase bone density and reduce the rate of fracture , HRT is especially beneficial for women who have undergone early menopause (before 45 years of age) those women are at more risk of osteoporosis. (Genant et al., 1997). HRT has an extremely good role in reducing the occurrence of all kinds of osteoporosis-related fractures, like hip and vertebral fractures, even in women not at a high risk of fracture (David et al., 2001). due to the fact the essential cause for postmenopausal osteoporosis is the loss of bone as a result of estrogen

deficiency, so HRT is one of the most essential treatment, in other hand based on the safety concerns raised by the results of new researches like women health Initiative study (WHI) and Million women's study HRT is not considered as the first-line treatment for osteoporosis by using different medical Societies and institutions (Gambacciani & Levancini, 2014). HRT has an important, favorable and big effect on bone density at all sites. And is capable of maintain and even increase BMD at all skeletal sites, such as lumbar spine, femoral neck and forearm in postmenopausal women (Wells et al., 2002). HRT may only consist of estrogens alone or estrogens combined with progestin. Estrogen is a sex hormone that is essential to female bone health as it promotes the activity of osteoblasts, which are cells that produce bone. When estrogen levels drop for the duration of menopause, the osteoblasts are not capable of successfully produce bone. HRT decreases bone turnover and increases BMD at all sites of skeletal in aged and late postmenopausal women and this helps to prevent osteoporosis due to the fact that HRT decreases fragility fracture risk by 20 – 35 % (Hulley et al., 2002).

Calcitonin therapy

Calcitonin is a hormone that is naturally produced within the thyroid, this peptide acts through specific receptors to strongly inhibit osteoclast function, it has been used within the treatment of osteoporosis for many years. Several controlled trials have reported that calcitonin stabilizes and in some cases produces a short-term increase in bone density on the lumbar spine level (Muñoz-Torres et al., 2004). the result of investigations showed that after the first year of therapy Calcitonin prevents bone loss at the spine and forearm by approximately 3 % and but there is no effect on bone loss on the hip, Calcitonin was not statistically different from placebo at preventing fractures of the spine and long bones, such as hip fractures. And the beneficial effect of calcitonin on bone density can be more in patients who have been taking corticosteroids for more than three months (Wells et al., 2000).

Calcitonin also used as a therapy for hypercalcemia and Paget's disorder, although it was used for approximately 30 years, it is much less widely used than bisphosphonates and estrogen . Calcitonin increase in bone mineral density and bone

strength as a result of exert its anti-resorptive effects via directly decreasing osteoclastic resorption Moreover, calcitonin seems to specifically target the most active osteoclasts, and in evaluation to most other antiresorptive agents it does not reduce the number of osteoclasts (Morten et al., 2008).

In addition, the physiological function of calcitonin in calcium homeostasis and bone remodeling, and its effects on bone cells, stays unclear. for instance, research using calcitonin-null mice indicate that it could be concerned in protecting the skeleton during durations of "calcium stress", such as growth, being pregnant, and lactation calcitonin protects osteoclasts from the effects of a nitric oxide-releasing compound, a highly effective apoptotic stimulus but, it can also interfere with bone remodeling by way of inhibiting bone formation even though no longer markedly in humans combined use of calcitonin and anti-resorptive agents with different modes of action may additionally overcome the side-effects experienced by a few patients taking bisphosphonates (Kuo et al., 2012).

Calcium supplementation

Calcium is one of the fundamental minerals that wanted for vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion, but with all of these functions just less than 1 % of calcium of the body is needed to support these metabolic functions. The other 99 % of the body's calcium is stored within the bones and teeth in which it supports their structure and function especially for continuous f bone remodeling into new bones which occur through regular resorption and deposition of calcium (Chavan et al., 2007). In a more recent study on approximately 7000 subjects older than 50 years of age, a calcium intake under 400 mg/ day was associated with decrease BMD and femoral cortical thickness, at the same time as a calcium intake above 1200mg/day became positively correlated with BMD. BMD (Chiodini & Bolland, 2018). The table 2.2 below shows the recommended calcium Intake provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies formerly National Academy of Sciences (NAS).

Table 2.2 Calcium Dietary Reference Intakes by Life Stage (Chavan et al., 2007)

Age	Adequate Intake	Estimated Average Requirement	Recommended Dietary Allowance	Tolerable Upper Intake Level
0-6 month	200 mg	--	--	1000 mg
6-12 month	260 mg	--	--	1500 mg
1–3 year	--	500 mg	700 mg	2500 mg
4–8 year	--	800 mg	1000 mg	2500 mg
9–13 year	--	1100 mg	1300 mg	3000 mg
14–18 year	--	1100 mg	1300 mg	3000 mg
19–30 year	--	800 mg	1000 mg	2500 mg
31–50 year	--	800 mg	1000 mg	2500 mg
51–70 year	--	For male 800 mg For female 1000 mg	For male 1000 mg For female 1200 mg	2000 mg
> 70 year	--	1000 mg	1200 mg	2000 mg

There is a different ingredient Foods that are highest in calcium include milk, fortified soy beverage, yogurt and tofu made with calcium sulfate. Nuts, seeds, legumes and vegetables are also sources of calcium. Table 2.3 shows the sources of Calcium (British Columbia, 2011).

Table 2.3. Food sources of calcium

Food	Serving size	Calcium amount
Milk and its products		
Buttermilk	250 Ml	300 mg
Cheese	50 g	370 mg
Kefir	250 ml	267 mg
Milk, evaporated	125 ml	367 mg
Milk, whole, 2%, 1%, skim	250 ml	291 to 316 mg
Yogurt	175 g	272 mg
Nuts, seeds and legumes		
Almond butter	30 mL	113 mg
Black beans, cooked	175 ml	34 mg
Sesame seeds kernels, dried	60 ml	23mg
White beans, cooked	175 ml	119 mg
Soy foods and beverages		
Soy beverage, unsweetened, fortified	250 mL	318 mg
Soy beverage, unsweetened, regular	250 mL	65 mg
Soybean, curd cheese	150 g	283 mg
Tempeh, cooked	150 g	144 mg
Tofu, made with calcium sulphate	150 g	525 mg
Vegetables and fruit		
Broccoli, boiled, drained	125 mL	33 mg
Chinese broccoli/gai lan, cooked	125 mL	46 mg
Chinese cabbage/bok choy, boiled, drained	125 mL	84 mg
Kale, boiled, drained	125 mL	49 mg
Mustard greens, boiled, drained	125 mL	87 mg
Okra, boiled, drained	125 mL	65 mg
Orange	1 medium	52 mg
Turnip greens, boiled, drained	125 mL	104 mg
Other		
Blackstrap molasses	15 mL	179 mg
Dried fish, smelt	35 g	560 mg
Salmon, pink, canned with bones	75 g	212 mg
Seaweed (agar), dry	125 mL	50 mg
Seaweed, wakame, raw	125 mL	63 mg

Vitamin D supplementation

Vitamin D or sunshine vitamin is produced through the body as a reaction to sun exposure, it could additionally be consumed in food or dietary supplements. maintaining healthful bones and teeth, regulation of calcium and maintenance of phosphorus levels within the blood are the reasons that make the most important requirement to have sufficient vitamin D due to the fact these reasons are extremely essential for keeping healthy bones Vitamin D is promotes calcium absorption from the bowel and allows mineralization of newly formed osteoid tissue in bone and plays an essential function in muscle function for this reasons it becomes essential for musculoskeletal health (Francis et al., 2006). However some researchers agree with that to help treatment of osteoporosis and prevent people from breaking a bone, it can be important to take both calcium supplements and vitamin D supplements collectively. Additionally, due to the fact the more people took calcium supplements along with the vitamin D, it's far difficult to understand exactly what effect the vitamin D was having (Bischoff-Ferrari, 2012). The recommended intakes of vitamin D during life had been updated by the U.S. Institutes of medicine (IOM) in 2010 and are currently shown in Table 2.4.

Table 2.4. Vitamin D Dietary recommended Intakes by Life Stage

Stage of life	Recommended vitamin D intake
Infants 0-12 months	400 IU (10 mcg).
Children 1-18 years	600 IU (10 mcg).
Adults to age 70	600 IU (10 mcg).
Adults over 70	800 IU (10 mcg).
Pregnant or lactating women	600 IU (10 mcg).

Just a few foods include vitamin D naturally include fish, liver and egg yolk. Most of the vitamin D in our food plan comes from foods with added vitamin D other foods like yogurt and soy beverage may additionally have added vitamin D. Table 2.5 suggests the sources of vitamin D (British Columbia, 2011) .

Table 2.5. Food Sources of vitamin D

Food	Serving size	Amount of vitamin D
Egg yolk, cooked 1egg	1 egg	0.8 mcg
Herring, Atlantic, cooked	75 g	4.025 mcg
Mackerel, Atlantic, cooked	75 g	1.95 mcg
Margarine	10 ml	1.5 mcg
Milk	250 ml	2.575 mcg
Salmon, Atlantic, wild, cooked	75 g	6.125 mcg
Salmon, chum, canned	75 g	5.05 mcg
Salmon, pink, canned	75 g	10.875 mcg
Salmon, sockeye, canned	75 g	13.925 mcg
Sardines, Atlantic, canned	75 g	1.75 mcg
Soy beverage, unsweetened, fortified	250 ml	2.175 mcg
Trout, cooked	75 g	3.7 mcg
Tuna, Bluefin, cooked	75 g	5.475 mcg
Tuna, canned, light	75 g	0.9 mcg
Tuna, yellow fin (albacore), cooked	75 g	2.65 mcg

Exercise (Physical Therapy)

Physical activity is one of the most effective treatments that assist BMD to increase and maintain. The previous studies showed that there may be a strong relationship between exercise and BMD, in particular in athletic people. However, people in which select an athletic way of life are more likely they have larger muscle groups and bones, even though there is a document that among older community-dwelling women there is an association of physical activity with a reduced risk for hip fracture. The proper evaluation of exercise as a preventative therapy for osteoporosis need to focus on the prevention of falls or osteoporotic fractures (Koike, 2006). Researches proved that bone loss decreases, BMD will keep, bone density increase within the younger adulthood though the magnitude of mechanical loading of the bone by a sufficient amount of continuous exercising including weight-bearing and aerobic

training. Therefore, also to increase muscle strength within the elderly, which certainly causes the prevention of falls and reduces the incidence of new fractures weight-bearing exercise must be recommended (Nakatsuka et al., 1994). The age of the person is detects how much the physical activity effects upon bone. Weight-bearing activity in children, such as jumping, will increase BMD by 5 % to 10 %. but in premenopausal women is suggested to increase BMD by 1 % to 5 % (Jennifer et al., 2004). The latest evidence showed that 1 to 2 years of mild to intensive exercise can growth postmenopausal bone mass, even though the quantities of bone gain are highly modest and site-specific However, it stays unclear that physical activity is also useful for bone health in postmenopausal and elderly. Women with osteoporosis who exercising endurance ability are usually decreased (Nakatsuka et al., 1994).

Bisphosphonates

Bisphosphonates are stable analogs of naturally occurring inorganic pyrophosphate that most commonly used for osteoporosis treatment. Bisphosphonates are different from their pharmacological properties including the time of onset, offset of impact, an affinity for bone mineral and inhibitory impact on osteoclastic bone resorption because it has a common chemical structure with side-chain variations Bisphosphonates can also administer orally or intravenously with a wide range of doses and dosing durations. Moreover, evidence showed that bisphosphonates have an effect on postmenopausal women with osteoporosis which reduces fracture risk and commonly bisphosphonates have a high-quality safety record (Lewiecki, 2010). Bisphosphonates are retained in bone for many years due to the fact they bind tightly to hydroxyapatite crystals, additionally bisphosphonates are powerful inhibitors of bone resorption. Bisphosphonates are released regionally by acidification, are taken up by osteoclasts, and impair the osteoclasts' ability to resorb bone during the process of bone resorption. The dosage of etidronate (carbon-substituted) is a long-term continuous management due to the fact inhibits bone resorption additionally impairs the mineralization of newly synthesized bone matrix (Lawrence et al.,1992).

Healthy life-style

Cigarette is terrible because it may interfere with calcium and decreases the quantity of absorption within the diets and that is lead to increase the risk of bone loss and risk of fracture additionally decreases the bone's ability to heal after a fracture. Excessive Alcohol interferes with the stability of calcium and reason hormone deficiencies people who drink heavily are more at risk of bone loss and fracture, because of each poor nutrition and improved risk of falling. For those reasons avoiding smoking and alcohol intake is one of the self-select manners to prevent osteoporosis.

2.2. Oxidative stress

An imbalance between the production of free radicals (reactive oxygen species ROS) and antioxidants is described as the oxidative stress (Betteridge, 2000). free radicals or (ROSs) are produced through normal metabolism of cells (Nahar et al., 2017). and environmental factors, such as air pollutants , cigarette smoke (Birben et al., 2012). also early findings of the studies suggest that environmental factors, such as high psychological stress and poor nutritional profile (e.g., low antioxidant and high fat intake), increase ROS production (Duck-Hee Kang, 2002).

Evidence show that free radicals and other ROS and RNS play a double role in living systems, they may be harmful and attack biological molecules such as lipids, proteins, and DNA and can cause oxidative damage and tissue dysfunction , and they may be useful in physiologic adaptation and in the regulation of intracellular signal transduction (Toshikazu & Yuji, 2002; Meo et al., 2016).

The main by-products which formed inside the cells of aerobic organisms is the ROS in which lead to Oxidative stress and may initiate autocatalytic reactions in such a way that converts the molecules into free radicals causing a series of damage (Rahman, 2007) Researches show that one of the progressive factors of many diseases like cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases is the effect oxidative stress (Pizzino et al., 2017). additionally evidences display that ROS is one of the most essential risk factors for osteoporosis as it associated with the activity and function of each the osteoblasts and osteoclasts cells and results in increase in the rate of bone loss (Stavros & Michael, 2010).

ROS consists of one or more unpaired electrons and they looking for another electron to fill their orbital and stabilize their electron balance for that reason ROS is classified as a member of highly reactive family, such as molecules contain oxygen and free radicals which includes hydroxyl (OH^-), superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen, and lipid peroxides (Rao et al., 2013).

2.3. Reactive oxygen species (ROS)

Reactive Oxygen Species (ROS) are highly reactive molecules or radical in which there chemical and physical properties are will know both on thermodynamic and kinetic points of view (Collin, 2019). Under aerobic situations living organisms reduce more than 90% of their oxygen immediately to water through cytochrome oxidase in electron-transport chain (ETC) via four-electron mechanisms without ROS release, the location of ETC components vary for eukaryotes and prokaryotes in which the system is represented via ETC, located in internal mitochondrial membrane however in prokaryotes located in plasmatic membrane , this ETC process operated at the same time with oxidative phosphorylation to produce energy in ATP form. the other 10% of oxygen is reduced through one-electron consecutive pathways in which result in convert oxygen molecule to superoxide anion radical (O_2) then non-radical hydrogen peroxide (H_2O_2) in which chemically more active than molecular oxygen yielded by using one-electron reduction with concomitant accepting of hydrogen atoms. (Fig. 2.7). This non-radical hydrogen peroxide (H_2O_2) then accept one extra electron and split up to hydroxyl radical (HO.) and hydroxyl anion (OH), in the last step of this reaction HO react with one more electron and hydrogen to yield water molecule (Lushchak, 2016).

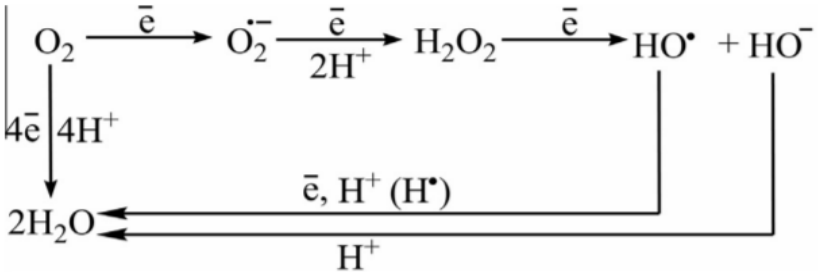


Figure 2.7. Reduction of molecular oxygen via four- and one-electron schemes (Lushchak, 2016).

The major molecules i.e., the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$), are called reactive oxygen species because they are oxygen-containing compounds with reactive properties. ROS may be radical or non-radical table 2.6 shows the two types of ROS, Unfastened radicals are molecules that contain as a minimum one unpaired valence electron at their outer shell, making them particularly reactive and brief-lived (Collin, 2019; Agarwal et al., 2017).

Table 2.6. radical and non-radical reactive oxygen species ROS (Agarwal et al., 2017)

Tow essential types of ROS	
Free radicals	Non-radicals
Hydroxyl radical (•OH) Superoxide anion ($\bullet O_2-$) Lipid peroxyl (•LOO−) Thiyl (•RS)	Hydrogen peroxide (H_2O_2) Singlet oxygen ($1O_2$) Ozone (O_3) Lipid peroxide (LOOH) Peroxynitrite (ONOO−)

There are many essential roles for ROS in which formed during normal metabolism of cells and it involves in many physiological processes such as increasing the activity of immune system, as one example pathogens kill by the (H_2O_2) in which released as a result of activation of Phagocytes as part of the immune system additionally ($^{\bullet}O_2^-$) have a function in preventing of infections. The non-phagocytic cells which contain NADPH oxidase isoform like fibroblast, endothelial cells and cardiac myocytes release ROS to regulating different intercellular signaling pathways (Agarwal et al., 2017).

ROS are a factor in the appearance of some of age-associated situations about 3-10 % of the oxygen transformed to ROS intermediates when used by tissues, in which these intermediates decreases the function of cells and tissue by oxidizing and degrading essential biological molecules, like proteins, lipids, and DNA , new evidence suggest that ROS produced by osteoclasts stimulates and facilitates resorption of bone tissue under ordinary physiological situations, and additionally when ROS have higher activity

than antioxidants lead to oxidative stress and results in bone loss and skeletal fragility, consequently a risk factor for osteoporosis (Smietana et al, 2010).

2.3.1. Formation and sources of reactive oxygen species ROS

The ROS is constituted of both endogenous and exogenous resources (Phaniendra et al., 2015) as shown in Fig 2.8

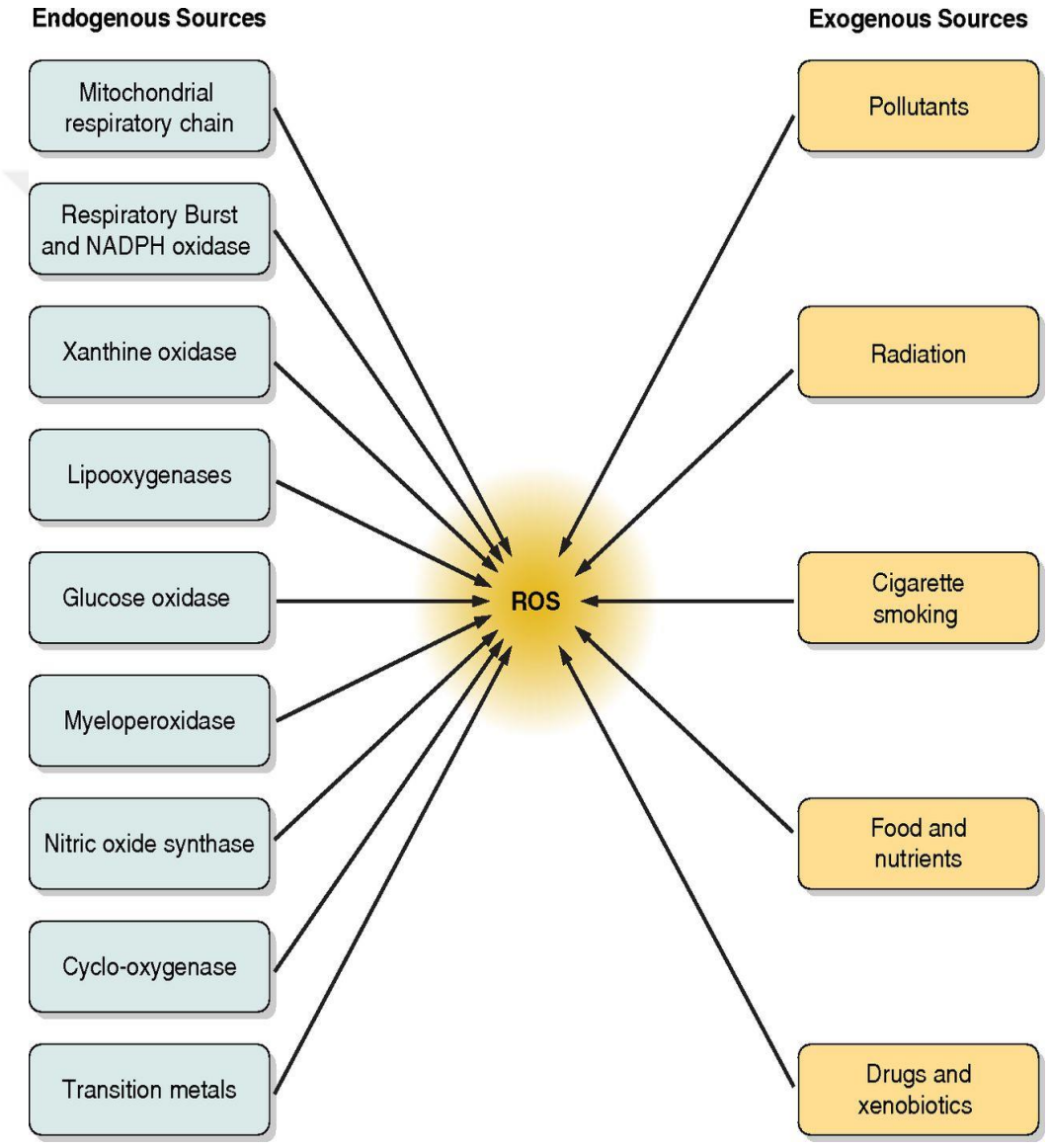


Figure 2.8. Endogenous and Exogenous factors leading to (ROS) generation (Bhattacharyya et al, 2014).

2.3.1.1. Endogenous sources of ROS

The primary resources of Endogenous ROS generation are mitochondria, the endoplasmic reticulum (ER), nuclei, cytosol, plasma membranes and also peroxisomes. However the essential site of ROS production in mammalian cells is the mitochondrial electron transport chain, and there are some vital enzymes that catalyze the chemical reactions of ROS-generation, including peroxidases, NADPH oxidase isoforms, myeloperoxidase (MPO), cyclooxygenases (COXs). lipoxygenases (LOXs), xanthine oxidase (XO), NADPH oxidase, nitric oxide synthase, and glucose oxidase (Bhattacharyya et al., 2014)

Mitochondrial Respiratory Chain

One of the most important sources of ROS is the mitochondrion in which approximately 1-3% of electrons leakage into oxygen within the process of energy transduction in the electron transport chain prematurely forming the radical superoxide, then Superoxide is released into the matrix of mitochondria.(Santo A et al., 2016) and then this superoxides are converted into hydrogen peroxide (H_2O_2) in which less reactive than superoxides through the reaction catalyzed by superoxide dismutase (SOD).but when transition metals such as iron and copper interacts with (H_2O_2) hydroxyl radicals (OH-) are formed which is the most reactive ROS, (Fenton's reaction)

$$2O_2 + 2H_2O_2 \longrightarrow O_2 + H_2O_2 + 2OH^-$$

$$2H_2O_2 \longrightarrow 2H_2O + O_2^-$$

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

Fenton's reaction (Noori, 2012).

The ROS produced via mitochondria can also cause to oxidative stress and lead damage the mitochondrial DNA, membranes, proteins and additionally reducing the ability of mitochondria to generate ATP to proceed all of their metabolic functions that are essential to the ordinary operations of most cells, such as The citric acid cycle,

amino acid metabolism, fatty acid oxidation, the ornithine cycle, synthesis of haem (Murphy, 2009).

Respiratory burst and NADPH oxidase

In the process of respiratory burst huge quantities of oxygen consumed through phagocytic cells throughout phagocytosis and release $O_2^{\bullet}$ into the extracellular space or phagosomes via the activation of NADPH oxidase. Relocation of the cytosolic additives to the cellular membrane leads to activate NADPH oxidase. NADPH oxidase including monocytes, eosinophils, macrophages and neutrophils are multicomponent enzyme present inside the phagosomes of phagocytes and plasma membrane (Babior et al.,1973).

$$NADPH + 2O_2 \xrightarrow{\textbf{NADPH oxidase}} O_2^{\bullet -} + NADP^{+} + H^{+}$$

Xanthine oxidase

Xanthine oxidase (XO) is an essential enzyme located inside the cytoplasm and additionally on the outer surface of the plasma membrane, is particularly expressed within the liver and small intestine. Superoxide radical and hydrogen peroxide are produced throughout the oxidation of hypoxanthine to xanthine and then into uric acid in which both reactions catalyzed via XO and in these reactions, oxygen acts as the electron acceptor.

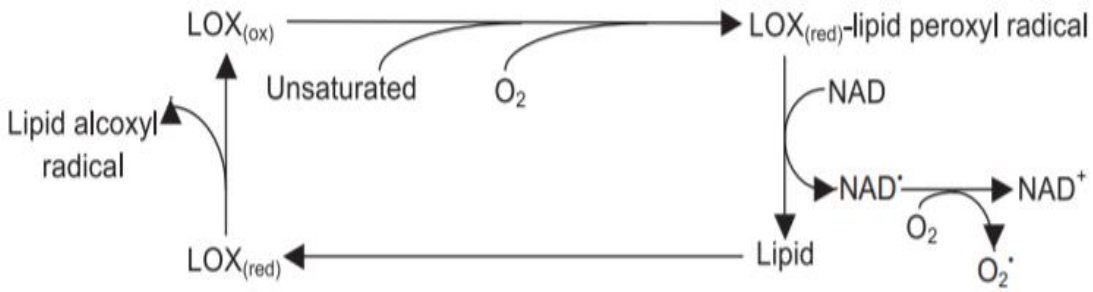
$$Hypoxanthine + 2O_2 + NADPH \xrightarrow{\textbf{XO}} xanthine + 2O_2^{\bullet -} + NADP^{+} + H^{+}$$

$$Xanthine + 2O_2 + NADPH \xrightarrow{\textbf{XO}} Uric\ Acid + 2O_2^{\bullet -} + NADP^{+} + H^{+}$$

$O_2^{\bullet}$ because of its short half-life is not a highly reactive free radical and is finally reduced to H_2O_2 (Kostić et al., 2015; Bhattacharyya et al., 2014).

Lipoxygenases

Lipoxygenases (LOX) are enzymes located in immune, tumor, and epithelial cells and it has an extraordinary role in lots of physiological functions of the cell which include skin sickness, tumorigenesis, and inflammation. And the oxygenation of most polyunsaturated fatty acids including arachidonic acid and linoleic acid are catalyzed by (LOXs) and hydroperoxyl derivatives consisting of hydroper oxyeicosatetraenoic acids (HPETEs) are yielded as a result of these oxygenation reaction in which then the other hydroxyl derivatives produced through reduction of (HPETEs) like leukotrienes (LT), lipoxins and hydroxyeicosatetraenoic acid (HETE) .ROS additionally generated from the reaction of oxidation of arachidonic acid catalyzed via LOX.



The substrates for LOX are varying in animals is arachidonic acid however in plants is linolenic or linoleic acids, The oxygenated lipids activate the mechanism of cellular signaling via particular cell surface receptors, provoke subsequent biological reactions Or are metabolized into effective lipid mediators (Mashima & Okuyama, 2015; Bhattacharyya et al., 2014).

Myeloperoxidase

A hem-enzyme Myeloperoxidase (MPO) is placed in macrophages, monocytes, and lysosomes of neutrophils. MPO catalyzes the generation of highly reactive HOCl through chlorination H_2O_2 additionally the oxidation of thiocyanate (SCN) Catalyzed via MPO for highly reactive ROS, hypothiocyanite (OSCN) generation. H_2O_2 reacts with HOCl and singlet oxygen ($^1O_2$) generates moreover with chloride ion (Cl).

$$H_2O_2 + Cl^- + H^+ \longrightarrow HOCl + H_2O$$

$$HOCl \xrightarrow{H_2O_2 \;\; H_2O} {}^1O_2 + H^+ + Cl^-$$

However ($^1O_2$) is not a free radical but because of its electronic structure has comparable properties with ROS (Bhattacharyya et al., 2014).

Cyclooxygenase

Membrane-bound, bifunctional Cyclooxygenase (COX) enzyme also referred to as PGH synthase and it has two essential roles, via its cyclooxygenase activity transform arachidonic acid to PGG2 and by its peroxidase activity transforms PGG_2 to PGH_2.

$$\text{Arachidonic acid (A.A)} + 2O_2 \xrightarrow{\textbf{COX}} PGG_2 \xrightarrow{\textbf{CO}} PGH_2$$

This PGH_2 is then transformed to stable prostanoids such as PGE_2 which biologically very active, additionally NAD and NADP radicals are generated through the peroxidase activity of COX in which those radicals can eventually generate O_2•, There are COX-1 and COX-2 isoforms in which identified COX, COX-1 is generally found in most tissues but COX-2 present with low or undetectable levels in most tissues and its expression can be increased through some physical stimuli, mitogenic factors, and inflammatory cytokines. The last researches display that COX has cytoprotective functions and when there is a high osmotic stress COX is induced in human gastric cancer cells and colon cancer cells (Bhattacharyya et al., 2014; Swindle, et al., 2007).

2.3.1.2. Exogenous or environmental sources of ROS.

There are numerous extracellular or environmental factors works as ROS-inducing agent like exposure to nanomaterials, Air pollutants (Dayem et al., 2017). both ionizing and nonionizing radiations, neutrons, x-rays, as well as rays, are examples of

ionizing radiations that all cause oxidative stress in which there particles have weak potential but they have high effect inside the body Ionizing radiation radiolysis H_2O to produce $HO\cdot$ or ROS by secondary reactions (Bhattacharyya et al., 2014) .

Chemotherapy is also the source of ROS generation via chemotherapeutic agents that caused to increasing lipid peroxidation and reduces antioxidants during chemotherapy. especially GSH levels within the tissue, chemotherapeutic agents that generate most of ROS are alkylating agents, anthracyclines such as daunorubicin and doxorubicin, epipodophyllotoxins such as teniposide and etoposide, and the camptothecins (Conklin, 2004).

Another source of ROS generation is cigarette smoke in which more than 7,000 oxidative agents and chemical compounds are present inside the cigarette, and approximately 1015 free radicals are found in one puff of tobacco smoke including chemicals that has high activity like epoxides, quinones, aldehydes and peroxides, the gas phase of cigarette smoke consists of peroxyl radicals, carbon-centered radicals, and •NO, however the tar phase carries nitrosamines, polycyclic fragrant hydrocarbons which is a stable compound additionally hydrogen peroxide (H_2O_2) and hydroxyl radicals ($HO^{\bullet}$) are produced through tar phase in the presence of iron.

Macronutrients including proteins, fats, and carbohydrates and micronutrients like vitamins and minerals are ingested via humans and they lead to producing H_2O_2 and $O_2^{\bullet}$, Fenton reaction generates ROS via dietary copper and iron and increasing the consumption of iron lead to increase the ROS generation, oxidative stress, lipid peroxidation. Inadequate amount of fatty acids present in the meals also results in generate ROS, in human's exposure to chronic acrylamide lead to an increase in the production of ROS then oxidative stress. When origin Lipids from animal and vegetables in microwave ovens heated caused to generate ROS. Additionally Chemical agents like quinones, heavy metals like chromium, arsenic, cadmium, mercury, and lead. Most drugs and Xenobiotics can all contribute to ROS generation and oxidative stress furthermore pesticides and organic solvents (Bhattacharyya et al., 2014).

2.3.2. The impact of ROS on bio molecules

DNA Damage

ROSs are produced by different sources in the cell either via exogenous agents or via cellular metabolism and these ROSs lead to damage DNA as a result of reacting with biomolecules in cells in which it also named as oxidative damage to DNA that is involved in many illnesses like carcinogenesis, aging, and mutagenesis, The major ROSs that participate in DNA damage in cells is hydrogen peroxide (H_2O_2), Superoxide anion ($O_2^{-\bullet}$), and Hydroxyl radicals ($^{\bullet}OH$), addition reactions and abstractions are the pathways for damaging DNA by free radicals in which lead to yielding radicals of carbon-centered sugar and radicals of heterocyclic bases like OH- or H-adduct and numerous products are yield by Further reactions of these radicals.

Oxidative damage to DNA can be measured by different analytical techniques such as liquid chromatography (LC), gas chromatography (GC) and mass spectrometry (MS) in which at the same time provide positive identification and measure numerous products, additionally accurate quantification. metabolically induced that oxidative stress is a major cause of the processes of aging and carcinogenesis due to the strong association between the mutation and DNA damage (Pero et al., 1990; Dizdaroglu et al., 2002).

however, damage of DNA through oxidative stress of ROS that produced via intracellular, results in DNA base modifications, single- and double-strand breaks, the formation of apurinic/apyrimidinic lesions wherein many of them are toxic and/or mutagenic , As a result with the ROS, the yielded DNA damage implicated within the etiology of disorder states and it is an immediate contributor to deleterious biological outcomes, for example, the presence of mutagenic 8-hydroxyguanine lesions in elderly and cancer cells (Salmon et al., 2004).

Protein Damage

Proteinaceous nature cells are approximately 70% of all oxidized molecules in oxidative stressed, and this indicated that the most prominent in vivo objectives of oxidants are protein. There are many posttranslational protein modifications as a result

of the impact of ROS which includes chlorination of side-chain amines, oxidation of sulfur-containing side chains, the formation of tryptophan, and additionally oxidation of histidines and as a result of irreversible modifications some intermediates are formed like sulfonic/sulfonic acids, methionine sulfone/sulfoximine , dityrosine and these modifications may also reason to fragmentation, destabilization, oligomerization, protein unfolding , aggregation and increasing degradation of proteins. However the formation of N-chlorination, methionine sulfoxide and Disulfide bond formation are reversible protein modifications in which commonly they occur in response to oxidative stress to adjust functions (Dahl et al., 2015).

The losses of protein functions and stop several biochemical approaches as a result of side-chain oxidation, backbone fragmentation or impaired protein folding indicates the consequences of ROS in proteins inside the methionine and cysteine are very effortlessly oxidizable, however via the activity of disulfite reductase, this reaction is reversed. Additionally, S-carboxymethyl cysteine and S-(2-Succinyl) cysteine are formed as a result of irreversible oxidation reaction of cysteine in which lead to the formation of fumarate and dicarbonyl groups that the marker for oxidative stress is the carbonyl by-product production due to the oxidation of the amino acid proline, arginine, threonine, and lysine through ROS. These proteins oxidized modifications are typically degraded and recognized inside the cells, but some of these modifications can result in dysfunctions of the cell. Brown-yellow pigment Lipofuscin as a physiological instance that is the product of oxidation (polymerization) of lipids and proteins catalyzed by iron it accumulates and it is used as an ageing marker due to the fact it is extraordinarily resistant to proteolysis (Rosângela et al., 2016).

Lipid Damage

Biologically there are two varieties of lipid peroxidation first one enzymatically wherein lipoxygenases and cyclooxygenase are involving within the fatty acid oxidation, and the second is lipid peroxidation in nonenzyme medium which the ROS, transition metal and nitrogen, and others are responsible for the reactions. The result of lipid peroxidation in high quantity is unfavorable to the cell and lead to inflammatory response due to the fact lipid hydroperoxides and aldehydes like Malondialdehyde, isoprostanes and 4-hydroxynonenal are formed as a result of the effect of ROS in

which leads to elevated cellular toxicity additionally caused to disrupts the structure and the function of lipid bilayers in which surrounding both the cells and inside the membranes of organelles specifically, the lipid peroxidation may regulate transportation, permeability, and fluidity of the membrane (Rosângela et al., 2016).

Evidence shows that resulting lipid peroxidation and oxidative stress are concerned in diverse and numerous pathological states which include atherosclerosis, irritation, cancer, and neurodegenerative illnesses. high reactive intermediates that formed as a result of oxidative stress result in generating lipoperoxyl radical (LOO•) through the altering membrane bilayers and peroxidation of polyunsaturated fatty acids, wherein then react with lipid to produce a lipid radical and a lipid hydroperoxides (LOOH) that is unstable compound and it generates new alkoxy and peroxyl radicals and additionally it is able to be decomposed to secondary products because of their short life, there are very nearby effects of free radicals produced as a result of lipid peroxidation, in other hand the products of lipid peroxides breakdown which includes malonaldehyde, hexanal, 4-hydroxynonenal and acrolein because of their extended half-life, high reactivity, their capacity to diffuse from their site of formation in comparison to free radicals may also function "oxidative stress second messengers," (Barrera, 2012)

Production of malonaldehyde (MDA)

One of the most dangerous effects of ROS is producing MDA as a result of peroxidation of polyunsaturated fatty acids in which used as an osteoclastic activity measurement parameter (Sheweita & Khoshhal, 2007). Within the production of aldehydes through lipid peroxidation, malonaldehyde (MDA) and 4-hydroxynonenal (HNE) are produced in high level, wherein MDA used as a degree of oxidative stress. in the process of peroxidation of polyunsaturated fatty acids oxidant, free radical or non-radical chemical species attack lipids containing carbon–carbon double bond(s) and they abstracted hydrogen from a carbon atom and oxygen insertion wherein lead to lipid hydroperoxides and peroxyl radicals production.as show in fig 2.9 (Barrera et al., 2018)

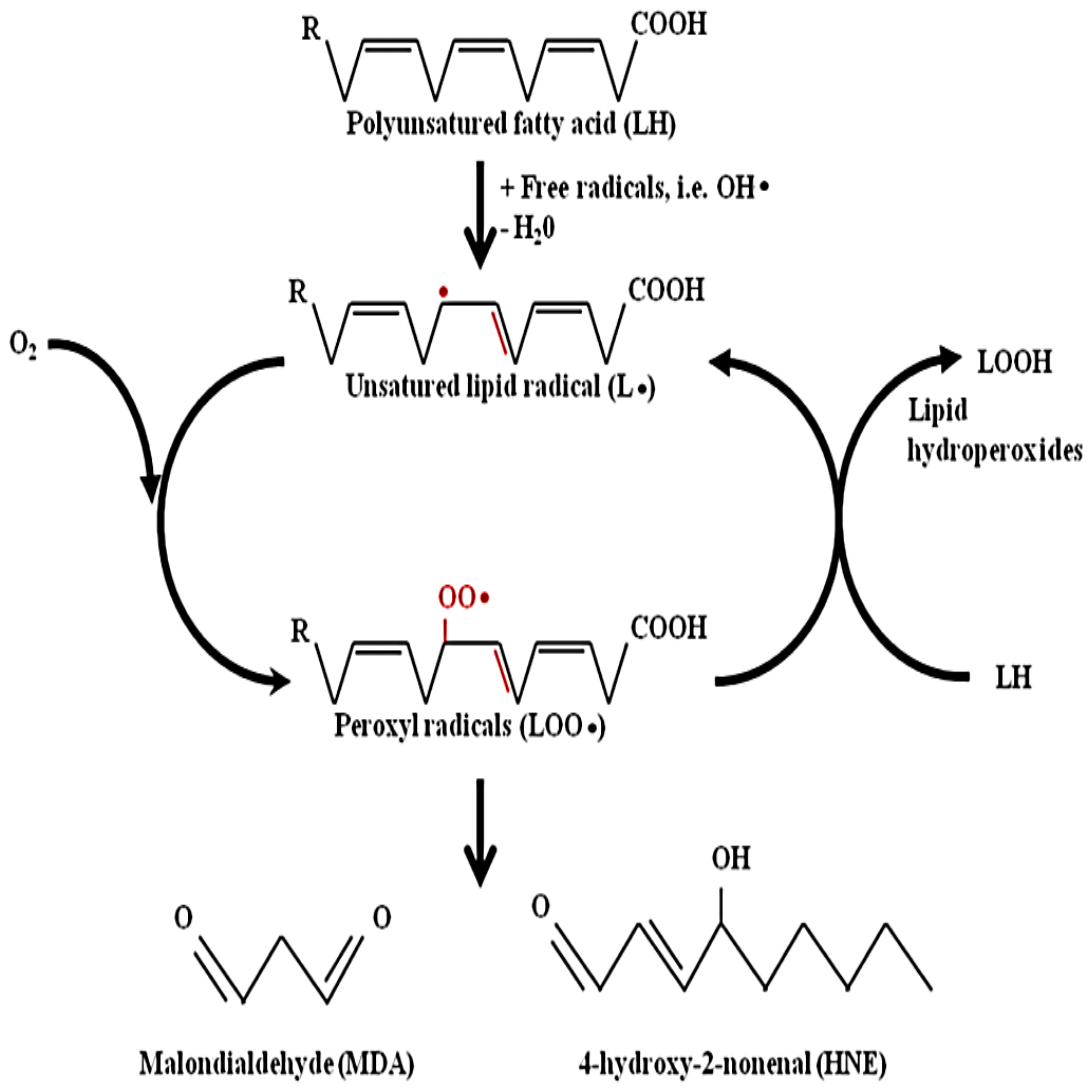


Figure 2.9: Formation Malondialdehyde (MDA) from polyunsaturated fatty acids.

The level of production of MDA increase with an increase in ROS, MDA level also used as a marker of the antioxidants and oxidative stress in cancerous patients (Gawel et al., 2004).

2.4. Antioxidants

ROS scavenger or an antioxidant, is a substance that prevent or reduce the oxidation of other molecules in the oxidation reaction electron transfers from a substance to an oxidizing agent, and some intermediates are produced which called free-radical or ROS that they destroys bio-cells by starting chain reactions, and this ROSs removed from this chain reaction by antioxidants and as a result reduce the effect of chain reaction , in many causes antioxidants play a reducing agent role like polyphenols or thiols (Arnér et al., 2001).

The antioxidant protection mechanism is one of the most important techniques besides the other techniques such as prevention of damage, physical protection mechanism towards damage, repair mechanism to relieve the oxidative damages that the human body system developed itself with it to reduce the incidence of the damaging effects in the body (Rahal et al., 2014).

Naturally antioxidants are present in most of nutritional sources like fruits, vegetables, and beverages and also antioxidants like flavonoids help bodies to decrease the hazard of mortality from coronary heart disorder and occurrence of myocardial infarction , Moreover, meta-analyses and epidemiological researches are recommended that many of illnesses like Cardiovascular illnesses, osteoporosis, diabetes, cancers, and neurodegenerative illnesses e.g., Alzheimer's disorder, may be preserving by long-term intake of plant antioxidants such as polyphenols (Sardarodiyan & Mohamadi, 2016).

The essential function of the antioxidant system is stabilizing the generation of radicals to assisting reduce oxidative damage inside the human body through the detection or prevention of the chain based on this function Gordon classified antioxidants into two vital types the first is the primary antioxidant mechanisms may consist of free radical scavengers or/and breaking the chain reaction and the secondary antioxidant mechanisms may consist of the production of undesirable volatiles to interrupting inhibition of lipid hydroperoxides, the metals deactivation, the elimination of singlet oxygen and the primary because of this, antioxidants can be described as

"*those substances that, in low quantities, act by preventing or greatly retarding the oxidation of easily oxidizable materials such as fats*" (Santos-Sánchez et al., 2019).

2.4.1. Classification of antioxidants

Halliwell and Guttering categorized antioxidants into three categories primary, secondary, and tertiary antioxidants wherein primary groups participate in prevention of oxidant formation, second groups are ROS scavengers and tertiary groups of antioxidants restore the oxidized molecules through different sources including consecutive or dietary antioxidants, and additionally enzymatic and non-enzymatic antioxidants are the general type of classification for antioxidants (Gowder & Mehta, 2016) as shown in Fig 2.10.

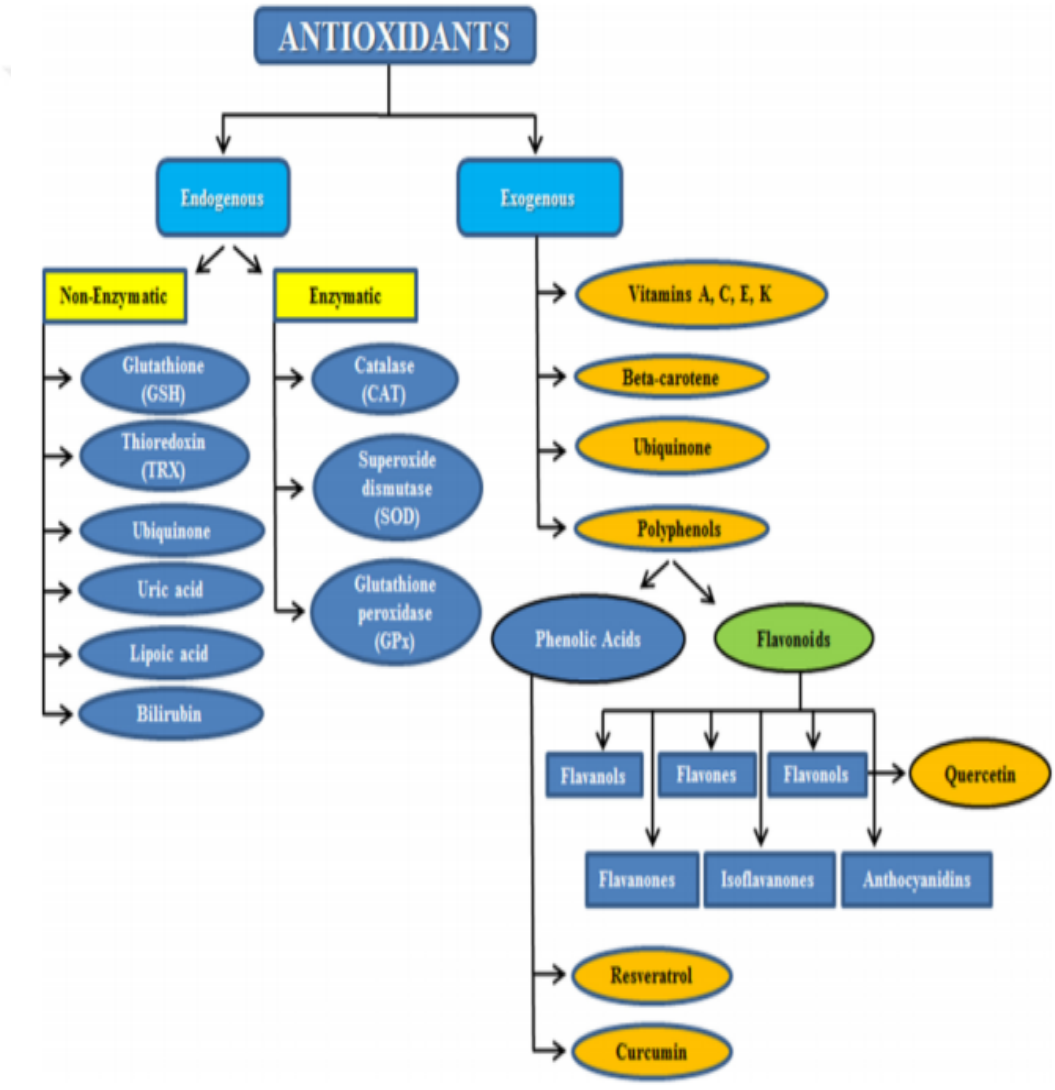


Figure 2.10. Antioxidants classification (Simioni et al., 2018).

however there are a few different classifications for antioxidants, as an example, based on the medium of work there are Hydrophilic antioxidants that consisting of, glutathione, uric acid and ascorbic acid which they react with oxidants within the blood plasma and cellular cytoplasm, and Hydrophobic antioxidants consisting of carotenes, α-tocopherol and ubiquinol which they protect cell membranes from lipid peroxidation and they synthesized inside the body or obtained from the diet , also based on their courses there are Endogenous and Exogenous antioxidants (Gowder & Mehta, 2016).

The Endogenous and exogenous antioxidants:

Endogenous antioxidants based on their actions against the ROS can be categorized into primary and secondary antioxidants. Primary antioxidants responsible for the deactivation of ROS into their intermediates like catalase (CAT), glutathione peroxidase (GPX), and Superoxide dismutase SOD, the primary antioxidant can be water-soluble such as glutathione, uric acid, ascorbate, etc. or lipid-soluble such as ubiquinols, tocopherols, and carotenoids, etc. Secondary antioxidant enzymes including glucose-6-phosphate dehydrogenase (G6PD), glutathione-s-transferase (GST), glutathione reductase (GSH), are responsible for detoxifying ROS, they constantly supplying glutathione and NADPH for primary antioxidants to keep their proper functioning via reducing the peroxides level, additionally some of metal ions increase the activity of enzymatic antioxidants like copper, selenium, zinc, manganese and iron (Gowder & Mehta, 2016).

There are exogenous antioxidants act besides the endogenous antioxidants wherein the body normally takes from the daily nutrients such as ascorbic acid (vitamin C), tocopherols (vitamin E), carotenoids (β-carotene), polyphenols and ubiquinone, this exogenous antioxidants act in different compartments and mechanisms and they neutralize free radicals , they restore oxidized membranes, they reduce ROS production, they are mainly free radical scavengers and additionally neutralize ROS via lipid metabolism, cholesteryl esters and short-chain free fatty acids (Simioni et al., 2018) .

2.4.1.1. Enzymatic antioxidants

The antioxidant enzymes are very essential in lowering lipid peroxidation and preserving the function and structure of cell membranes by decreasing the H_2O_2 and lipid hydroperoxides level. Additionally, the human body uses enzymatic antioxidant mechanisms to defend itself from ROS. The enzymatic antioxidant examples are SOD, CAT, peroxiredoxin, and GSHPx as shown in Table 2.7 (Balasaheb & Pal, 2015).

Table 2.7. Location and the reaction of enzymatic antioxidants

Enzymatic antioxidant	Location	Reaction
Mn/Cu/Zn SOD	Mitochondrial matrix (Mn SOD) cytosol (Cu/Zn SOD)	$O2^{\bullet -} \rightarrow H_2O_2$
CAT	Peroxisomes cytosol	$2\ H_2O_2 \rightarrow O_2 + H_2O$
GSHPx	Cytosol	$H_2O_2 + GSH \rightarrow GSSG + H_2O$

Superoxide dismutase (SOD)

Superoxide dismutase (SOD) is a highly reactive enzymatic antioxidant that located in the mitochondria and cytosol, in which catalyzes the dismutation of $O_2^{\bullet -}$ to O_2 and less-reactive species H_2O_2 in presence of the metal ion cofactors such as copper-zinc (Zn) (Cu), or manganese (Mn) which they undergo oxidation and reduction fig 2.11. (Balasaheb & Pal, 2015; Flora, 2009).

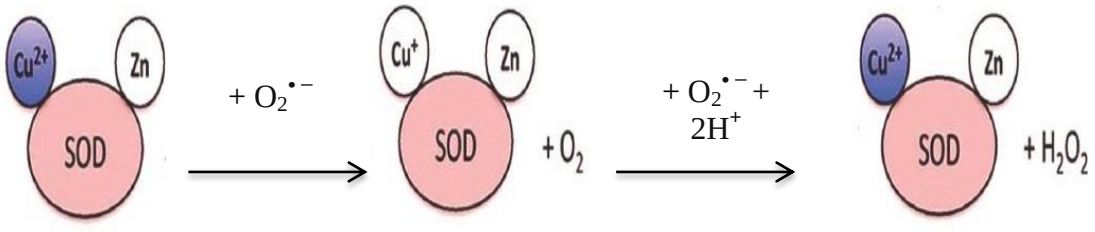


Figure 2.11. catalytic mechanism for dismutation of O_2^- by SOD (Younus, 2018).

Superoxide dismutase has 4 different isomers based on metal cofactors , a metalloprotein binding Zn and Cu ions to SOD which are present within the cytosol of the cell are known as SOD1 , whilst SOD associated with the Mg or Fe ions known as SOD2 in which they normally localized within the mitochondria, SOD3 is also associated with the Cu and Zn but located in extracellular with high affinity for heparin and heparin sulfates , the last isomer for SOD is SOD4 wherein associated with Ni and located in numerous aerobic microorganism that discovered in soil of class of Streptomyces (Aguilar et al., 2016).

SODs are consist of a completely essential antioxidant defense towards ROS inside the body in which they discovered in every kingdom of life and the physiological importance and therapeutic ability of SOD have been achieved through numerous studies in which it can prevent precancerous cellular modifications and serve as an anti-inflammatory agent.

The normal level of SOD decrease with the increasing age and hence the older individuals become greater at risk of ROS related illnesses, additionally SOD is used for personal care products and in cosmetics as an anti-aging ingredient due to its potential to reduce the impact of ROS on the skin also used for preventing fine lines, age spots and wrinkles in many causes SOD used to assist softens scar tissue, protects towards UV rays and decreases other signs and symptoms of aging. moreover, there is an essential link among SOD and numerous health problems in human which includes postcholecystectomy pain syndrome, malignant breast disorder, cystic fibrosis, RBC-related problems, steroid-sensitive nephrotic syndrome, cancer, also researchers determined the association between Alzheimer’s disease and the activity of SOD (Younus, 2018).

Catalase (CAT)

Catalase (CAT) is enzymatic antioxidants that founded within the organelle cell known as peroxisome and it is present in the cells of animals, aerobic microorganism and plants, conversion of hydrogen peroxide to an oxygen molecule and water effectively promotes via Catalase (Balasaheb & Pal, 2015; Flora et al., 2009). And the

reaction takes place in two stages with using cofactors either manganese or iron. In the first stage H_2O_2 oxidized the hem group to Fe +4 and produce water molecule:

Catalase - Fe^{+3} + H_2O_2 → (O = Fe^{+4} - Enzyme) Compound I + H_2O

Then in the second stage the highly-oxidizing Fe +4 form reacts with the second H_2O_2 releasing water and an oxygen molecule:

(O = Fe^{+4} - Enzyme) Compound I + H_2O_2 → catalase - Fe^{+3} + $2H_2O$ + O_2

As a result two molecules of highly reactive ROS which is H_2O_2 converted to H2O and oxygen molecules (Gowder & Mehta, 2016).

Catalase enzyme consists of 4 identical tetrahedrally organized protein subunits, every subunit includes a molecule of NADPH and a hem group and this NADPH molecule protects the enzyme from deactivation via H_2O_2 , CAT is one of the energetic antioxidants however its highest activity appear whilst it is inside the liver and erythrocytes .In numerous organs, CAT has been considered as biomarkers of oxidative stress in conjunction with other antioxidant enzymes. The levels of many antioxidant enzymes, including catalase, had been established that decline with age for long time (Gowder & Mehta, 2016; Aguilar et al., 2016).

Glutathione S-transferase (GST)

(GST) is a multifunctional isoenzymes antioxidants that detoxifies large number of endogenous and exogenous cellular compounds, and also the nucleophilic attack of (GSH) at the electrophilic centers of substrates is catalyzed through GSTs such as poisonous compounds, insecticides, organic hydroperoxides and metabolites , GSTs play an essential role towards therapeutic drugs cancer causing agents, , and various types of ROS (Kim et al., 2017). GSTs catalyze the general reaction of:

$$GSH + R\text{-}X \xrightarrow{GST} GSR + HX$$

GSTs have two essential roles in this enzymatic reaction. First by binding both GSH and the electrophilic substrate to the active site of the enzyme, Brings the

substrate close to the glutathione (GSH) and second role is the Activating the sulfhydryl group on GSH, thus opens the way for nucleophilic attack of GSH on the electrophilic substrate (R-X) (Hossain et al., 2006).

Additionally GSTs responsible for biosynthesis regulation and intracellular hormone transportation. GSTs are divided into numerous of subfamilies: alpha, delta, sigma, omega, epsilon, kappa, pi, mu, zeta, and theta based on their characteristics (Kim et al., 2017). Every GST subunit is a protein dimer in which contains two-component standalone catalyst site. The first component formed from the conservation of amino-acid residues in the amino terminal of the polypeptide that's a binding site (the G site) specific for GSH or a closely related homolog. And the second component in that structurally changeable and formed from residues in the carboxy-terminal of the polypeptide that is a site that binds the hydrophobic substrate (the H site) There are numerous different reactions that catalyze through GSTs such as substitution reactions aromatic nucleophilic, epoxide ring openings, isomerizations, reversible additions to α, β-unsaturated aldehydes and ketones, for some GSTs, peroxidase reactions (Hossain et al., 2006).

Glutathione peroxidases (GPX)

Is an enzymatic antioxidant that contains selenium (Se) in which its located the extracellular and cytoplasm of human tissues and its function is reducing H_2O_2 and lipid peroxides to water and lipid alcohols, respectively also oxidizes glutathione to glutathione disulfide and it has high reactivity towards oxidative stress of ROS (Touyz et al., 2007; Balasaheb & Pal, 2015).

$$2GSH + H_2O_2 \text{ (or R-OOH)} \xrightarrow{GPx} GSSG + 2H_2O \text{ (or R-OH)}$$

Cytosolic organelles uses GPx as a protective against unfavorable outcomes of the hydroperoxides formed as a result of normal aerobic metabolism, any deficiency in GPx or glutathione makes a cell at a risk to damage for this reason and to protect body cells GPx protects only towards low levels of H_2O_2 in comparison with other

antioxidants that protects towards high level of H_2O_2formed during respiration (Michiels et al., 1994).

In mammals 5 unique isoenzymes of GPx are discovered in the base of their localization and primary structure and the first one is GPX1 or cellular GPX is an enzyme that depends on selenium and placed within the cytosol and mitochondria, second one is GPx2 or gastrointestinal GPX is a cytosolic enzyme that is located in the epithelium of the gastrointestinal tract, third one is GPx3 or extracellular plasma GPx is mainly located in the kidney and is released into the blood circulation, fourth one is GPx4 or Phospholipid hydroperoxide GPx is placed within the most tissues and last one is GPx5 or epididymis-specific secretory GPx (Rahal et al., 2006).

The structure of all isomers of GPx antioxidant enzymes consists of four identical tetramer subunits and every subunit in the active site has a selenocysteine which directly participates in the 2 electron reduction of the peroxide substrate and to regenerating the reduced form of selenocysteine GSH utilized by GPx as the ultimate electron donor (Ceballos-Picot et al., 1992; Forstrom et al., 1978).

Detoxification of lipid peroxides and H_2O_2 cannot takes place in the absence of sufficient glutathione levels or GPx activity and it could be they convert to lipid peroxyl radicals and OH-radicals, respectively, through transition metals (Fe2+) (Touyz et al., 2007)

2.4.1.2. Non-enzymatic antioxidants

The natural antioxidants and the synthetic antioxidants are the two essential varieties of non-enzymatic antioxidants (Balasaheb & Pal, 2015). non-enzymatic antioxidant system consists of antioxidants that trap free radicals and avoid the reaction of free radical initiation through neutralizing or picking them up via electron-donating, as a result of this process the antioxidants itself become a free radical but with the less reactivity in comparison with the initial free radical and the other antioxidants more easily can neutralize free radicalom antioxidants , the example of nonenzymatic antioxidants or ROS scavengers are in the table with their location and function in the body (Santos-Sánchez et al., 2019).

Table 2.8. Location and function of the essential non-enzymatic antioxidants

Non-Enzymatic antioxidant	Function	Location
Vitamin C	Acts as a free radical scavenger and recycles vitamin E	Aqueous phase of cell
Vitamin E	Major chain-breaking antioxidant in cell membrane	Cell membrane
Uric acid	Scavenger of OH radicals	Product of purine metabolism
Carotenoids	Scavengers of ROS and singlet oxygen quencher	Membrane tissue
Glutathione	Serves multiple roles in the cellular antioxidant defense	Non-protein thiol in cell
Lipoic acid	Effectual in recycling vitamin C, and also a functional glutathione substitute	Endogenous thiol
Metals ions sequestration: transferrin, ferritin, lactoferrin	Scavenger of free radical and inhibitor of lipid peroxidation	Mitochondria and cytosol
Nitric oxide	Chelating of metal ions, and responsible for Fenton reactions	Mitochondria and cytosol
Ubiquinone	Reduced form serve as functional antioxidants	Mitochondria
Bilirubin	Extracellular antioxidant	Product of hem metabolism in blood

Glutathione (GSH)

GSH (L-γ-glutamyl-L-cysteinyl-glycine) is a non-protein thiol antioxidant, that defense towards the oxidative stress of ROS also it has many functions within the cells such as stopping the generation of oxidants, scavenging free radicals, and decreasing oxidant reactivity .biologically one of the most essential anti-oxidative protection mechanisms against ROS is the GSH metabolism which in the presence of tripeptide glutathione (GSH) glutathione peroxidases GPx enzyme reduces peroxides through

adding 2 electrons which lead to decomposes of peroxides to water (or alcohol) while GSH concurrently oxidized (Flora et al., 2009; Aguilar et al, 2016).

The overall reaction takes place in two stages, in the first stage lipid peroxides or H_2O_2chemically catalyzes by GPx to produce alcohols and water by glutathione (GSH) which forms glutathione disulfide (GSSG).

$$2GSH + H_2O_2 \text{ (or R-OOH)} \xrightarrow{GPx} GSSG + 2H_2O \text{ (or R-OH)}$$

Then in the second stage the reduction of GSSG back to GSH in the presence of NADPH catalyzes by Glutathione reductase.

$$GSSG + H^+ + NADPH \xrightarrow{\text{Glutathione reductase}} 2GSH + NADP^+$$ (Mattmiller & Sordillo, 2013).

For protection towards oxidative damage of radicals and ROS, GSH exerts mitochondria protection through the contribution of a collection of nutrients that may immediately or circuitously protect mitochondria and enhance mitochondrial function (Aguilar et al., 2016). GSH has a primary function within the regulation of the intracellular redox state of vascular cells through presenting decreasing equivalents for many biochemical pathways (Touyz et al., 2007).

2.5. Oxidative stress in bone remodeling

Metabolically Bone is an energetic tissue that continuously undergoes remodeling process through two counteracting processes, bone resorption concerning BMD degradation is carried out through osteoclasts and bone formation carried out via osteoblasts and osteocytes in which to preserve bone mass and integrity must be balanced among resorption and formation of bone (Cicek & Cakmak, 2018).

Remodeling process is the process of interaction between osteoclasts, osteoblasts, osteocytes which are three major bone cells and many molecular agents such as growth factors, cytokines and hormones. remodeling process which is a physiological process of bone wherein needs about six months to complete in which

osteoclasts remove old or damaged bone cells that's eventually replaced with new bone cells formed through osteoblasts even as the osteocytes maintains mechanical load via the transduction of signals also preserving ordinary ranges of mineralization and repairing microfractures and microdamage

After every remodeling cycle healthful bones prevent extensive changes in mechanical strength or bone mass by tightly maintain and regulate. The balance between osteoclast and osteoblast activity alters during the bone remodeling process due to the oxidative stress, this may cause to several metabolic bone disorders and disorders of skeletal system such as osteoporosis in which BMD and bone mass reduce and this makes more prone to fracture and weakness of bone.

Latest clinical evidences have proven that the system of ROS and/or antioxidant may involve in the pathogenesis of bone loss. The separation of pre-osteoclasts in osteoclasts and bone resorption strengthen activated by Oxidative stress, H_2O_2 increases the number of osteoclasts and there activates also increases the level of tartrate-resistant acid phosphatase when added to mononuclear cells of human. Fig.2.12

ROS in excessive levels result in increased the bone remodeling process and bone mass decrease with consequent alteration this is all because the fact that ROS block and decreases the process of differentiation , the activity of osteoblast, osteogenesis and additionally decreases the mineralization. The mentioned factors result in formulate a spectrum of responses that ranging from growth, differentiation, proliferation, arrest to cellular death through activating high variety signalling pathways Fig.2.12

ROS favors the osteoclastogenesis through inducing the apoptosis of osteoblasts and osteocytes also cells derived from mature osteoblasts and localized within the bone matrix. This is because of activation of large number of signalling pathways via ROS in particular mitogen-activated protein kinase (MAPKs) including c-Jun-N terminal kinase (JNK), extracellular signal-regulated kinases (ERK1/2) and MAPK which might be concerned in osteoblast or osteocytes apoptosis (Domazetovic et al., 2017).

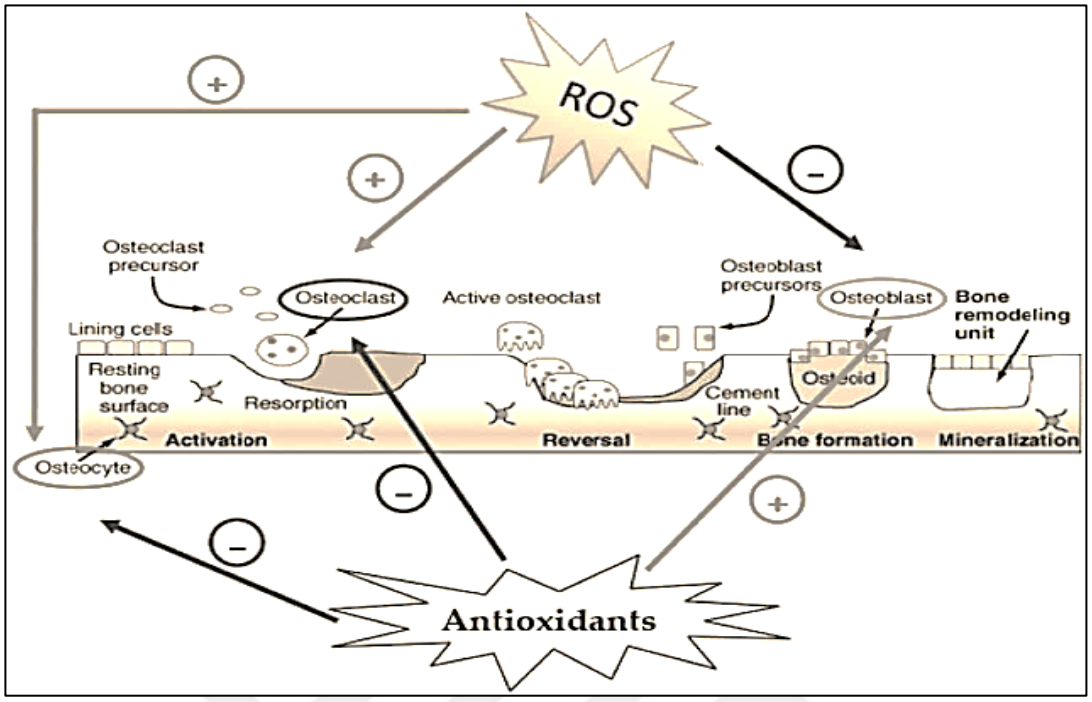


Figure 2.12. ROS and antioxidants effect on the activity of essential bone cells (osteoblasts, osteoclasts and osteocytes) in bone remodeling process.

ROS increases the activity of osteoclast differentiation and osteocyte apoptosis (+). Whilst decreases the activity of osteoblast (−) inducing bone resorption.
Antioxidants increases the activity of osteoblast differentiation (+) .Whilst decreases the activity of osteoclast and osteocyte apoptosis (−) inducing bone formation.

On the other hand, antioxidants have extraordinary effects, which participate in osteoblasts differentiation and bone formation additionally preserving essential osteocytes that participate in osteogenesis and osteoblast activity at the same time as decreases osteoclast activity and its differentiation (Domazetovic et al., 2017).

Antioxidants that reduce the manufacturing of ROS have a high medical value in the treatment of osteoporosis due to the fact ROS is the crucial cause for Osteoporosis and has a vital role in the promotion of this disease (Laher, 2014).

The principle mechanism action of the most vital endogenous and exogenous antioxidants is the preventing or inhibiting the molecules that lead to oxidative damage within the cell, primary antioxidants are able to scavenge ROS via donation of hydrogen and they are essentially chain breakers, however Secondary antioxidants are peroxide decomposers, metallic chelators, UV radiation absorbers, oxidative enzyme

inhibitors and singlet oxygen quenchers wherein by using this characteristics reduce the impact of ROS (Pisoschi et al., 2015).

Non-enzymatic vitamins C and E decreases the risk of fractures the incidence of osteoporosis because they have an essential role in the differentiation of mesenchyme cell into osteoblasts production of collagen and also bone mineralization(Zinnuroglu et al., 2012). Biological researches showing that thiol and non-thiol antioxidants have an essential role in the reducing oxidant effects in which participate in mineralization process, reduction of osteoclast activity ,activation of osteoblast differentiation and generally they work as a scavengers of ROS, additionally in combination with GSH-reductase eliminate GSSG in which produced in the reduction reactions also maintaining GSH/GSSG normal level (Romagnoli et al., 2013).

There are a number of endogenous protection mechanisms can fight free radical attack or oxidative stress through promoting antioxidant defenses such as endogenous antioxidant enzymes consisting of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) also the metal chelating proteins . Exogenous antioxidants also have a function in the scavenge of ROS wherein they come from nutritional sources such as vegetables and fruits that contain numerous phytonutrient antioxidants such as water-soluble antioxidant polyphenols, lipid-soluble lycopene, vitamins including C and E and the carotinoids effective antioxidant (Matés et al., 1999; Rao & Rao, 2007).

3. MATERIALS AND METHODS

3.1. Material

In this thesis investigation, we took blood from 33 healthy individuals, and 33 patients with osteoporosis in both male and female. From each of healthy and patient individuals, we took 4ml of blood from an antecubital venous vein and added 2 ml to the biochemistry tube.

3.1.1. Devices and materials

Spectrophotometer

pH-meter

Vortex

Thermostatic Water Bath

Refrigerated Centrifuge

Oven

Deep freeze

Sensitive balance

Stopwatch

Adjustable Automotive Pipettes

Automated Pipette Tip

Glass pipette

Serum Storage Tubes

Deep Freezing Tubes

Spectrophotometer cuvette

Magnetic stirrer

Beaker

Flask

Erlenmeyer flask

3.1.2. Reagents and chemicals

KH_2PO_4

Na_2HPO_4

NaOH

H_2O_2

Phosphate buffer

EDTA-$Na_2.2H_2O$

BHT

TBA

TCA

NaCl

NaH_2PO_4

$Na_3C_6H_5O_7$ (Sodium citrate)

DTNB (5, 5-dithio-bis-(2-nitrobenzoic acid)

GSH

CDNB (1-Chloro, 2, 4-dinitrobenzene)

95% Ethanol

KH_2PO_4

3.2. Method

The study starts, brachial vein blood samples (4cc) were taken from the cases in the patient group (osteoporosis) ranging from 50-75 years of age in both male and female and the control groups. Blood samples carry up into biochemistry laboratory tubes. The tubes serum were separated from plasma by centrifugation in "Nuve NF 800 centrifuge" at (5000 rpm) for 5 minutes and obtained serums were conserved (at 20 ℃) until they are processing. When the adequate numbers of samples were obtained, serum malondialdehyde (MDA) levels and, reduced glutathione (GSH), Glutathione S-transferase (GST) and catalase (CAT) activities were spectrophotometrically measured in the Biochemistry laboratories of the Department of Chemistry, Faculty of Science, and University of Yüzüncü Yil.

3.3. Analysis of The Sample

3.3.1. Determination of catalase (CAT) activity

In this study Catalase CAT activity was determined by using Aeibi method, in which used hydrogen peroxidase substrate H_2O_2 because CAT reacts with a known quantity of H_2O_2. The method of determining CAT is :

For blank tube: 1.4 ml of 30 mM hydrogen peroxide (H_2O_2) was placed into the empty tube and 0.1 ml of phosphate buffer was adding.

For sample tube: 1.4 ml of 30 mM hydrogen peroxide (H_2O_2) was placed into the empty tube and 0.1 ml of sample was adding.

The absorbance then measured two times at 240 nm with thirty-second intervals by spectrophotometri divice. (Aeibi, 1948).

Activity = (2.3 / Δx) x [(log A1 / log A2)].

Activity: Calculated in U / L.

Δx = 30 seconds

2.3 = 1 μmol optical density of H_2O_2 in 1 cm light path.

3.3.2. Determination of reduced glutathione (GSH) activity:

The reduced glutathione (GSH) was measured as the final product of the reaction was performed .that turned into the formation of the yellow color, of obtained clear liquid of sulfhydryl groups and DTNB (5 ', 5' - (dithiobis 2-nitrobenzoic acid).measurement of the reduced glutathione level within the EDTA blood was done in 412 nm wavelength in the spectrophotometer within 24 hours (Beutler et al., 1963).

Activity (mg / ml) = [(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 yellow color molar extinction coefficient in which formed during interaction of GSH and DTNB.

3.3.3. Determination of glutathione s-transferase (GST) activity

Glutathione *S*-transferase (GST) activity assays in insects are usually performed by spectrophotometric kinetic measurements of conjugated product formation with substrates such as reduced glutathione (GSH) and 1-chloro-2,4-dinitrobenzene (CDNB). This requires a spectrophotometer that can measure absorbance in the UV range and microcentrifugation to remove the particulates from crude homogenates which absorb light at 340 nm (Vontas et al., 2000).

GST Activity (M / ml) = [ΔOD / min) / 10] x V_{Total} /V_{sample}

10 = 1 dansmol CDNB optical density value of 1 cm light path.

Min = 4 minutes

V_{Total} = Total volume

V_{sample} = Serum volume

3.3.4. Determination of malondialdehyde (MDA) level

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

C = F * 6.41 * A

C: Concentration.

F: Dilution factor.

A: Absorbance.

3.4. Statistical data analysis

The defining statistics for the studied parameters were expressed in standard deviation. T-Test was used in cases where normal distribution condition was achieved and Mann Whitney U test statistic was used in cases where normal distribution condition was not achieved. Statistical significance level was taken as $p < 0.05$ in the calculations and SPSS statistical package program was used for the calculations.

4. RESULTS

The results obtained in the present study were from total number of 66 subjects out of which 33 were healthy controls and 33 osteoporosis cases. There was a statistical significant difference in the mean GSH, GST, MDA and CAT levels in the osteoporosis patients compared with the healthy controls. And this difference in the statistical significant shows the importance of our achieved results.

When reduced glutathione (GSH) level was examined (Table 3.1) , the relationship between patient group levels (0.0005 ± 0.00034 μmol/L) and control group levels (0.0016 ± 0.00010 μmol/L) was found to have a statistically significant relationship ($p<0.05$).

In contrast, the relationship between patient group and control (GST) Glutathione S-transferase enzyme activity results (0.0035 ± 0.00231 U / L and 0.0386 ± 0.00399 U / L, respectively) (Table 3.1), were found to be statistically insignificant ($p < 0.05$).

Furthermore, malondialdehyde (MDA) level was examined, we achieve values that shows the correlation between patient and control group levels (4.2079 ± 0.21151 μmol/L and 2.1122 ± 0.32730 μmol/L, respectively) as in (Table 3.1), was statistically significant ($p<0.05$).

In other hand when catalase (CAT) enzyme activity was examined (Table 3.1), the relationship between patient group levels (0.0766 ± 0,.00026 U/L) and control group levels (0.2513 ± 0.01382 U/L) was found to have a statistically significant relationship ($p<0.05$).

Table 3.1. Descriptive statistics and comparison results for GSH, GST, MDA and CAT

Parameter	Controls Mean ± S.D (n=33)	Patients Mean ± S.D (n=33)	P
GSH (μmol/L)	0.0016 ± 0.00010	0.0005 ± 0.00034	P < 0.001
GST (U/L)	0.0386 ± 0.00399	0.0035 ± 0.00231	P < 0.001
MDA (μmol/L)	2.1122 ± 0.32730	4.2079 ± 0.21151	P < 0.001
CAT (U/L)	0.2513 ± 0.01382	0.0766 ± 0.00026	P < 0.001

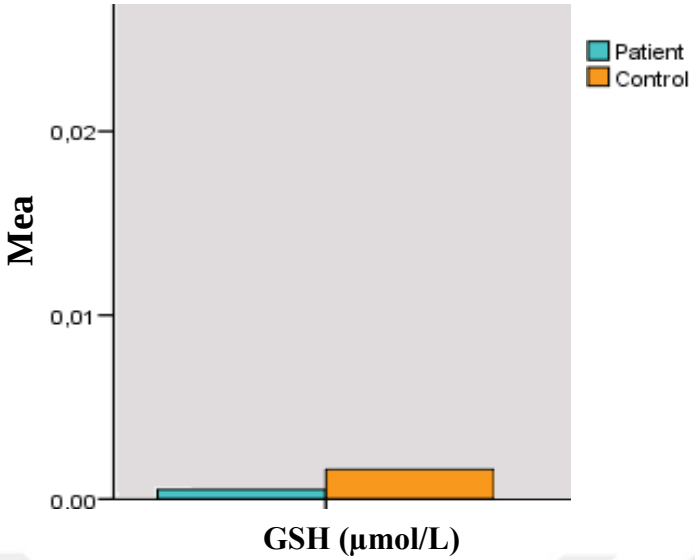


Figure 3.1. The level of GSH compared between patient and control group.

Reduced glutathione (GSH) level was examined, we achieved a value that indicates the correlation between patient group and control group (0.0005 ± 0.00034 µmol/L ; 0.0016 ± 0.00010 µmol/L) as in fig 3.1 , where these value were statistically significant ($p<0.05$).

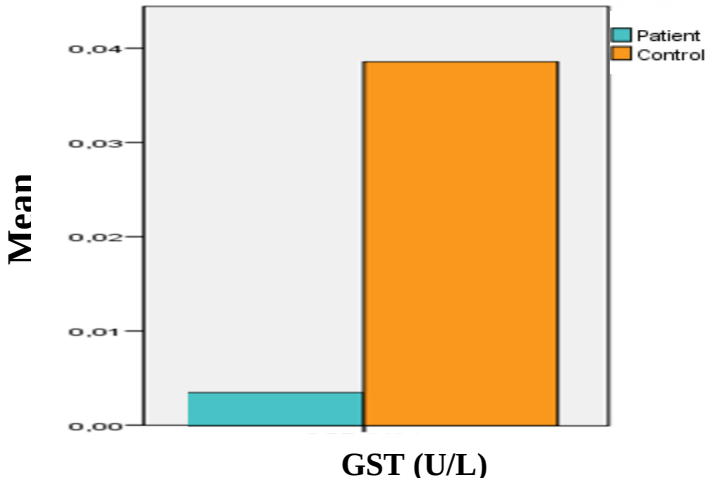


Figure 3.2. The level of GST enzyme compared between patient and control group.

Glutathione S-transferase (GST) enzyme activity was examined fig 3.2 , and the result shows the relation between patient group and control group (0.0035 ± 0.00231 U/L and 0.0386 ± 0.00399 U / L, respectively) which is statistically significant ($p<0.05$).

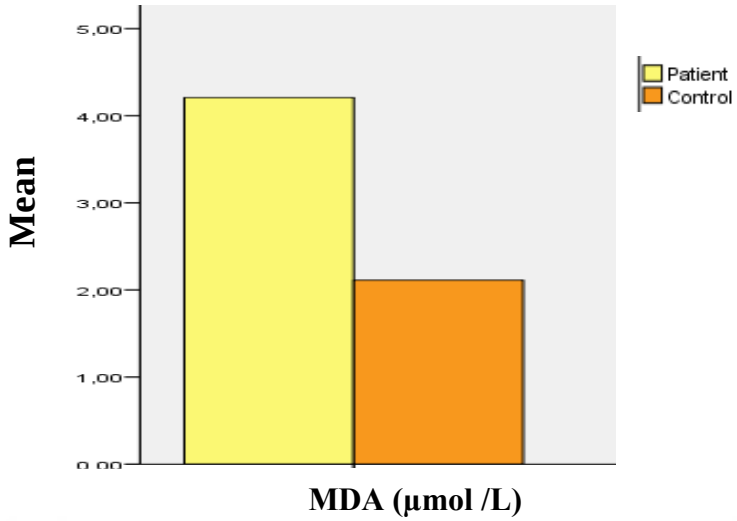


Figure 3.3. The level of MDA compared between patient and control group.

The comparison of MDA malonaldehyde levels of patient and control group gives the statistically significant relationship ($p<0.05$) between patient and control group levels (4.2079 ± 0.21151 µmol/L and 2.1122 ± 0.32730 µmol/L, respectively) as in fig 3.3.

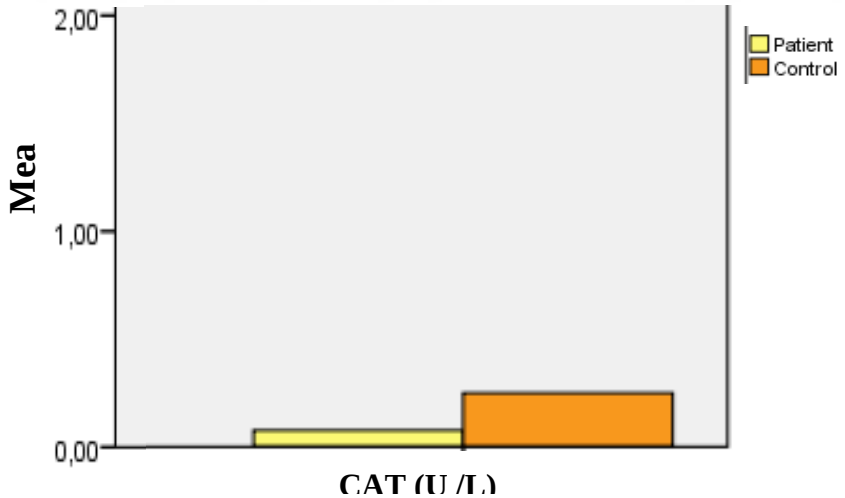


Figure 3.4. The activity of CAT compared between patient and control group.

Finally, catalase CAT enzyme activity was analysed, as in fig 3.4 and the result shows the statistically significant relationship ($p<0.05$) between osteoporosis patient groups level (0.0766 ± 0.00026 U/L) and control group levels (0.2513 ± 0.01382 U/L).

5. DISCUSSION AND CONCLUSION

Osteoporosis is a disorder characterized by means of low bone mass and microarchitectural deterioration of bone tissue and its effects many millions of human beings around the world and caused to greater bone fragility and consequent increase in fracture risk. Fragility fractures are most commonplace at the wrist, spinal vertebrae and hip, osteoporotic fractures are a main reason of morbidity and incapacity inside the aged and, within the case of hip fractures, can cause untimely loss of life. Similarly, they impose a large economic burden on health services, costing many billions of dollars every year (Prentice, 2004).

Osteoporosis is often underrecognized and undertreated because it's affecting 200 million individuals worldwide and it is silent disease until it affects the person in the form of fracture also its one of the most common metabolic bone disorder thus treatment for osteoporosis is essential medically and non-medically, some of treatments are calcium, vitamin D, calcitonin, parathyroid hormone, bisphosphonates, estrogen and balance and exercise training programs, additionally medical and non-medical treatments should be used together to minimize the risks for patients with osteoporosis (Lin & Lane, 2004).

The etiopathogenesis of osteoporosis is related to the number of free radicals formation, free radicals are endlessly produced in the human bodies which occur as part of normal cell metabolism especially by biochemical redox reactions involving oxygen (Chavan et al., 2007).

When free radicals are produce in high amount and they eliminate the ability of antioxidant defense system to remove these oxidants and antioxidants can not prevent or counterate free radicals forming in the first place at this time free radicals can change the integrety of the cells and they can damage many of biomolucules such as proteins, DNA and lipids and this cause to many diseases such as neurodegenerative diseases, diabetes, atherosclerosis and cancer also reasearches implying that oxidative stress is participate at least in the part of aging pathophysiological processes and it was proposed that ROS could be responseblie for the development of osteoporosis. Aditionally several in animal and vitro studies have proven that oxidative stress or free

radicals decreases bone formation by reducing survival of osteoblasts and the differentiation and also ROS increases the bone resorption by activating osteoclasts (Kang, 2012).

In addition, there are many factors that lead to increase the production of ROS and at the same time they lead to increase the risk factor of osteoporosis such as high amount alcohol intake, nutrients deficiency, smoking, low antioxidant reputation, excessive sports activities interest and immoderate caffeine intake are the major risk factors (Rao et al., 2013). but the most effectable risk factor which is world wide problem is the vitamin D deficiency and there is some other factors that have less effect comparing with others like thalassemia, diastolic blood pressure, tea consumption and soy intake additionally zinc (Zn) level are investigated by Some studies with bone markers and found some relations with osteoporosis (Abdollahi et al., 2005).

Free radicals are efficiently scavenged by antioxidants and when there is an imbalance between production and scavenging of the same free radical, oxidative stress will occurs and leads to oxidative deterioration of lipids or polyunsaturated fatty acids, thus compromising cellular function and antioxidant status of the body. When the number of free radicals increase also the osteoclastic activity and lipid peroxidation increases in which indicated by increase in the levels of malondialdehyde (MDA) , in another way there is a decrease of the antioxidant status of the body, reflected by low levels of glutathione peroxidase (GHPx), glutathione reductase (GHR) and superoxide dismutase (SOD). And also Alkaline Phosphatase (ALP), Inorganic Phosphorus (Pi) and Free or ionic calcium (Ca^{+2}) also undergo alteration in the osteoporosis scenario which are The Osteoblastic markers, Vitamins E and C are antioxidant vitamins are exert their osteoprotective effect by curbing the excessive free radicals formation and thereby controlling the MDA levels, which indicate the lipid peroxidation in the body (Chavan et al., 2007). Antioxidants have extraordinary effects, which participate in osteoblasts differentiation and bone formation, additionally preserving essential osteocytes that participate in osteogenesis and osteoblast activity at the same time as decreases osteoclast activity and its differentiation (Domazetovic et al., 2017).

Antioxidants that reduce the manufacturing of ROS have a high medical value in the treatment of osteoporosis (Laher, 2014)

The principle mechanism action of the most vital endogenous and exogenous antioxidants is the preventing or inhibiting the molecules that lead to oxidative damage within the cell, primary antioxidants are able to scavenge ROS via donation of hydrogen and they are essentially chain breakers, however , secondary antioxidants are peroxide decomposers, metallic chelators, UV radiation absorbers, oxidative enzyme inhibitors and singlet oxygen quenchers wherein by using this characteristics reduce the impact of ROS (Pisoschi et al., 2015).

Non-enzymatic vitamins C and E decreases the risk of fractures the incidence of osteoporosis because they have an essential role in the differentiation of mesenchyme cell into osteoblasts production of collagen and also bone mineralization (Zinnuroglu et al., 2012).

Biological researches showing that thiol and nonthiol antioxidants have an essential role in the reducing oxidant effects in which participate in mineralization process, reduction of osteoclast activity ,activation of osteoblast differentiation and generally they work as a scavengers of ROS (Romagnoli et al., 2013).

There are a number of endogenous protection mechanisms can fight free radical attack or oxidative stress through promoting antioxidant defenses such as endogenous antioxidant enzymes consisting of superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) also the metal chelating proteins . Exogenous antioxidants also have a function in the scavenge of ROS wherein they come from nutritional sources such as vegetables and fruits that contain numerous phytonutrient antioxidants such as watersoluble antioxidant polyphenols, lipid-soluble lycopene, vitamins including C and E and the carotinoids effective antioxidan (Matés et al., 1999; Rao et al., 2007)

In conclusion, in this study, antioxidants such as GSH, GST and CAT were decreased and the increase of malondialdehyde (MDA) level indicates that the lipid peroxidation level was increased as in patients associated with osteoporosis and the results show that oxidative stress has related to osteoporosis and may increase risks of osteoporosis.

As a conclusion of this study, in patients associated with osteoporosis the level of malonaldehyde (MDA) which is the end product of lipid peroxidation, and it indicates that the level of oxidative stress is also increased in other hand the level of antioxidants GSH, GST and CAT are decreased when they compared with healthy controls ($P<0.05$). In osteoporosis patients, many of biomolecules are damaged such as DNA, lipids, carbohydrates, and proteins due to oxidative stress of high concentration of oxidants which are released by stress-activated macrophages and neutrophils. Also many cells and tissues are damage as result of reaction of MDA and Lipid peroxidation with unsaturated fatty acids (released of cell membranes).

In this study, the resulting level of catalase CAT activity was statistically and significantly reduced when it is compared to the controls group in osteoporosis ($P<0.05$). This reduced catalase activity in the osteoporosis patients may also have taken place due to catalase being inactivated via H_2O_2. Both of these also show decreased catalase activity in osteoporosis patients serum and this reduce may be happen due to the converting H_2O_2 into H_2O and O_2. Consequently, it preserves the cells from the dangerous effects of collected hydrogen peroxide. This end result is following other findings.

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

The mean level of glutathione S-transferase (GST) activity showed a statistically significant decrease in osteoporosis cases when compared to the controls group ($P<0.05$). The decreased GST activity may be due to the fact that every GST subunit is a protein dimer and by increasing the oxidants many of proteins are

breakdown thus in osteoporosis patients the amount of protein sub units is low due to effect of oxidants also GST will decrease , also GST is a multifunctional antioxidant that detoxifies large number of endogenous and exogenous cellular compounds, and also the nucleophilic attack of (GSH) at the electrophilic centers of substrates is catalyzed through GSTs such as poisonous compounds, insecticides, organic hydroperoxides and metabolites , GSTs play an essential role towards therapeutic drugs cancer causing agents, and various types of ROS (Dayem et al, 2017).

In conclusion, in this study, antioxidant activitys such as GST, CAT and GSH were decreased and increased lipid peroxidation level such as malondialdehyde (MDA) was increased in patients with osteoporosis and the results show that oxidative stress has related to osteoporosis and may increase risks of osteoporosis. In the present study, it shows that oxidative stress affects tissue cellular damage very well in osteoporosis patients. Smoking, alcohol intake, vitamin D and insufficient exercise are the main factors associated with it. ROS are produced in normal metabolism and living cells.

Lipids, proteins and DNA are exposed oxidative damage when the ROS level in the cell increases. Other problems include DNA damage, loss of enzyme activity and inhibition of protein synthesis that leads to cell death. ROS is a key product in cells and contributes to the regulation of oxidation and reduction and signal transmission pathways. Further studies are essential to investigate antioxidant enzymes and oxidative stress (OS) status in osteoporosis

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EXTENDED TURKISH SUMMARY
(GENİŞLETİLMİŞ TÜRKÇE ÖZET)

OSTEOPOROZLU HASTALARDA BAZI ANTİOKSİDANT AKTİVİTESİNİN VE OKSİDATİF STRES DÜZEYLERİNİN BELİRLENMESİ

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ÖZ

Osteoporoz, kemiklerin güç, yoğunluk ve kalitelerini kaybettiği, ayrıca kemiğin daha gözenekli ve kırılgan hale geldiği bir durumdur. Osteoporoz için en etkili risk faktörleri kalsiyum ve D vitamini eksikliği, sigara, yüksek miktarda alkol alımı, besin eksikliği ve yetersiz egzersizdir. Bu çalışmada Van Yüzüncü Yıl Üniversitesi Tıp Fakültesi Ortopedi ve Travmatoloji Anabilim dalı osteoporozlu hastalarından kan serum örnekleri toplandı. Bu çalışmanın amacı oksidatif stress göstergesi olan malondialdehit (MDA) düzeyi ve bazı antioksidan aktiviteleri belirlemektir. Osteoporoz hastalarının kan serumunda lipid peroksidasyonu düzeyi olan malondialdehit asit (MDA) düzeyi, antioksidanlar olarak redükte glutatyon (GSH) düzeyi , katalaz (CAT) ve glutatyon S-transferaz (GST) aktiviteleri ölçüldü. CAT, GSH ve GST aktiviteleri hasta gruplarında sağlıklı kontrol grubuna göre anlamlı olarak azaldı ($p < 0.05$), ancak MDA düzeyleri sağlıklı kontrol grubuna göre anlamlı olarak daha yüksek bulundu ($p < 0.05$). Sonuç olarak, antioksidanlar ve oksidatif stres arasındaki herhangi bir dengesizliğin osteoporoz hastalığı gelişmesinin nedeni olabilir.

1.GİRİŞ

Osteoporoz, kemiklerin güçlerini kaybettikleri ve kırılma olasılıklarının daha yüksek olduğu bir durumdur. Kemik gücünün azalması nedeniyle oluşan kırıklar 'kırılganlık kırıkları' olarak tanımlanır ve bunların çoğu osteoporoz nedeniyle olabilir. Vücudun farklı bileşenlerinde osteoporoz sonucu kırılganlık kırıkları meydana gelebilse de, bilekler, kalçalar ve omurga genellikle etkilenen maksimum bölgelerdir (James, 2016).

Osteoporoz hastalığından dolayı neredeyse dünya çapında 2 yüz milyondan fazla insan özelikle Avrupa, Amerika Birleşik Devletleri ve Japonya'da ise yetmiş beş milyon insan etkilenmiştir. 50 yaşından büyük yaşlarda her 2 kadından 1'i ve her 5 erkekten 1'i sonunda osteoporotik kırıklar yaşamaktadır, dünyadaki kalça kırığı kadınlarda% 240, erkeklerde% 310 oranında 2050 yılına kadar öngörülüyor. Yapılan çalışmalarda, osteoporoz 2010 ve 2050 yılları arasındaki dönemde 8.1 milyon kırık (% 78 kadın,% 22 erkek) olduğu bildirilmiştir (Rao et al., 2013).

Osteoporozun etyopatogenezi, serbest radikal oluşumu nedeniyle ilgilidir, serbest radikaller, özellikle oksijen içeren biyokimyasal redoks reaksiyonları ile normal hücre metabolizmasının bir parçası olarak meydana gelen insan vücudunda üretilir.

Serbest radikaller, aynı serbest radikalin üretimi ve atılması arasında bir dengesizlik olduğunda oluşur, bunun sonucunda oksidatif stres meydana gelir. Serbest radikaler, lipitlerin veya çoklu doymamış yağ asitlerinin oksidatif bozulmasına yol açar, böylece hücresel fonksiyon ve vücudun antioksidan durumunu tehlikeye atar. Serbest radikallerin sayısı da arttığında, malondialdehit (MDA) seviyelerindeki artışla gösterilen osteoklastik aktivite ve lipit peroksidasyonu artar, başka bir değişle vücudun antioksidan durumunun azalması, düşük glutatyon seviyeleri ile ortaya çıkar. Başlıca antioksidanlar glutatyon peroksidaz (GHPx), glutatyon redüktaz (GHR) ve süperoksit dismutaz (SOD) dır. Ayrıca Alkalin Fosfataz (ALP), İnorganik Fosfor (Pi) ve Serbest veya iyonik kalsiyum (Ca^{+2}) da osteoblastik markerlerdir. E ve C antioksidan vitaminler alındığında osteoporoz düzeyinde değişiklik oluşur (Chavan et al., 2007).

Buna ek olarak, sigara içmek, yüksek miktarda alkol alımı, düşük antioksidan alınmı, besin eksikliği, aşırı spor aktiviteleri, hareketsiz yaşam ve fazla kafein alımı osteoporoz için başlıca risk faktörleridir (Rao et al., 2013). D vitamini eksikliği osteoporoz risk faktörleri, dünyanın farklı bölgelerinde% 4 ila 80 arasında olduğu bildirilmiştir (Abdollahi et al., 2005).

2. MATERYAL VE YÖNTEM

2.1. Materyal

Bu Araştırmada sağlıklı 33 erkek ve kadın ve 33 osteoporozlu hastasından kan alındı. Sağlıklı ve hasta bireylerin her birinden, antekubital venöz venden 4 ml kan alındı ve biyokimya tüpüne 2 ml, serum tüpüne ise 2 ml ilave edildi.

2.1.1. Cihazlar ve Malzemeler

Spektrofotometrea
pH ölçer
Vortex
Thermo ile belirtilen Su Banyosu
Soğutmalı santrifüj
Fırın
Derin dondurucu
Hassas terazi
Kronometre
Ayarlanabilir Otomotiv Pipetleri
Otomatik Pipet Ucu
Cam pipet
Serum Saklama Tüpleri
Derin Dondurucu Tüpler
Spektrofotometre küveti
Manyetik karıştırıcı

Deney şişesi

Erlenmeyer şişesi

2.1.2. Reaktifler ve Kimyasallar

Potasyum dihidrojen fosfat

Disodyum fosfat

Sodyum hidroksit

Hidrojen peroksit

fosfat tamponu

Etilendiamintetraasetikasit, disodyum dihidrat

Butil hidroksil toluen

Tiyobarbitürik asit

Trikoasetik asit

Sodyum klorit

Sodyum dihidrojen fosfat

Sodyum sitrat

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

Glutatyon

2,4-Dinitroklorobenzen

% 95 Etanol

Potasyum dihidrojen fosfat

2.2.Yöntem

Bu çalışmada, osteoporoz tanısı teşhisi konulmuş 33 hasta ve 33 sağlıklı bireyden oluşan ve yaşları 18-65 arası bireylerden seçildi. Biyokimyasal parametreler serum örnekleri ile belirlendi. Bu çalışma için kan örneklerinin alınmasından önce, Van Yüzüncü Yıl Üniversitesi Tıp Fakültesinden Etik Kurul onayı alındı. Hasta ve sağlıklı bireylerden usulüne uygun olarak venözden 4 ml kan alındı ve 2000 rpm/dk da 5 dakika santrifüj edildi ve ardından serumlar plazmadan ayrıldı. Ayrılan serumlar, redükte glutatyon (GSH) , Glutatyon S-transferaz (GST), katalaz (CAT) ve malondialdehit (MDA) seviyelerini belirlemek için kullanıldı.

Katalaz (CAT) aktivitesi tayini

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

Kullanılan çözeltiler:

1. 30 mM H_2O_2'nin hazırlanışı: 10 ml bidistile suyun içine, % 30'lik H_2O_2'den 34 µl alınarak konuldu (% 35'lik H_2O_2'den 25,8 µl alınarak konuldu).
2. 50 mM Fosfat Tamponunun hazırlanışı: 6.81 g KH_2PO_4 ve 7.1 g $Na2HPO_4$ bidistile suda çözülerek, tamponun pH'ı 1N NaOH ile 7.4'e ayarlandı ve hacim 1 litreye tamamlandı.

Aktivite Hesabı:

E.Ü.= (2,3 / Δx) x [(log A1 / log A2)] Aktivite; U/L cinsinden hesaplandı.

Δx= 30 saniye

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

Redükte glutatyon (GSH) aktivitesi tayini

Kullanılan çözeltiler:

1. Fosfat tamponu: 0.3 M disodyum fosfat bidistile su ile hazırlandı.
2. Ellman's ayıracı:; %1 sodyum sitrat, 100 ml'ye bidistile su içinde eritildi. İçerisine 40 mg DTNB (5',5'-(2-ditiobis nitrobenzoik asit) eklendi.

GSH tayin yöntemi:

1-) 200 µl serum üzerine 800 µl fosfat tamponu eklendi. 412 nm'de ilk absorbans (OD1) kaydedildi. Aynı tübe 100 µl Ellman's ayıracı ilave edildi, 2.absorbans (OD2) kaydedildi.

Hesaplama:

Glutatyon derişimi mmol/g protein biriminden hesaplandı.

C / 1000 = (OD2-OD1) / 13600 x E1 x 5/2 x ½

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

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

1000: mmol'e dönüşüm katsayısı.

C: mmol / glutatyon (mg/dl)

OD1: DTNB ilave edilmeden önce 412 nm dalga boyunda ölçülecek optik dansite.

OD2: DTNB ilave edildikten sonra 412 nm dalga boyunda ölçülecek optik dansite.

Glutatyon-s-transferaz aktivitesi tayini

Ayıraçlar:

1. 20 mM GSH: 6,1 mg GSH Saf su ile 1 ml'ye tamamlandı. Gerekli miktarda günlük olarak hazırlandı.(50 numune)

2. 20 mM CDNB: 4,05 mg CDNB % 95'lik etanolle 1 ml'ye tamamlandı. Gerekli miktarda günlük olarak hazırlandı.(50 numune)

3. 0.2 M NaK Fosfat Tamponu (pH 6.5):Disodyum fosfat 1,19 g,Potasyum fosfat2,25 g EDTA 29,2 mg; Saf su ile 100 ml'ye tamamlandı. pH doymuş sodyum hidroksit ile ayarlandı

Eritrositte çalışırken: Eritrosit % 0.9 NaCI ile 1/10 seyreltildi, hemoliz edildi. Hemolizat 4˚C'de 5000 rpm'de 10 dk. Santrifüj edildi.

Deneyin yapılışı:

1-) Deney tüpüne 440 µl tampon ve 20 µl serum konuldu.

2-) Vortekslendi ve 37 ˚C'de 10 dk.inkübe edildi.

3-) İnkübasyondan sonra tüplere 20 µl GSH ilave edildi.

4-) 10 sn. sonra 100 µl CDNB eklendi, vortekslendi.

340 nm'de 1. Ve 5. Dakikalarda absorbans ölçüldü.

Aktivite hesabı:

GST Aktivitesi (Ü/ml) = [(ΔOD/ dak) / 10] X (Vtoplam / Vörnek)

ΔOD = Optik Dansite Değişimi

Dak: 4 dakika

10 = 1 µmol CDNB ‘nin 1 cm’lik ışık yolunda verdiği optik dansite değeri

VToplam = Toplam hacim

VÖrnek = Serum hacmi

Malondialdehit (MDA) düzeyi tayini

Kullanılan çözeltiler:

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

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

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

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

5-) % 30’luk TCA çözeltisi (trikloroasetik asit) : 30 gr TCA, 100 ml distile suda eritildi.

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

Deneyin yapılışı:

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

Malondialdehit düzeyi hesaplaması:

C= konsantrasyon

F=Seyreltme faktörü

A=Absorbans

C= F x 6.41 x A

Düzey hesabı; µmol/L olarak hesaplandı.

İstatistiksel Analiz

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

3- SONUÇLAR

Osteoporozdaki MDA, CAT, GST ve GSH seviyeleri, sağlıklı kontrollerle karşılaştırıldığında istatistiksel olarak anlamlı bir farklılık gösterdi. Malondialdehit (MDA) düzeyi incelendiğinde (Tablo 3-1), hasta grubu ile kontrol grubu düzeyleri arasında sırasıyla (4.2079 ± 0.21151 µmol/L and 2.1122 ± 0.32730 µmol/L) istatistiksel olarak anlamlı bir ilişki olduğu saptandı ($p < 0.05$).

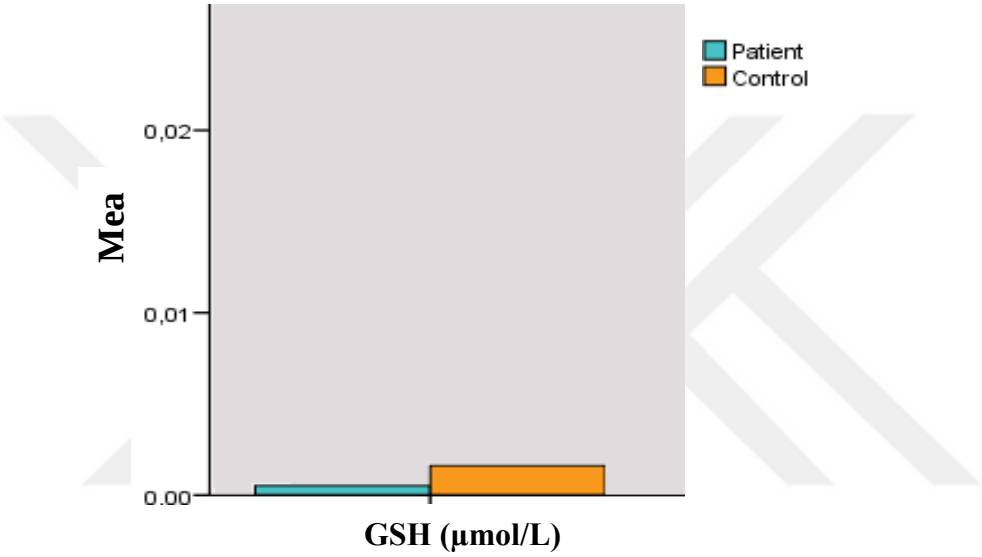
Buna karşılık, hasta grubu ile kontrol grubu arasında redükte glutatyon (GSH) arasında (sırasıyla 0.0005 ± 0.00034 µmol/L ve 0.0016 ± 0.00010 µmol/L) (Tablo 3-1) istatistiksel olarak anlamlı bulundu ($p < 0.05$).

Ayrıca, Glutatyon S-transferaz (GST) aktivitesi (Tablo 3-1) , hasta grubu ile kontrol grubu (sırasıyla 0.0035 ± 0.00231 U / L ve 0.0386 ± 0.00399 U / L) arasındaki korelasyonun da istatistiksel olarak önemli olduğu bulundu ($p < 0.05$).

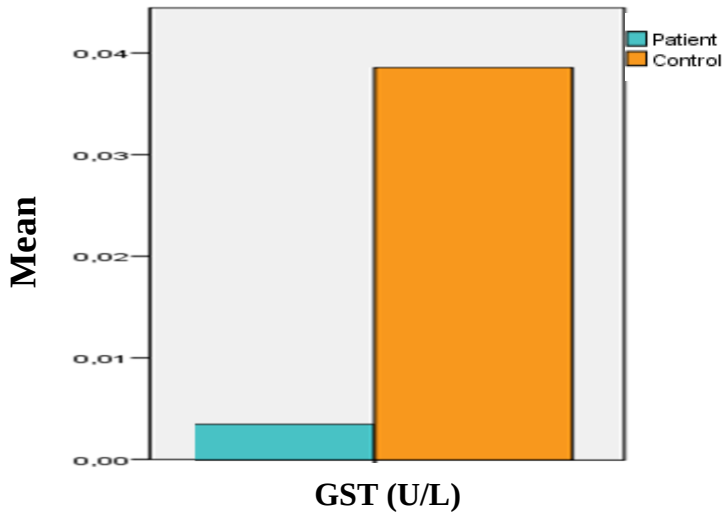
Öte yandan, katalaz (CAT) enzim aktivitesi (Tablo 3-1), hasta ve kontrol grubu seviyelerinin (sırasıyla 0.0766 ± 0.00026 U / L ve 0.2513 ± 0.01382 µmol / L) istatistiksel olarak anlamlı olduğunu göstermiştir ($p < 0.05$).

Tablo 3.1. Kontrol ve hasta gruplarında osteoporoz belirteçlerinin karşılaştırılması

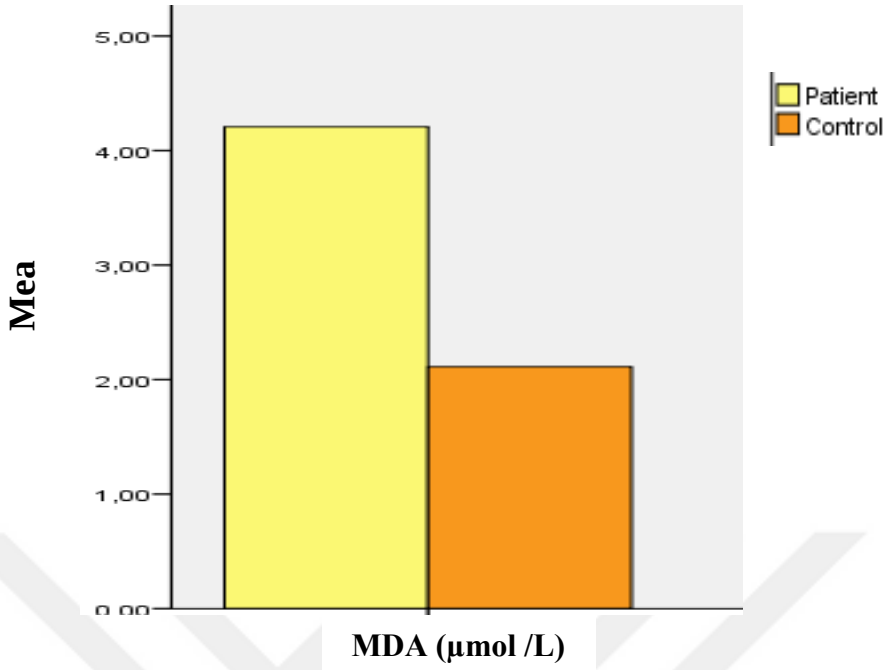
Parameterler	kontrol Ortalama ± S.S (n=33)	Hasta Ortalama ± S.S (n=33)	P
GSH (µmol/L)	0.0016 ± 0.00010	0.0005 ± 0.00034	P < 0.001
GST (U/L)	0.0386 ± 0.00399	0.0035 ± 0.00231	P < 0.001
MDA (µmol/L)	2.1122 ± 0.32730	4.2079 ± 0.21151	P < 0.001
CAT (U/L)	0.2513 ± 0.01382	0.0766 ± 0.00026	P < 0.001



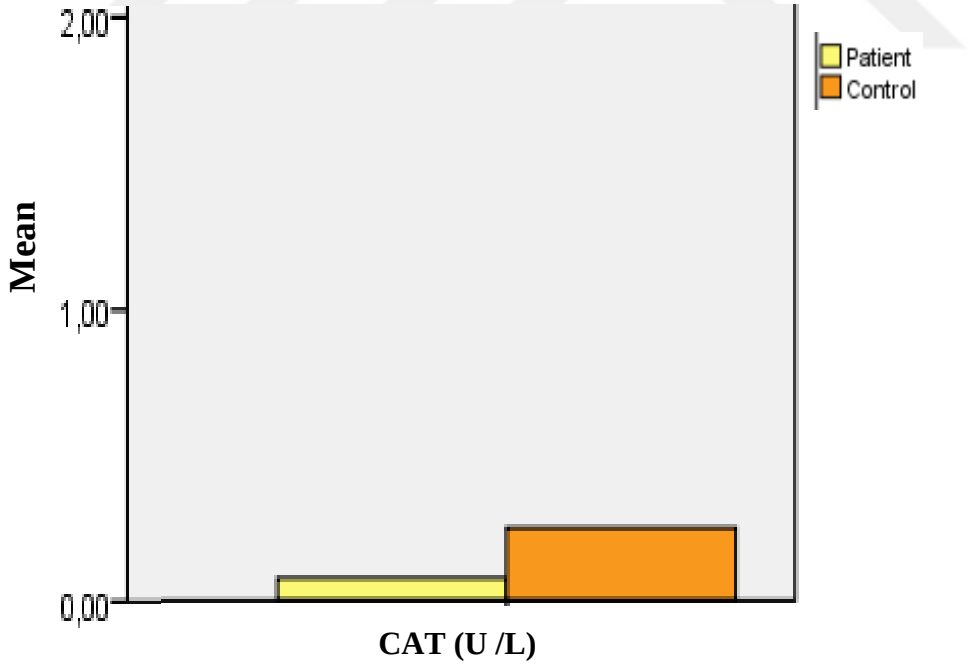
Şekil 3-1 . Hasta ve sağlıklı kontrol grubu arasındaki karşılaştırılan GSH seviyesi.



Şekil 3-2. Hasta ve sağlıklı kontrol grubu arasındaki karşılaştırılan GST seviyesi.



Şekil 3-3. Hasta ve sağlıklı kontrol grubu arasındaki karşılaştırılan MDA düzeyi.



Şekil 3-4. Hasta ve sağlıklı kontrol grubu arasındaki karşılaştırılan CAT seviyesi

4. TARTIŞMA VE SONUÇ

Bu çalışmanın amacı osteoporoz hastalarında oksidatif stress göstergesi olan malondialdehit (MDA) düzeyi ve bazı antioksidan aktiviteleri belirlemektir. Osteoporoz hastalarının kan serumunda lipid peroksidasyonu ürünü olan malondialdehit asit düzeyi (MDA) ile redükte glutatyon (GSH), katalaz (CAT) ve glutatyon S-transferaz (GST) gibi antioksidanlar ölçüldü.

Çalışmanın bulguları, lipid-peroksidasyonunun bir götergesi olan MDA'nın, osteoporoz hastalarda kontrol grubuna kıyasla MDA açısından istatistiksel olarak anlamlı şekilde arttığını göstermiştir ($p<0.05$). Osteoporoz hastalarında, oksidatif olan yüksek konsantrasyonlardaki oksidanlar, stresle aktive olan makrofajlar ve nötrofiller tarafından salınır. Bu, lipidlerin, proteinlerin, karbonhidratların ve DNA'nın zarar görmesine neden olabilir. Lipid peroksidasyonu ve MDA ile reaksiyona giren hücre zarlarının doymamış yağ asitleri serbest bırakılır. Oksidatif stres belirteci olarak işlev görür ve iltihaplanma etkilerini artıran immünojenik moleküllerin daha fazla üretimine yol açan lisin kalıntılarıyla reaksiyona girer

ROS oluşumu devam ettikçe, osteoporoz hastalığı geniş ölçüde yayılarak dokulara gereksiz zarar verebilir. ROS atakları membran lipitleri, çoklu doymamış yağ asitleri ve hücre fonksiyonunu bozarak meydana gelir ve lipit peroksidasyonuna neden olur. Ek olarak, malondialdehit gibi lipit peroksidasyonunun nihai ürünü, oksitlenmiş lipitlerin ayrışmasıyla üretilecektir.

Glutatyon S-transferaz (GST) aktivitesinin ortalama seviyesi, kontrol grubuna kıyasla osteoporoz vakalarında istatistiksel olarak anlamlı bir düşüş gösterdi ($P <0.05$). Azalan GST aktivitesi, her GST alt biriminin bir protein dimeri olması ve oksidanların arttırılmasıyla birçok proteinin parçalanması nedeniyle osteoporoz hastalarında oksidanların etkisine bağlı olarak protein alt birim miktarının düşük olması da GST miktarını azalacaktır.

Bu tez çalışmada CAT aktivitesi, osteoporoz hastaları kontrol grubuyla karşılaştırıldığında istatistiksel olarak ve anlamlı bir şekilde azaldı ($P<0.05$). Osteoporoz grubundaki bu azalan katalaz aktivitesi, katalazın H_20_2 ile etkisizleştirilmesinden dolayı meydana gelmiş olabilir. Osteoporoz hastasının azalmış

katalaz seviyesi, artan inflamasyonla açıklanabilir. Katalaz H_20_2'nin H_2O ve O_2'ye değişmesine nedenle olur. Sonuç olarak, hücreleri biriken hidrojen peroksitin zararlı etkilerinden korur. Bu sonuç diğer yapılan litaratür bulgularla uyumludur

Bu çalışmada GSH düzeyi osteoporoz kontrol grubuyla karşılaştırıldığında istatistiksel olarak anlamlı derecede azaldığı tesbit edildi ($P < 0.05$). Glutatyonun azaltılması bir flavoenzimdir ve GSSH'nin glukoz-6-fosfat dehidrojenaz tarafından sağlanan GSH'ye indirgenmesinde rol oynayan NADPH'ye bağlıdır

Sonuç olarak, bu çalışmada osteoporoz hastalarda CAT GSH ve GST gibi antioksidanların azaldığı ve malondialdehit asit düzeyinin (MDA) ise arttığı saptanmıştır. Bulunan bu sonuçlar, oksidatif stresin osteoporoz ile ilgili olarak hastalığının olumsuz yönde etkilerini artırabileceğini göstermektedir. Ek olarak, oksidatif stresin, osteoporoz hastalarında dokunun hücresel hasarını çok iyi etkilediğini göstermektedir.

CURRICULUM VITAE

Abdulrahman Abdulrazzaq ISMAEL was born in 1993, Erbil / Iraq. He finished primary and secondary education in Erbil. He started the study of BSc degree in the University of Salahaddin in 2011, College of Science Chemistry Department and graduated in 2015. He started the study of master degree in department of Chemistry (Biochemistry) in the in the Institute of Science of Van Yüzüncü Yıl University in Turkey.

T.C
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Yukarıda başlığı/konusu belirlenen tez çalışmamın Kapak sayfası, Giriş, Ana bölümler ve Sonuç bölümlerinden oluşan toplam 80 sayfalık kısmına ilişkin, 23/12/2019 tarihinde şahsım/tez danışmanım tarafından yerleştirilen intihal tespit programından aşağıda belirtilen filtreleme uygulanarak alınmış olan orijinallik raporuna göre, tezimin benzerlik oranı % 16 (Yüzde on altı) dır.

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