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

DETERMINATION OF OXIDATIVE STRESS LEVEL (MALONDIALDEHYDE), SOME ANTIOXIDANT ACTIVITIES (CATALASE AND REDUCED GLUTATHIONE) AND IMA (ISCHEMIA MODIFIED ALBUMIN) IN GOUT PATIENTS

M. Sc. THESIS

PREPARED BY: Nawar Yaseen MOHSIN
SUPERVISOR: Prof. Dr. Halit DEMİR
Co-Supervisor: Assist.Prof. Dr. Sezai ÖZKAN

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ACCEPTANCE and APPROVAL PAGE

This thesis entitled “DETERMINATION OF OXIDATIVE STRESS LEVEL (MALONDIALDEHYDE), SOME ANTIOXIDANT ACTIVITIES (CATALASE AND REDUCED GLUTATHIONE) AND IMA (ISCHEMIA MODIFIED ALBUMIN) IN GOUT PATIENTS” and prepared by Nawar Yaseen Mohsin under consultation of Prof. Dr. Halit Demir and Assist.Prof. Dr. Sezai ÖZKAN in Department of Chemistry, on date of 20/10/2020 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.

Chair: Prof. Dr. Halit DEMİR Signature:

Member: Prof. Dr. Suat EKİN Signature:

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This thesis has been approved by the committee of The Institute of Natural and Applied Science on ..……../........../…..…... with decision number ....

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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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Nawar Yaseen MOHSIN

ABSTRACT

DETERMINATION OF OXIDATIVE STRESS LEVEL (MALONDIALDEHYDE), SOME ANTIOXIDANT ACTIVITIES (CATALASE AND REDUCED GLUTATHIONE) AND IMA (ISCHEMIA MODIFIED ALBUMIN) IN GOUT PATIENTS

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Gout is a may be and complex arthritis type that could affect anyone. It is characterized by the feeling of sudden and extreme bouts of pain, swelling and redness in the joints, particularly the joint at the base of the broad toe. Gout when forming uric acid crystals around the joint, causing inflammation and acute pain-(gout attack), uric acid crystals are formed in people whose uric acid level is high. The body produces uric acid as part of the purine process, a substance that is naturally present in the body and in certain types of food. Throughout this research, samples of blood serum from gout patients were obtained from the Van Yüzüncü Yıl University Hospital Orthopedics and Traumatology Clinic. This study was aimed at identifying the levels of lipid peroxidation malondialdehyde (MDA) and antioxidants such as reduced glutathione (GSH), catalase (CAT) and Ischemia Modified Albumin (IMA) in the blood serum of gouty patients. CAT and GSH activities were significantly decreased in the patient groups compared to the (healthy control group) ($p<0.001$). MDA levels were significantly higher in the group of patients relative to the balanced control group is ($p<0.001$). In conclusion, this study, oxidative stress may play an important-role in the development of gout disease.

Keywords: CAT, GSH, IMA, MDA, UA.

ÖZET

GUT HASTALARINDA OKSİDATİF STRES DÜZEYİ (MALONDİALDEHİT), BAZI ANTİOKSİDAN AKTİVİTELERİN (KATALAZ VE REDÜKTE GLUTATYON) VE İMA (İSKEMİ MODİFİYE ALBUMİN) DÜZEYİNİN BELİRLENMESİ

MOHSIN, Nawar Yaseen
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Gut, herkesi etkileyebilecek yaygın ve kompleks bir artrit şeklidir. Gut, eklemlerde ani ve şiddetli ağrı, şişme ve kızarıklık hissi ile ortaya çıkmaktadır, genellikle ayak başparmağının dibinde eklemde ürik asit kristalleri birikerek iltihaplanma ve akut ağrıya neden olan bir hastalıktır. Ürik asit seviyesi yüksek olan kişilerde ürik asit kristalleri oluşur. Vücutta ve belirli yiyecek türlerinde doğal olarak bulunan bir madde olan pürin işleminin bir parçası olarak ürik asit üretir. Bu çalışmada, Van Yüzüncü Yıl Üniversitesi Hastanesi Ortopedi ve Travmatoloji Kliniğinden gut hastalarından kan serumu örnekleri alındı. Bu çalışmanın amacı, gut hastalarının kan serumunda İskemi modifiye albümin (İMA) ile lipid peroksidasyonu olan malondialdehit (MDA) ve indirgenmiş glutatyon (GSH), katalaz (CAT) gibi antioksidanların aktivitelerini belirlemekti. Hasta grupları sağlıklı kontrol grubuna göre CAT ve GSH aktiviteleri anlamlı olarak azaldığı tesbit edildi ($p < 0.001$). Hasta grubu sağlıklı kontrol grubuna göre MDA düzeyleri ise anlamlı olarak yüksek bulundu ($p < 0.001$). Sonuç olarak, oksidatif stres, gut hastalığının gelişiminde önemli bir rol oynayabilir.

Anahtar kelimeler: CAT, GSH, IMA, MDA, UA.

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November 2020

Nawar Yaseen MOHSIN

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SYMBOLS AND ABBREVIATIONS

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

Symbols	Description
Cu	Cupper
Fe	Iron
Zn	Zinc
O2	Oxygen
O3	Ozone
O2-	Superoxide
OH-	Hydroxyl
ºC	Centigrade temperature
min	Minute
g	Gram
hr	Hour
L	Liter
Ml	Milliliter
µl	Microliter
mg	Milligram
mM	Mill molar
M	Molar
N	Normal
rpm	Round/Minute
µg	Microgram
µm	Micrometer
β	Beta
α	Alfa
U/L	Unit/liter

Abbrevations	**Description**
TLRs	Toll-like reseptors
ATP	Adenine triphosphate
CAT	Catalase
DNA	Deoxyribo nucleic acid
RNA	Ribonucleic acid
EDTA	Ethylenediamine tetra acetic acid
GPx	Glutathione peroxidase
GSH	Reduced glutathione
SYK	Spleen tyrosine kinase
H_2O_2	Hydrogen peroxide
Kcal	kilocalorie
MSU	Mono sodum urate
MDA	Malondialdehyde
NADPH	Nicotinamide adenine dinucleotide phosphate
NO_2	Nitrogen dioxide
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
RSS	Reactive sulphur species
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
UV	Ultraviolet
GSH	Reduced glutathione
NLR	Nod-like reseptor
Mn	Manganese
GSSG	Oxidized glutathione
KOH	Potassium hydroxide
Na_2CO_3	Sodium carbonate
BSA	Bovine Serum Albumin
NH4 (SO4)	Ammonium sulphate
$CuCI_2$	Copper chloride

Abbrevations	Description
NaOH	Sodium hydroxide
DTNB	5,5,dithiobis-(2-nitrobenzoic acid)
BHT	Butylhydroxytoluene solution
TBA	Thiobarbituric acid solution
TCA	Trichloro acetic acid solution
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-Sulphonic acid)
PYD	Pyrindomain
ASC	Apoptosis associated speck linke protine
IL1β	Inter leukin one beta
TNF-a	Tumer necrosis fctor alpha
NETs	Neutrophil extracellular trap structures

INTRODUCTION

Gout is the most serious type of inflammatory arthritis caused by the accumulation in and around the peripheral joints of uric acid monosodium crystals (García Melchor and Sabrià Leal 2014). While there is a clear correlation between hyperuricemia and gout, only a minority of hyperuricemia sufferers experience gout (van Leeuwen et al., 2018). This is likely because soluble uric acid is not gout etiological, but the development of monosodium urate crystals inside the joint and its contact with the surface membrane of articular cells contributes to gout inflammation marked by a rapid onset of intense joint pain (classically the first metatarsophalangeal injury), swelling and erythema. (Ridi and Tallima, 2017; Vaidya et al., 2018).

The occurrence of gout in the general public ranges from (0.1%) to around (10%) and the rate of occurrence ranges from 0.3 to 6 cases per 1,000 individuals (Mohammed et al., 2019). The risk of gout has increased among men and the elderly (Rees et al., 2014). In addition, obesity, hypertension, and the use of certain medications are risk factors for incident gout (Beydoun et al., 2018). Also, the consumption of purine-rich foods, high fructose foods along with alcohol intake is positively linked with gout and/or hyperuricemia (Beydoun et al., 2018). Furthermore, genetic variation contributes significantly to the prevalence of gout across different ethnic groups (Robinson, 2018). Several genetic variants loci linked to serum urate levels in various genes (Dong et al., 2018).

1.1. Aims of the Study

1. Study some biochemical parameters (Reducted glutathione, catalase, and malondialdehyde) and some pathogenic signs (ischemia modified albumin) in gout patients.
2. Investigate each function of the free radicals and antioxidant variants in gout occurrence.
3. Analyze the association between the oxidizing stress in gout patients and biochemical parameters.
4. Explain the effect of oxidative stress on the human body.

2. LITERATURE REVIEW

2.1 The Gout

Gout is a type of inflammatory arthritis, arises after chronic hyperuricemia that permits monosodium deposition urates in and around the joints with the subsequent painful recurrent flares and tissue damage (Zhu et al., 2011; Vargas-Santos and Neogi, 2017).Inflammatory arthritis is a common form and thought to affect men more than women. In general, women become more susceptible to gout after menopause (Zhu et al., 2011). Gout usually occurs as recurrent attacks resulting in painful, red, and swollen joints, the big toe joint is commonly affected, but other joints including fingers, knees, heels, and wrists may also be affected (Schlesinger, 2010).Chronic hyperuricemia predisposes individuals to gout, concentrations of urate above the solubility threshold induces Monosodium urate (MSU) crystals formed in synovial joint fluid, soft tissues (Martillo et al., 2014). The clinical characteristics of gout are caused by the inflammatory response to urate crystals with monosodium (Saigal and Agrawal, 2015). They induce episodes that are initially infrequent, affecting the foot joint, and well responsive to anti-inflammatory medications such as colchicine and nonsteroidal anti-inflammatory drug (Moolenburgh et al., 2006). These do not, however, prevent urate deposition from progressing, so frequent and widespread attacks can develop, permanent damage to the joint may result from gout erosions, and tophi can occur. Therefore, optimal management is required to provide a gradient for the crystal resorption (Wong, 2005).

Many people with hyperuricemia developing no gout or even crystals of uric acid (Perez-Ruiz et al., 2015). While hyperuricemia is the main pathogenic defect in gout, only 5% of people with hyperuricemia above (9 mg/dL) developing to gout (Ragab et al., 2017). Accordingly, it is important in the incidence of gout in Other factors such as genetic predisposition account for gout incidence (Suresh, 2005).

2.2. The Gout Pathophysiology

2.2.1. Hyperuricemia and crystals formation

Uric acids are the final product of endogenous and exogenous purine-metabolism, it is a weak acid found as ionized form (urate) at normal physiological states. Humans lack the expression of uricase (Nyborg et al., 2016), an enzyme that catalyzes the conversion of urate into a more soluble product called allantoin that could be eliminated easily by the kidney so that under normal physiological states human serum urate levels are close to its limit of solubility. Seventy percent of uric-acid is excreted by the kidneys, while the rest is excreted by the gut (Roddy et al., 2013). In the kidney secretion and reabsorption occurs along the proximal-renal tubules in which 10% of filtered urate being excreted, this process is controlled by a group of apical and basolateral expressed transporters(Abhishek et al., 2017).Overproduction of urate or underexcretion or both result in increased uric acid levels (Saigal and Agrawal, 2015). Hyperuricemia is characterized as a level of uric acid serum which is higher than 7.0 mg/dL in postmenopausal women and men, while in premenopausal women, hyperuricemia occurred when uric acid exceeds 6.0 mg/dL (Kopke and Greeff, 2010).

Hyperuricemia reduces urate solubility and permits uric acid supersaturation and MSU crystal nucleation and growth (Gonzalez, 2012; Chhana et al., 2015). Factors that influence urate solubility include synovial fluid temperature, pH, the concentration of the salt, and cartilage matrix components that may contribute to crystal-forming processes (Chhana et al., 2015; Ragab et al., 2017). The process is improved by the acidic and cold environment (George and Minter, 2019). During the asymptomatic gout stage of hyperuricemia, the crystals are deposited first in supersaturated body fluids, then in tissues and later around joints (Pascual et al., 2015). Nucleation occurs when monosodium urate molecules have clustered, followed by crystal growth in which stable mass formed that is Unlikely to dissolve inside the solvent (Chhana et al., 2015).

Considered hyperuricemia is a risk factor for gout, however, not all patients with hyperuricemia develop monosodium urate (MSU) crystals or gout (Perrin et al. 2011; Borghi 2017).

Clinical stages for progression of gout include asymptomatic hyperuricemia, Followed by an acute gout attack or persistent gout, then intercritical gout and, lastly, chronic topical gout (Kim et al., 2003).

2.2.2. The immune response (flares)

The innate immune system provides the initial non-specific defense response to monosodium urate crystals (Liu-Bryan, 2010). Monosodium urate crystals are pro-inflammatory that can induce, amplify, and sustain an acute inflammation (Terkeltaub, 2017). Major cells reacting to crystals are leukocytes, especially neutrophils and macrophages, also endothelial cells, synoviocytes, and mast cells are involved (So and Busso, 2009; Abhishek et al., 2017; Busso and So, 2010).

Mechanisms describe the interaction between crystals and cells include phagocytosis which is fundamental for immune cell activation. In macrophages toll-like receptors (TLRs) are sensors recognizing danger signals and infections, also play an essential role in triggering اثار the immune response (Busso and Ea, 2011). Also, direct interaction between crystals and cell membranes may lead to cell induction by the activation of spleen tyrosine kinase (Syk) which triggers either cellular response or internalization of these crystals (Popa-Nita et al., 2009).

In the case of gouty arthritis, the inflammation begins when the macrophages in the joint space phagocytized MSU crystals, this triggers the assembly and activation of protein complex within the cytosol of the macrophage called an inflammasome (Figure.1-1), this complex consists of NLRP3 protein, also known as cryopyrin, is a member of the nod-like receptor proteins (NLR) Family that are cytosolic pattern-recognition receptors (PRRs) activated by signals that have reached the cytosol of the cell, this type consists of 3 domains: central nucleotide binding and oligomerization domain (NACHT) leucine-rich domain (LRR), and pyrin-domain (PYD).

- Apoptosis associated speck like protein containing a CARD (ASC) protein, an adaptor protein that connects the NLRP3 with caspase-1 via its domains.
- Caspase-1, a member of the family of inflammatory caspases which catalyze the cleavage of inactive interleukin one beta (IL-1β) pro molecule into active IL-1b (So and Martinon, 2017).

Upon activation of inflammasome oligomerization of the NLRP3 protein and its recruitment to a complex of adaptor proteins and effector enzymes will take place, then IL-1β which is a pro-inflammatory cytokine that has different types of actions on cells and tissues will be released from the cell, IL 1β release regulates cells apoptosis, differentiation, and proliferation in gouty arthritis leading to expression of a wide range of inflamming mediators for instance interleukin-six IL-6 and tumor necrosis factor alpha (TNF-a) that are directly responsible for the neutrophil influx to the synovium, to create an inflammatory response at the location of crystal deposition, and thereby control acute inflammatory episodes (Joosten et al., 2010; Kingsbury et al., 2011; Gonzalez, 2012; Jin et al., 2012; Szekanecz et al., 2019).

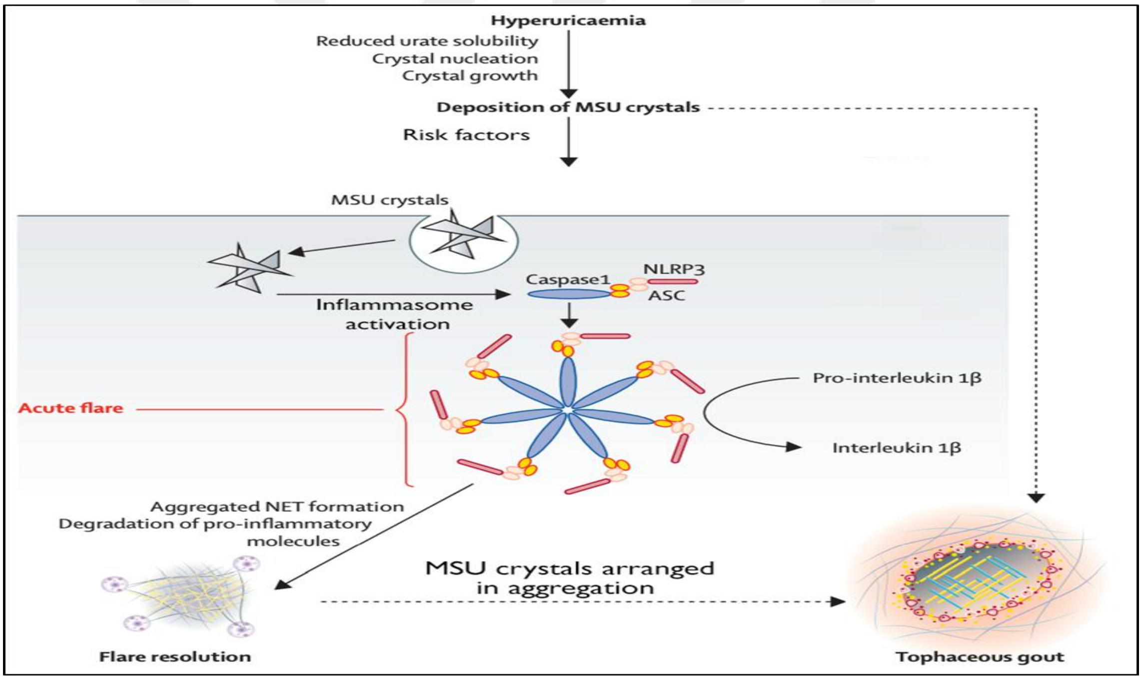

Figure 2.1. Progression from hyperuricemia to the clinical manifestations of gout.

The first acute gout attack occurs when MSU crystals are thrown into the joint space from the articular cartilage, initiating an inflammatory cascade, the starting point begins with the phagocytosis of MSU crystals(Doherty, 2009), producing reactive oxygen species ROS, followed by the release of active IL-1β from monocytes and macrophages(So and Martinon, 2017), ending with attacks or (flares) of severe joint pain associated with swelling and erythema that commonly affect first metatarsophalangeal joint (“podagra”), other joints such as mid foot, ankle, knees, finger-joints, and wrist may also be affected(Joosten et al., 2010; Roddy, 2011; Gonzalez, 2012).

The action mediates the resolution phase of the acute gouty inflammation neutrophil extracellular trap structures (NETs) that are extracellular structures consisting of cell contents (protein) attached to the chromatic frame (DNA)(So and Martinon, 2017).formed by neutrophils during a process called NETosis as a result of reactive oxygen species, NETs degrade inflammatory mediators (Apel et al., 2018). In addition, Anti-inflammatory cytokines played a crucial role in stopping the inflammatory cycle, thus, resolving inflammation (Schauer et al., 2014).

2.2.3. Tophaceous Gout

Chronic hyperuricemia and ineffective treatment of gout result in increased urate crystals deposition in the joint leading to the formation of aggregation of large MSU crystals with infiltrated immune cells that are known as tophi (Chisnall, 2014). Pro inflammatory-cytokines for example (IL-1β) and TNF-α are produced within the tophus that explains the “state of chronic inflammation and resolution” (Owen L & Gonzalez,2017). Typically, tophi do not cause pain but if left untreated, over time, they can increase in size and result in bone erosion, affecting joint mobility and later causing musculoskelet al disability (Patel et al., 2010; Dalbeth and Gow, 2007).

2.3. Uric Acid

Uric acid $C_5H_4N_4O_3$ is a weak organic acid produced as a product of purine metabolism mainly by the liver (de Oliveira and Burini, 2012), at the normal physiological condition (PH 7.4) uric acid circulates in an ionized form known as the monovalent sodium salt (urate) or the salt of uric acid (George and Minter, 2017). In the urinary tract where PH falls into 5.75, it occurs as a non-ionized form of uric acid (Pasalic et al., 2012). The normal range of the uric acid in human’s blood is “1.5 to 6.0 mg/dL in women and 2.5 to 7.0 mg/dL in men”. It has poor water-solubility as well as in plasmas, it will reach plasma saturation if its concentration is greater than (6.8 mg/dL) which may increase the risk of urate crystal formation (Jin et al., 2012). It is known that the physiological concentration of urate in humans is close to its limit of solubility (Álvarez-Lario and Macarrón-Vicente, 2010; Jin et al., 2012; Grassi et al., 2013; Maiuolo et al., 2016; Paul et al., 2017).

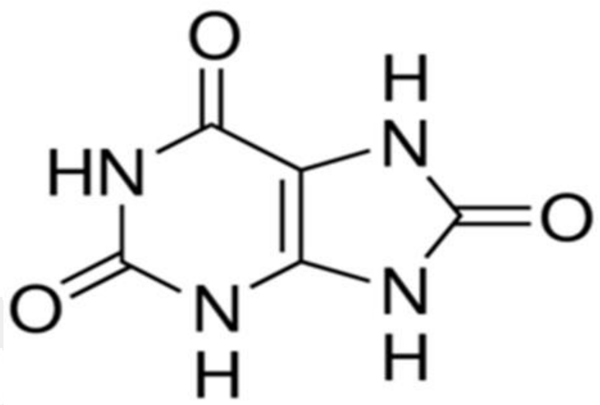

Figure 2.2 Uric acid $C_5H_4N_4O_3$.

Uric acid contains two double bonds and considered an excellent antioxidant, comprising about 60% of total plasma antioxidant capacity (Vukovic et al., 2009). “An antioxidant is any molecule that is stable enough to neutralize a free-radical by donating an electron thus reducing its ability to cause damage by its scavenging property, while free radicals are unstable molecules that are highly reactive and contain an unpaired electron in their atomic orbital, able to accept or donate an electron”(Aprioku, 2013). The most important free radicals containing oxygen are hydrogen-peroxide, radical superoxide-anion, hydroxyl radical, single oxygen, hypochlorite, radical nitric oxide, and radical peroxynitrite, these can initiate a chain of cell damage by affecting lipids, nucleic acids, and proteins (Lobo et al., 2010).

Uric acid acts as an effective scavenger of free radicals and a chelator of metal ions, it is known to stabilize vitamin C in serum by its chelating property and have peroxy-nitrite neutralizing activity (Vukovic et al., 2009). In addition, it reduces the damage caused by reactive oxygen species, so it has an important role under both normal physiological conditions and oxidative stress (Bobulescu and Moe, 2012).

On the other hand, increasing uric acid levels is known to be a prognostic factor for renal disease, diabetes mellitus, cardiovascular disease, and inflammation (Batta et al., 2016).

2.3.1. Uric acid production

Uric acid is produced mostly by the liver as a result of the metabolic route of the purines nucleotides, a component of nucleic acids (Maiuolo et al., 2016). Uric acid levels influenced by endogenous and exogenous purines content, endogenous purines are derived from de novo purine nucleotide synthesis, salvage synthesis of purine nucleotides, and nucleic acid breakdown, while exogenous purines are obtained mainly from the diet (El Ridi and Tallima, 2017).

De novo purines synthesis consists of ten highly conserved steps starting with phospho-ribosyl-pyro-phosphate (PRPP) obtained from pentose phosphate pathway with adenosine triphosphate (ATP) and ending with inosine monophosphate (IMP), is mediated by 6 different enzymes, required five molecules of ATP and is regulated by feedback inhibition (Pedley and Benkovic, 2017). The produced IMP is then used to generate adenosine monophosphate (AMP) and guanine monophosphate (GMP) that are the main intermediates in this pathway, These are either used for the inclusion in nucleic acids or the formation of uric acid into xanthine (Jiménez and Puig, 2012; Yin et al., 2018).

For uric acid production, deaminase enzyme is used to convert AMP to IMP which is then dephosphorylated by nucleosidase to form inosine, and nucleotidase enzyme that catalyzes the conversion of GMP to guanosine, inosine is then converted into hypoxanthine while guanosine is converted into guanine by the action of purine nucleoside phosphorylase (Jin et al., 2012). Next through oxidation of hypoxanthine through xanthine oxidase and deamination of guanine by guanine deaminase, both hypoxanthine and guanine are converted into xanthin. In the end, xanthine oxidase is reduced to form uric-acid (Mandal and Mount, 2015; Maiuolo et al., 2016). (Figure.1-2)

The *de novo* biosynthetic pathway is essential to refill the purine demand in dividing cells, while under normal physiological conditions most of the cellular purines are derived from the recycling of degraded bases using the salvage pathway. During the salvage pathway, hypoxanthine and guanine can be reutilized via the enzyme hypoxanthine-guanine phosphoribosyltransferase (HGPT) to produce IMP and GMP, respectively, and then used in nucleic acid production, this will decrease the precursors for uric acid production (Pedley and Benkovic, 2017).

Increased cell breakdown (e.g., hemolysis, tumor growth, and large tumor necrosis) leads to increased adenosine, inosine, and guanosine levels (Gustafsson and Unwin, 2013). In addition, ATP breakdown also leads to ADP and AMP production, which can result in increased urate levels(Moffatt and Ashihara, 2002; Pasalic et al., 2012).

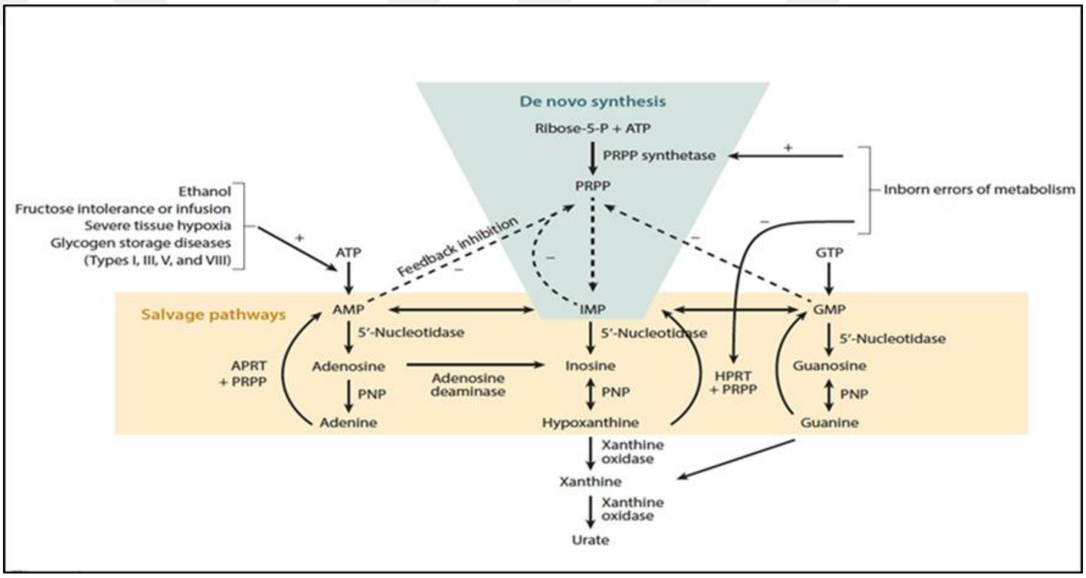

Figure 2.3. Urate production pathways (Mandal and Mount, 2015).

2.3.2. Uric acid metabolism

The metabolism of uric-acide is a complex process regulated by many factors that act on its production, which accomplished mainly by the liver and on its excretion by kidney and intestine (Chaudhary et al., 2013) . (Figure 2-4).

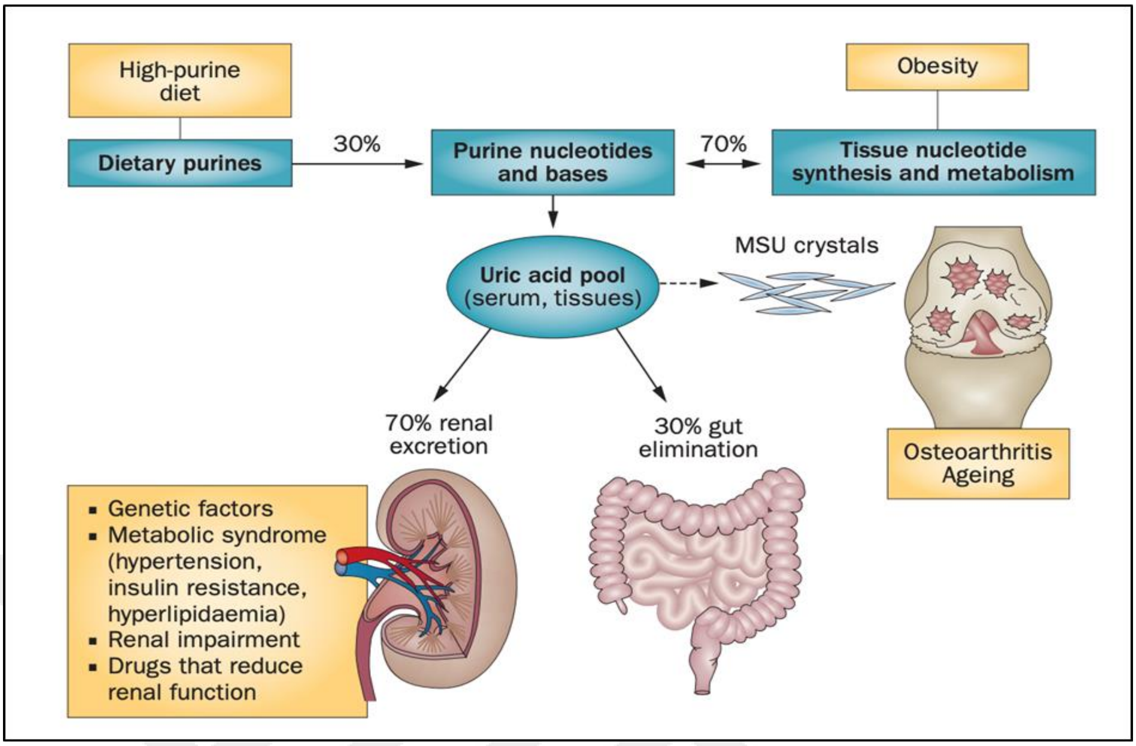

Figure 2.4. Pattern-recognition receptors (Rees et al., 2014).

2.3.3. Uric acid elimination

The kidney has an important function in controlling of urate levels in the body as it is responsible for 70% of urate elimination, while the remaining part is secreted into the intestine, where it is further metabolized by a gut bacteria in a process known as urinalysis (Bobulescu and Moe, 2012; Li et al., 2018).

Renal handling of urate involves four components system that includes glomerular filtration, secretion, reabsorption, and post secretory reabsorption (Kutzing and Firestein, 2008; Sakurai, 2013) (Figure. 1-4). These processes are not necessarily linear and the role of reabsorption appears greater than secretion (Jalal, 2016). Less than 5% of circulating urate is bound to protein, while the remaining is not bound and easily filtered through the glomerulus into the system of renal tubules (proximal tubules, a loop of Henle and distal tubules)(Martillo et al., 2014). Under normal conditions, almost all the filtered urate is reabsorbed by tubules back to blood while only 5% to 10% is excreted in urine(de Oliveira and Burini, 2012; Gustafsson and Unwin, 2013; Chittoor et al., 2017). In general, Reabsorption occurs in the proximal-tubule segment (S1), secretion at the (S2) segment of the proximal tubule, and Post-secretory reabsorption occurs at the (S3) proximal tubule of segment (Anzai and Enduo,2012).

Molecules known as transporters are needed to permit the import/export of uric acid through the epithelium of renal proximal convoluted tubules (Hyndman et al., 2016; Pérez, 2018). Several transporters such as URAT1 and GLUT9 have been identified in recent years, these are known to play an important roles in uric acid transport across renal tubules (Bobulescu and Moe, 2012).Due to the absence of uricase and the presence of efficient reabsorption of urate throughout renal tubules, urate levels in humans are generally higher than those in other mammals (Anzai et al., 2007).

It has been discovered that defects in urate transporters can result in hyperuricemia which is considered as a prognosis factor for gout, hypertension, and cardiovascular diseases. The mechanism that describes renal urate handling is complex due to bidirectional transport through renal tubules and the species differences (Rafey, 2003).

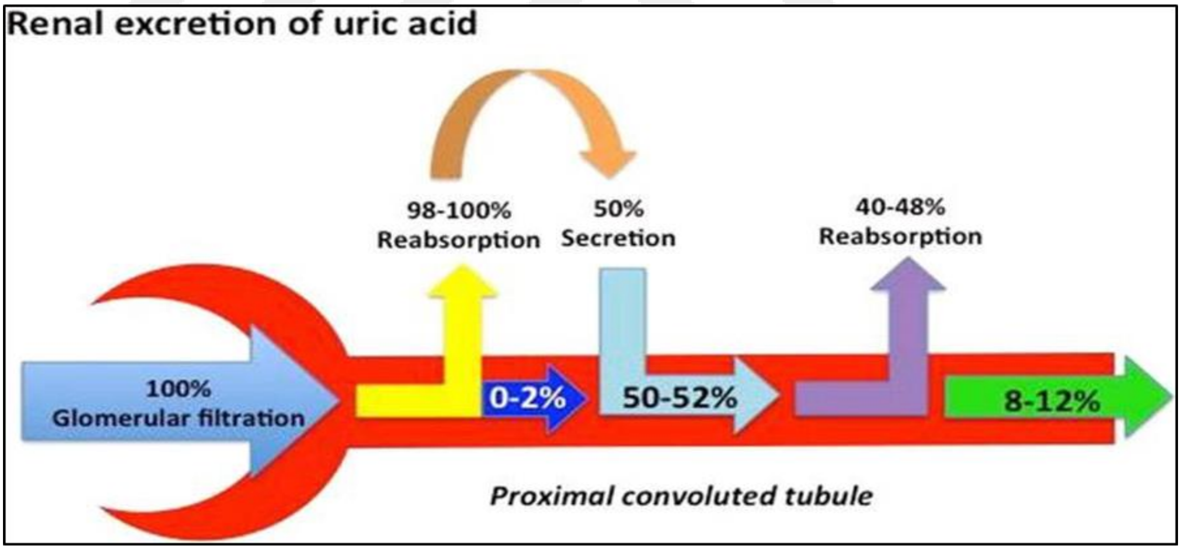

Figure 2.5. Renal excretion of uric acid. (Ragab et al., 2017).

2.4. Gout Risk Factors

2.4.1. Age and gender

Gout rarely occurs in children because of the low uric acid level 3-4 mg/dl and high elimination of uric acid. In adults, urate level in men is higher than in women, it increases in men at puberty and the uricosuric effect of estrogen leads to decrease urate level in pre-menopause women (Chisnall, 2014; MacFarlane and Kim, 2014). As a result, the prevalence of gout among women is significantly lower than among men (Zhu et al., 2011).

Gout incidence is increased in the elderly as the urate levels rise due to decreased efficiency of the kidney, in addition to the use of some medications and the presence of other diseases that predispose to gout (Doherty, 2009).

2.4.2. Consumption of alcohol

Consumption of alcohol is considered as a risk factor for gout as it affects uric acid production and excretion. Alcohol intake increases lactate production that induces ketoacidosis during which keto acids compete with uric acid excretion (Neogi et al., 2014). Also, keto acids enhance the activation of the URAT1 anion exchange function. Thus, increasing uric acid reabsorption and decreasing its excretion (Zhang et al., 2018). Ingestion of alcohol increases urate production via accelerating the degradation of ATP to AMP during the convention of acetate into acetyl coenzyme A (Acetyl-CoA) during the metabolism of ethanol (Choi, 2012). In addition, alcohol affects Allopurinol activity (a drug used for gout treatment) by inhibiting the conversion of pro-drug allopurinol to its active metabolite oxypurinol (Fam, 2002; Choi, 2012).

It has been found that the risk of gout differs according to the type of alcohol beverage. Among alcoholic beverages, beer has the greatest risk as it is rich with the purine guanosine (Burns and Wortmann, 2013).

2.4.3. Obesity

The definition of obesity as a body-mass index (BMI) of greater than (30 kg/m2). It is considered a public health problem that increases with higher consumption of meat, seafood, and fat, combined with inactivity (Lee et al., 2006; Juraschek et al., 2013).

Obesity is known to associate with insulin resistance in which cells become less sensitive to a hormone secreted by pancreatic β-cells known as insulin. This hormone stimulates glucose uptake by cells thus regulate glucose level in the blood (Li et al., 2013). It has been found that Inflammatory mediators found in adipose tissue can reduce insulin signaling and thus decreasing glucose uptake (Gregor and Hotamisligil, 2011).

It has been found that obesity and high body fats are associated with gout (McAdams DeMarco et al., 2011). This is possibly due to obesity-associated insulin resistance (Chisnall, 2014). Uric acid increases in people with insulin resistance as insulin enhance urate transporter activity in renal proximal tubules, thus increasing urate reabsorption and decreasing excretion (Kushiyama et al., 2014). Also, it was suggested that there is an association between hepatic fatty acid synthesis and de novo synthesis of purines, thus the production of uric acid increased (de Oliveira and Burini, 2012).

Several have studies suggested that weight gain over time is associated with increasing serum urate levels and the opposite effect observed with weight loss as urate level decreased over time (Tanaka et al., 2015; Ali et al., 2018; Grygiel-Górniak et al., 2018).

2.4.4. Nature of the diet followed

Purine-rich foods such as seafood, red meat along with high fructose consumption are known to associate with the risk of gout, while purine-rich vegetable intake such as peas, lentils, beans, spinach, mushrooms, and cauliflower has no association (Zhang et al., 2012; Roddy and Choi, 2014).

Increased daily consumption of meat and seafood is associated with a significantly elevated risk of incident gout as meat contains high purine content as well as animal meat contains saturated fats that are associated with insulin resistance, these raise serum urate levels and decrease renal excretion (Teng et al., 2015; Liu et al., 2018).

It has been found that high consumption of fructose, sweetened beverages and fruit juices were associated with an increased risk of gout (Kakutani-Hatayama et al., 2017). This is because fructose is known as the only carbohydrate that influences uric acid levels as its phosphorylation requires ATP which lead to depleting phosphate, resulting in activation of AMP deaminase that converts AMP to inosine monophosphate and subsequent formation of uric acid by hypoxanthine xanthine pathway (Jamnik et al., 2016). Furthermore, fructose is associated with insulin resistance and obesity that are closely related to the risk of gout (Kakutani-Hatayama et al., 2017; Beydoun et al., 2018).

2.4.5. Hypertension

It has been found that the risk of gout is increased by the effect of hypertension (Singh et al., 2011; Roddy and Choi, 2014; McAdams DeMarco et al., 2012), possibly because hypertension can cause poor kidney function, also hypertension is considered as a part of metabolic syndrome which has a significant association with gout(Sun et al., 2018). Besides, the use of some antihypertensive drugs could be associated with the risk of gout (Choi, 2012; McAdams DeMarco et al., 2012).

The association between gout and hypertension is bi-directional as some authors reported that uric acid is responsible for elevated blood pressure by inhibiting bioavailability of nitric- oxide (NO), a vasodilator, and others noted that hypertension is a risk factor for hyperuricemia (Choi, 2012; Bardin and Richette, 2017).

2.4.6. Diabetes disease

The association between gout and diabetes is complex as patients with diabetes have a lower risk of developing gout (Rodriguez, Soriano, and Choi 2010; Pan et al., 2016), while gout is also a risk factors for the developing (diabetes type2) (Li et al., 2013; Pan et al., 2016; Bardin and Richette, 2017; Rho et al., 2016).

Some studies suggested that the association of type 2 diabetes with gout is duration-dependent and depends on type 2 diabetes-associated comorbidities such as high BMI hypertension and decreased kidney efficiency, while type 1 diabetes showed a lower risk of gout (Rodriguez, Soriano, and Choi 2010; Wijnands et al., 2015; Pan et al., 2016). Others reported that when blood glucose increased above 10 mmol/L, a case known as glycosuria is associated with low uric acid levels in individuals with diabetes thus have a low risk of gout(Rodriguez, Soriano, and Choi 2010). In addition, diabetes results in impaired inflammatory response by affecting inflammatory mediators and mast cell degranulation and immune cell adhesion and other processes that reduce the inflammatory response to urate crystals and they play a defensive role against the possibility of gout (Pan et al., 2016).

It was found that the risk of gout may increase in individuals with diabetes type 2 that is possibly due to the presence comorbidities that act as risk factors for gout and caused mainly by diabetes (Wijnands et al., 2015).

2.4.7. Consumption of a drugs

The use of some medications is known to be a risk factors for gout as they increase serum urate levels through affecting urate transporters such as URAT1 and GLUT9, thus reducing renal excretion of uric acid(Kuo et al., 2015).

The diuretics are considered as the most common drugs that associate with the risk of gout (Singh et al., 2011; Bruderer et al., 2014; Krishnan, 2012).

Other Medications, including low-dose aspirin and drugs used for organ transplantation, are known to increase the developing risk of gout (Zhang et al., 2014). However, the risk of gout is dose-dependent in the case of aspirin as it has uricosuric when taken at high doses (>3g/day). Besides, it can reduce uric acid renal excretion at low doses (1-2g/day)(Singh et al., 2011; Ragab et al., 2017; Ben Salem et al., 2017).

Also, using some antihypertensive drugs results in rising serum urate levels such as b-Blockers (Choi, 2012; Kuo et al., 2015). Furthermore, the use of cytotoxic drugs in the treatment of certain types of cancer that result in cell destruction and subsequent release of uric acid can cause gouty arthritis (Kopke and Greeff, 2014).

2.4.8. The genetics effect

Urate levels vary among individuals and that attributes to genetic and environmental factors, about 60% of this phenotypic variation is caused by heritable factors (Richette and Bardin, 2010). Many studies showed that serum urate level is genetically determined (Meng et al., 2015). Several genes have been identified to have an important roles in the development of gout, such as genes encoding for enzymes involved in the purine metabolism or for urate transporters protein (Kolz et al., 2009).

Monogenic disorders caused by a mutation in a single gene are thought to be associated with hyperuricemia and gout. For example, hypoxanthine-guanine phosphoribosyltransferase (HGPRT) deficiency also known as Lesch– Nyhan syndrome, and the (5-phosphoribosyl-1-pyrophosphate synthetase) (PRPS1) super-activity are rare x-linked diseases that are associated with changes in enzymes that play an important role in purine metabolism leading to hyperuricemia as (HGPRT) catalyzes the formation of inosine monophosphate (IMP) and guanosine monophosphate (GMP) during the salvage pathway, while PRPS1 is used in *de novo* purine synthesis and thus increasing uric acid levels (Jin et al., 2012; Kuo et al., 2015). Also, variation in genes encoded for urate transporters have a great effect on urate levels (Choi, 2010), such transporters include URAT1, ABCG2, and SLC2A9 (Hollis-Moffatt et al., 2009; Phipps-Green et al., 2010; Urano et al., 2010).

Genetic studies had given more and more knowledge about the pathophysiology of hyperuricemia and gout (Choi, 2010). Even variants with weak effects are important as they could be used as a drug target in the treatment of diseases (Chisnall, 2014).

2.5. Urate Transporters

2.5.1. ABCG2 transport

ATP-binding cassette, sub-family G, member 2 (ABCG2) is a member of ABC transporter family that is found in all organisms (Dassa and Bouige, 2001), it is known to be involved in the transportation of a variety of substances through the cell membrane (Dawson and Locher, 2006).

ABCG2 is expressed in the brush membranes of renal proximal tubules cells, the apical membrane of the intestinal epithelium and liver hepatocytes where it is involved in urate secretion (Hosomi et al., 2012). Polymorphism in the ABCG2 gene located on chromosome 4 is found to associate with raised urate level as it reduces renal urate secretion (Woodward et al., 2009; Zu Schwabedissen and Kroemer, 2011)

The loss or reduction in ABCG2 activity in renal tubules will provide a raise in renal urate reabsorption and a reduction in urate renal excretion which is considered to be the underlying hyperuricemic mechanism in the majority of gout patients (Mandal and Mount, 2015).

2.5.2. URAT1 transport

Urate transporter 1, solute carrier family 22, member 12 (SLC22A12) is a protein that is one portion of the Organic Anion Transporter (OAT) family. It is known that URAT1 regulates urate levels in humans (Riches and P.L, 2012). By achieving non-voltage dependent interchange of urate from glomerular filtrate with organic anions in the lumen across the apical membrane of proximal renal tubules, URAT1 is expressed on the apical membranes of the renal tubular cells (Taniguchi and Kamatani, 2008; Shin et al., 2011).

A polymorphism of this gene located on chromosome 11q13 (Enomoto et al., 2002) has been associated with impaired excretion of uric acid and hyperuricemia in German Caucasians (Thuy Duong et al., 2019), while in Japanese URAT1 mutation was shown to protect from the development of hyperuricemia and gout(Vazquez-Mellado et al., 2007).

2.5.3. Glut9 transport

Glucose transporter 9, solute carrier family 2 member 9 (SLC2A9), a member of the hexose transporters GLUT family proteins, was initially identified as fructose and glucose transporter protein, and then studies showed that it is a voltage-dependent urate transporter (Mueckler and Thorens, 2013). GLUT9 is known as high affinity-low capacity glucose/fructose transporter that has structural similarities to the other facultative glucose transporter (GLUT) family members for instance 12 transmembrane helices, amino-cytoplasm, carboxy termini and an N-linked glycosylation site (Clémençon et al., 2014).

It has been reported that this protein handles the reabsorption of urate at renal tubule epithelial cell back into circulation in a voltage-dependent manner, it also has a significant part in urate homeostasis in the liver (Clémençon et al., 2014), GLUT9 is considered as electrogenic transporter as its activation is driven primarily by the electrochemical gradient of the cell (Xu et al., 2017; Benn et al., 2018).

In humans SLC2A9 (solute carrier family 2 member 9) gene that is located at 4p16.1 region in chromosome 4, encodes the glut9 protein, and due to the alternative splicing of the gene triggered by transcriptional regulation of two different upstream promoters of the gene, two splice variants are produced known as the short and the long isoforms, the RNA of the short variant (GLUT9S) is 215kb long, consisting of 13 exons and encoding for 512 amino acid residues protein, while the longer splice variant is about 195kb, consisting of 12 exons and encoding for a 540-amino acid residue protein, these variants differ in the splicing of the N-terminus and both expressed in kidney and placenta, with the long isoform, that is also expressed in the liver and leukocytes (Hurba et al., 2014; Kimura et al., 2014; Szablewski, 2017). In the kidney, the long isoform of SLC2A9 is expressed in the basolateral membrane (blood side) of the epithelial cells of the renal proximal tubules in humans in which N-terminal amino acid sequence of this isoform involves in the basolateral sorting of the protein while the short isoform is expressed in the apical membrane (urine side) of the collecting tubule (Kimura et al., 2014; Xu et al., 2017).

It has been reported that about 3% of the uric acid level variation could be caused by a polymorphism in the glut9 gene (Dalbeth et al., 2017). Loss-function-mutations of this gene are found to associate with hypouricemia by decreasing urate reabsorption on the renal tubules (Zhu et al., 2018). Many genetic variants within this gene have been reported to associate with hyperuricemia and gout susceptibility (Guan et al., 2011).

2.6. Single Nucleotide Polymorphism (SNP)

The change in the DNA sequence that is common among population, groups, or individuals with more than 1% frequency is known as genetic polymorphism (Su et al., 2015). The most common form of this variation called single nucleotide polymorphism (SNP) in which one nucleotide: adenine (A), thymine (T), cytosine (C) or guanine (G) is replaced by another, the alteration is bi-allelic where each allele could present in 1 of 2 alternatives throughout the genome (Sripichai and Fucharoen, 2007).

In human, several millions of SNPs have been identified, SNPs are very abundant throughout the genome, they tend to become more frequent in non-coding regions than in coding regions, it occurs in the coding region, thus may either be synonymously leading to no change in protein product or be a non-synonymous that alter the amino acid which results in codon change (missense) or changes the termination codon (nonsense), SNPs also may occur in non-coding regions and are useful in evolutionary studies (Teama, 2018). Thus, many SNPs could lead to diseases as they change the function or structure of the encoded protein and these SNPs are used in diagnostic purposes (Popa et al., 2009). Furthermore, they could potentially serve as drug targets for the treatment of diseases as they affect drug metabolism and or delivery. In addition, SNPs may affect the risk of common disease and predispose people to many diseases (Goto et al., 2001; Syvänen, 2001; Shastry, 2009; Ismail and Essawi, 2012; Adusumalli et al., 2019). Sripichai and Fucharoen, 2007

SNPs raised by nucleotide substitution may be a transition that involves the replacement of one purine base by another or pyrimidine base by another, they may also result from a transversion in which the substitution occurs between a purine and a pyrimidine occurs (Weber et al., 2002).

2.7. Treatment

The main goals in gout management are:

- Reducing pain.
- Resolving joint inflammation.
- Achieving SUA <6mg/dL.

- Reducing the chance of other acute attacks.

The choice of drugs depending on patient comorbidities and the stage of the Gout (Khanna et al. 2012; Engel et al. 2017).For the relief of pain and inflammation reduction, non-steroidal anti-inflammatory drugs (NSAIDs), colchicine, or corticosteroids are commonly used, where NSAIDs inhibit cyclooxygenase isoenzymes (COX-1) and (COX-2), thus blocking prostaglandin G2 production, a compound that mediates inflammatory response (Brune and Patrignani, 2015). Colchicine is known to have an anti-proliferative effect as it interferes with microtubule and causes cell growth arrest. So it interferes with leukocyte function, preventing lysosomal degranulation and chemotaxis(Dalbeth et al., 2014). Also, it inhibits crystal-induced NLRP3 inflammasome assembly and thus prevents IL-1β production (Burns and Wortmann, 2012). In the case of acute gout limited to single-joint the application of corticosteroids is effective as it is known to inhibit (pro inflammatory-Cytokines) for example Interleukin1(IL-1) and tumor necrosis factor (TNF-α) and this involved in reducing inflammation by different roles, it is also used in patients with resistance to both of NSAID and colchicine (Schlesinger, 2017). After treatment of acute gout, urate-lowering drugs (ULT) are used to prevent another attack and decrease urate levels so that dissolving of urate crystals can occur, three choices for ULT are available: xanthine-oxidase inhibitors (allopurinol, oxypurinol, and febuxostat) which reduce uric acid production by inhibiting the enzyme that catalyzes the oxidation of the (hypoxanthine to xanthine) and (xanthine to uric acid) (Benn et al., 2018). Uricosuric drugs (probenecid, sulfinpyrazone, and benzbromarone) that increase uric acid excretion by the selective inhibition of renal tubular urate transporters such as GLUT9 and URAT1 (Benn et al., 2018). Uricases are also used to enhance systemic-hydrolysis of uric acid into allantoin that can easily be cleared by the kidney into the urine (Sattui and Gaffo, 2016; Schlesinger, 2017).

2.8. Oxidative Stress

Cells undergo oxidative stress because of one of three causes: the first is an increase in the age of oxidants, the second is a decrease in the production of antioxidants and the third is an inability to restore oxidant harm. The damage of the cell is acted upon Reactive Oxygen Species (ROS). The reactive oxygen species source in vivo is oxygen absorbing air, given the fact that the fatty acids (ROS) are also formed by “peroxisomal β-oxidation”, (microsomal-cytochromeP450)“digestion of xenobiotic compounds, incitement of the phagocytosis by pathogens or lipopolysaccharides, arginine-digestion, and a tissue-specific enzyme” (Fies et al., 1999: Schafer & Buettner, 2001). The action of superoxide dismutase (SOD), catalase CAT, or glutathione-peroxidase (GSH) (Hayes and McLELLAN, 1999) cleanses reactive oxygen species (ROS) from the cells under the ordinary state. The primary damage to cells arising from the reactive oxygen species caused macromolecules to shift, for example, Membrane lipid polyunsaturated unsaturated fats, essential protein, and DNA. In fact, oxidative pressure and reactive oxygen species have also been found in disease states such as inflammation (Rheumatoid Arthritis RA) (Hitchon CA, El-Gabalawy HS) Alzheimer's disease (Christen, 2000) and the pathologies brought about by diabetes.

2.8.1. The accumulation of oxygen-derived free radicals (oxidativestress)

Cell damage caused by free radicals, in particular (receptive oxygen species ROS), is an insignificant portion of cells hurt under various circumstances Pathological, such as compound and radiation injury, ischemia-reperfusion injury (prompted by the rebuilding of the bloodstream in ischemic tissue), cell maturing, and microbial slaughtering by the phagocytes. Free radicals are synthetic species in an external system with a single unpaired electron. Vitality made by this unsteady design is discharged through responses with neighboring particles, for example, inorganic or natural concoction – proteins, lipids, sugar, nucleic acids a considerable lot of what are the cell layer and center parts. Free radicals also begin autocatalytic responses, whereby atoms in which they react are themselves converted into free-radicals and then disperse the chain of damage. Reactive oxygen species (ROS) is a kind of oxygen-determined free extreme whose role is settled in cell injury (reactive oxygen species ROS). They are frequently produced in the cells during the mitochondrial breath and period of regeneration, but they are debased and removed by cell-safe frameworks. Cells should then maintain a stable state in which free radicals should briefly add at low fixations but do not cause damage. Where (Reactive Oxygen Species ROS) is formed increments or the searching frameworks are incapable, the effect is an excess of such free radicals, which causes conditions called oxidation stress. Oxidative stress became caught up in a wide variety of pathological treatments, including cell death, malignancy, maturation, and certain degenerative disorders, such as Alzheimer's disease. (Reactive oxygen species ROS) are likewise created in enormous sums by leukocytes, especially neutrophils and macrophage, as middle people for devastating organisms, dead tissue, and other undesirable substances. Thusly, injury brought about by these responsive mixes regularly goes with fiery responses, during which leukocytes are enlisted and enacted (Doherty et al., 2006). We are addressing the age and evacuation of (reactive oxygen species ROS) in the following article, and how they contribute to cell injury. The attributes of the most important absolute free radicals.

Free radicals in cells may be generated in a few different ways. The diminishing oxidation reaction that occurs during conventional metabolic procedures. sub-atomic O_2 is reduced by the conversion of (four-electrons) to H_2 to produce two water particles during a normal intake. This transformation is catalyzed by the oxidative compounds ER, cytosol, mitochondria, peroxisomes, and lysosomes small amounts of quite decreased intermediates are formed in this process in which distinctive numbers of electrons are transferred from (O_2), which include superoxide anion (O_2, one electron), hydrogen peroxide (H_2O_2, two electrons) and hydroxyl particulate matter (OH, electrons).

Brilliant energy conservation (e.g., bright light, x-ray); For example, ionizing-radiation may hydrolyze water into free radicals (OH) and hydrogen (H). During aggravation, rapid explosions of reactive oxygen species (ROS) are supplied in actuated leukocytes. This happens through an unambiguously regulated response in the multiprotein complex of the plasma layer which uses NADPH oxidase for redox response. In addition, other intracellular oxidase produce O_2.

The enzymatic metabolism of exogenous synthetics or drugs can generate free radicals that are not reactive oxygen species (ROS) but have comparable impacts (e.g., CCL_4 can produce CCL_3.Transition metals, for example, contribute iron and copper or recognize free electrons during intracellular responses and catalyze free extreme development, as in the Fenton response)($\mathrm{H_2O_2 + Fe_2^+ \rightarrow Fe_3^+ + OH + OH^-}$). Since the vast majority of the free intracellular sign is in the state of ferric (Fe_3^+), interest in the Fenton response must be diminished to the ferrous (Fe_2^+) structure. O_2 can improve this decline, and consequently Iron and O_2 wells can manage oxidative cell damage.

Nitric oxide (NO), a major chemical mediator produced by endothelial cells, macrophages, neurons and other types of cells, can function as a free-radical agent and can also be converted into extremely reactive peroxynitrite-anion ($ONOO^-$) and NO_2 and NO_3.

Free-radicals are inalienably unstable and unexpectedly turn high and wide. It is, for example, unstable and unexpectedly rots (dismutase) into O_2 and H_2O_2 in the sight of light. Numerous non-enzymatic and enzymatic devices have also been developed by cells to expel free radicals and thus restrict damage. Antioxidants can block free radicals from starting out. Sources are Vitamins A and E soluble in lipids in the cytosol, as well as ascorbic acid and glutathione.

As we have seen, iron and copper can catalyze the formation of reactive oxygen species (ROS). These reactive metal levels are reduced by binding ions to store and transport proteins (e.g., transferrin, ferritin, lactoferrin, and ceruloplasmine), thus reducing the formation of reactive oxygen species (ROS).

A variety of the enzymes function as free-radical scavenging mechanisms, breaking down H_2O_2 and O_2. These proteins, and they include:

1) Catalase used in peroxisomes breaks down H_2O_2. ($2H_2O_2 \rightarrow O_2 + 2H_2O$).
2) Superoxide-dismutases (SOD) exist in many types of cells and transform O_2 to H_2O_2 ($2O_2 + 2H \rightarrow H_2O_2 + O_2$). This group contains both manganese SOD present in the mitochondria and copper, zinc-SOD found in cytosol.

3) Glutathione peroxidase also defends against injury by free radical catalysis breakdown ($H_2O_2 + 2GSSG_{(glutathion\ homodimer)} + H_2O$) or ($2OH + 2GSSG + 2H_2O$). The intracellular ratio of oxidized glutathione (GSSG) to reduced glutathione (GSH) is an important indicator of the cell's detoxification capacity.

Trigger Pathological Free radicals, ROS and other free-radicals have wide-ranging consequences but cell damage is especially important in many respects to reactions. Membranes of peroxidated lipids O_2 free radicals can induce lipid peroxidation in the plasma and organelayers. Oxidative harm is initiated when O_2 inferred free radicals, especially OH, invade the dual bodies of unsaturated lipid fat layers.

The lipids release radical bonds, create peroxides which are unstable and reactive themselves, followed by an autocatalytic chain reaction (called propagation), which can cause widespread damage to the membrane. The proteins are modified oxidatively. Free radicals promote amino-acid side chains oxidation, protein-including protein crosslinks (e.g. Disulfide bonds), and protein spine oxidation. Oxidative protein modification can affect the complex positions of chemicals, disrupt the affirmation of auxiliary protein, and update proteasomal manipulation of unfurled or folded-proteins, triggering a cell-wide uproar.

Free radicals can induce breaks in single- and double-strand DNA, cross-linking of DNA strands and adduct formation. The damage of oxidative DNA has been involved in cell ageing and cell malignancies.

The standard view of free-radicals was that they induced necrosis cell damage and death, and in reality, producing reactive oxygen species (ROS) is a continuous preface to putrefaction. It presents some such free radicals may also induce apoptosis, in either case. An variety of cell receptors and biochemical intermediates have also discovered a role of ROS in motioning. To say the facts, as one theory reveals, the significant activities stem from its ability to improve the development of harmful enzymes rather than direct macromol O_2 ecular damage. Such theoretically lethal atoms represent considerable physiological functions (Jansen et al., 2011).

Malondialdehyde (MDA) is a normally happening result of lipid peroxidations, it is an exceptionally responsive three-carbon dialdehyde item as a side-effect of polyunsaturated fat peroxidation and furthermore during arachidonic-acid digestion for the combination of prostaglandin (Marnett, 1999). Malondialdehyde (MDA) can be formed in human platelets during the catalysis of cyclooxygenase (COX), arising from thromboxane synthase catalyzed prostaglandin-endoperoxide (PGH2), and independently into the liver cell (PGH2) (Sundy et al., 2008).

2.8.2. The risks of oxidative stress

Oxidative stress is a dangerous phenomenon resulting from free radicals and oxidants. ROS is working on the alteration of the cell membranes and other structures such as deoxyribonucleic acid (DNA), lipids, lipoproteins, proteins (Young and Woodside, 2001; Droge, 2002).

ROS at normal conditions is maintained by different enzyme systems and is a normal fact in the body. Where it is sharing these systems at low levels contribute to the vivo balance between oxidation and reduction. Therefore, oxidative stress is also known as a disorder between the antioxidants and prooxidants in the body (Rahal et al., 2014). And as oxidative stress occur because of an imbalance between the formation of reactive oxygen species (ROS), reactive nitrogen species (RNS), and the capacity of the vital system to dispose of these reactive mediums. Dangerous substances and compounds arise as a result of biochemical modifications by ROS and RNS, like lipid peroxidation, which action on oxidizing proteins, lipids, and DNA damage (Kukongviriyapan et al., 2016).

Oxidative stress is also an ongoing process in vivo body resulting from normal cellular procedures. As was mentioned before, there is a balance status in biological processes between ROS and antioxidant production (Rahal et al., 2014). This balance can be lost due to some causes but, and oxidative stress production can surpass normal physiological levels, which in the role can cause cellular damage (Saral et al., 2005).

Oxidative stress can occur on short term result trauma, certain toxins, Infections, overworking, all of that cause to light injuries to different tissues. Tissues forme several enzymes that contribute to radical production like xanthine oxidase, lipogenase, cyclooxygenase, when occurring damage. They work stimulation phagocytes, lead more iron and copper ions to be liberated into circulation, and disable electron transport series. When these effects continue for a long interval of time, the ROS/antioxidant balance can be lost, that in the role can cause to develop different kinds of cancer, or lead present cancer to prevalence readily. Moreover, some studies were shown a relationship between ROS and diabetes mellitus and various age-associated diseases as such the Parkinson’s disease (Rao et al., 2006). ROS also causes chronic fatigue, atherosclerosis, Alzheimer’s, malaria disease (Figure 2.6) (Islam and Shekhar, 2015).

The most important endogenous reasons that cause increased reactive oxygen species (ROS) production include (Elevation of concentration, inflammation and increased mitochondrial leakage), and exogenous (active exercise, diet, social, environmental contamination from smoking, chronic inflammation, psychological and emotional stress, among others). The causes of decreased antioxidant defenses include decreased endogenous antioxidant enzymes and reduced intake or consumption of antioxidants from food (Poljsak et al., 2013).

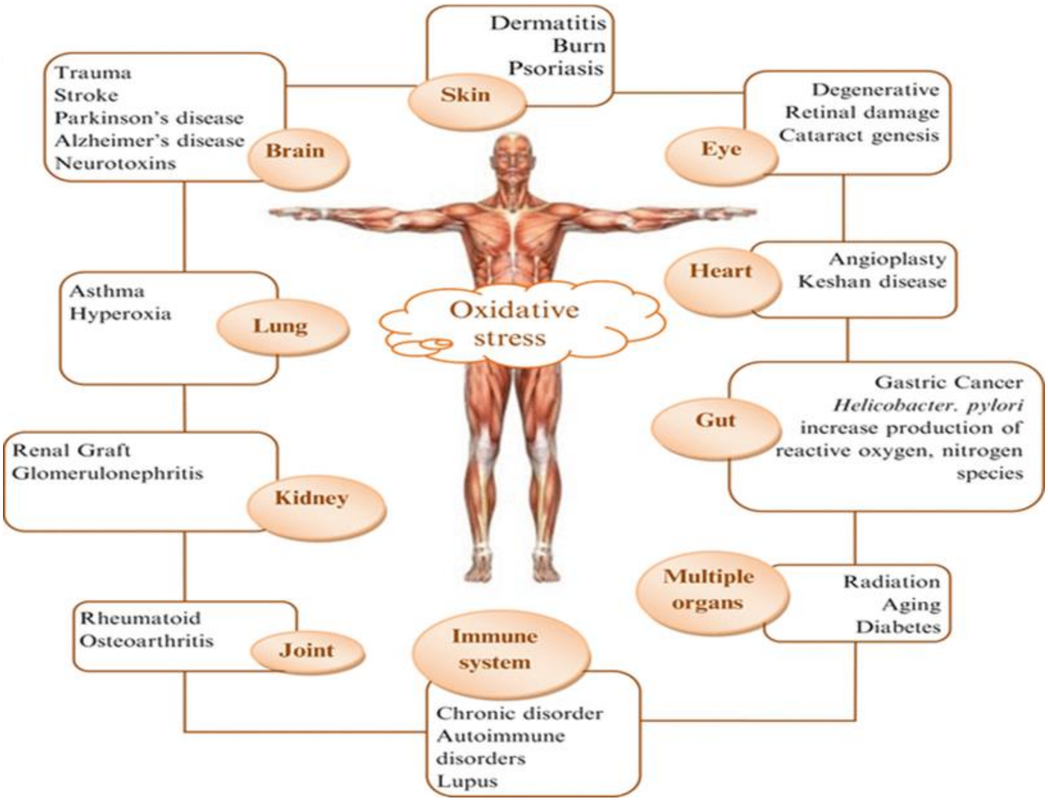

Figure 2.6. Oxidative stress on the human health (Islam and Shekhar, 2015).

2.8.2.1. Nucleic acid (DNA) damage

Oxidative DNA lesions are formed due to constant oxidative damage to DNA, causing major problems and mutations. The body has lots of methods to prevent these damages by using antioxidants or DNA repair enzymes (Willcox et al., 2004; Halliwell, 2007). If not functioned properly and provided sufficient quantities, oxidative stress can attack DNA and causes some of chronic and degenerative disorders, ageing and certain acute conditions (trauma, stroke, and cancer). Reactive oxygen-species (ROS) are formed through a variety of events and pathways. Some reports suggest that every human cell can is exposed to for nearly 1.5×105 oxidative infections daily from hydroxyl-radicals and the other reactive-species (Beckman and Ames, 1997). Hydroxyl radicals can attack to components DNA and react with it, causing damage both the pyrimidine and purine bases and also the deoxyribose-backbone (Dizdaroglu et al., 2002).

ROS-induced DNA damage involves single or double stranded DNA breaks, purine, pyrimidine, or deoxyribose modifications, and DNA cross-links. DNA harm can lead either in curb or agitation of transcription, induction of signal transduction course, genomic instability, and replication troubles, all of which are concerned with carcinogenesis (Marnett, 2000; Cooke et al., 2003). The progress of cancer in the humans is a complicated procedure including cellular and molecular changes mediated by variety endogenous and exogenous catalysts. It is well discerned that oxidative DNA harm is answerable for cancer development (Valko et al., 2004; Valko et al., 2007). Chromosomal defects and oncogene activation is the beginning of carcinogenesis, and the reason for this is the free radicals. The formation of hydroxylated based is considered from the important cases and common that infection DNA, and be an indication of chemical carcinogenesis (Valko et al., 2004; Halliwell, 2007).

Genetic mutations and altering normal gene transcription becomes because of adduct formation with normal cell evolution. Base and sugar lesions, strand breaks, DNA protein cross links, and base free sites are the most modifications and changes that occur on the DNA structure. For example, smoking and chronic infection resulting from noninfectious injuries like asbestos are reasons for oxidative DNA damage that can cause to the development of the lung cancer and other tumors (Valko et al., 2004; Willcox et al., 2004).

2.8.2.2. The lipid peroxidation

Lipid peroxidation is considered one of the dangerous conditions that damage cells, cause differences in them and pathological states in adults and babies. It occurs naturally in the body and small amounts because of the influence that it plays (reactive oxygen-species, hydroxyl radical, hydrogen peroxide, etc). It can be produced via the action of phagocytes (Mylonas and Kouretas, 1999). The elimination of the end-compounds of such lipid peroxidation reactions and membrane lipids causes significant damage to viability cells and even tissues.

The great damage that happens to the tissues, results from self-propagating chain reaction of lipid peroxidation causing initial oxidation of only a few lipid molecules. Despite much research on lipid peroxide it has not yet been arrived at a sufficient result about how much affect lipid peroxide is doing on pathological conditions, but it has been affected in several diseases such as atherosclerosis, inflammatory bowel disease (IBD), retinopathy of prematurity (ROP), bronchopulmonary dysplasia BPD, Parkinson's disease, kidney disease, asthma, preeclampsia and others (Mylonas and Kouretas, 1999).

Peroxyl radicals react with lipids, causing dehydrogenation of fat and production hydroperoxides, lead to an increase propagate the free-radical pathway. Hydroperoxides effect on cells and destroy it. Also, it can interact with transition met als important in the body such as iron or copper to formation aldehydes like malondialdehydes, which will harm cell membranes (Halliwell and Gutteridge, 1990). Cell membrane phospholipids are more likely to oxides, thus be entered in free radical chain reactions as a result of constant damage that it's exposed by free radicals. Some of the fatty acids are polyunsaturated, possessing a methylene group between two double bonds that gives the fatty acid high sensitive to oxidation. The high concentration of polyunsaturated fatty acids in phospholipids lets them enter in free radical chain reactions (Wang et al., 1996).

2.8.2.3. Oxidative damage to protein

Proteins are oxidatively exposed in several methods: free radical-mediated peptide cleavage, oxidative amendment of amino acid, and protein production crosslinkage through reaction with lipid peroxidation compounds. Proteins are that including amino acids such as cysteine, methionine, histidine, and arginine show to be the most affected to oxidation (Freeman and Crapo, 1982). Oxidative stress is working on oxidizing amino acids that are in proteins, causing the formation of protein-protein cross-linkages, causing a change in the formation of the protein and modify of functioning, Lack of enzyme activity, lack of receptor function and conveying protein (Butterfield et al., 1998).

Reactive oxygen species (ROS) can ruin proteins and cause of produce carbonyls and alteration on amino acids like the formation of methionine sulfoxide, protein-carbonyls, and protein peroxide. Significant changes occur as a result of oxidations to which proteins are exposed, causing an effect on signal transduction mechanism, heat, enzyme action, and proteolysis susceptibility. Also it's possible to occur increasing in levels of protein carbonyls in many diseases such as, Alzheimer’s disease(Smith et al., 1991), cataractogenesis (Garland et al., 1988), Parkinson's disease (Floor and Wetzel, 1998), Rheumatoid Arthritis (Chapman et al., 1989), progeria, atherosclerosis, respiratory destroys syndrome, Werner’s syndrome, ageing(Smith et al., 1991), and diabetes (Jones and Hothersall, 1993).

3. MATERIALS AND METHODS

In this study, blood serum samples were collected from gout patients in the Orthopedics and Traumatology Clinic of Van Yüzüncü Yıl University Hospital. Their gout ages ranged between 13 and 87 years. The study also included 33 controls with ages ranging between 14 to 88 years. The period of specimen collection started from December 2019 to March 2020. Personal information for each patient was obtained, including name, age, weight, height, medications, and chronic diseases.

3.1. Experimental Design

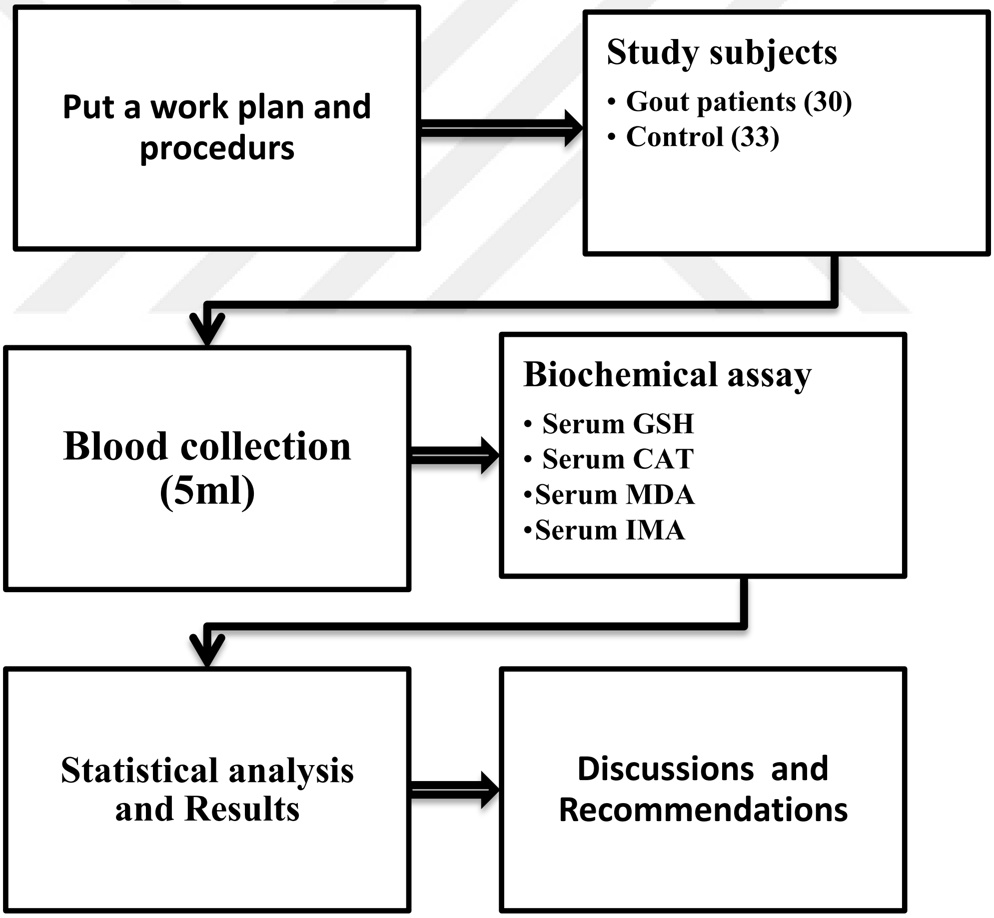

Figure 3.1. A chart showing the stages of work and their arrangement.

3.1.1. Equipments

The equipments used throughout the laboratory work are presented is:

Bench centrifuge

Deep freeze

Electrophoresis equipment

Water bath

Micro-centrifuge

Vortex mixer

Oven

Gel tubes

Refrigerated Centrifuge

Test Tubes

Sensitive balance

Stopwatch

Pipette

Micro Pipette

Spectrophotometer cuvette

Adjustable Automotive Pipettes

Glass pipette

Automated Pipette Tip

PH-meter

Flask

Beakers

Magnet

Filter paper

3.2. Chemical and Biological Materials

The chemical and biological materials used in the laboratory work are listed as:

3.2.1. Reagents and chemical

Absolute ethanol

Butyl Hydroxy toluene B.H.T.

Sodium hydroxid

Thiobarbituric acid T.B.A.

Trichloroacetic acid T.C.A.

5,5-2dithiobis nitrobenzoic acid

D.T.N.B.

Hydrogen peroxide

buffer Phosphat

E.D.T.A.

nitro blue tetrazolium (N.B.T)

Xanthine

Nitro blue tetrazolium

Xanthine oxidase

Water

3.3. Methods

3.3.1. Sample collection and creparation

Each individual was subjected to a venous blood collection. Five milliliters of blood was withdrawn with sterile, disposable syringe and transferred into gel tubes. Then trnsfering to centrifuged at 5000 rpm for 10 min, half of the separated sera was used in reduced glutathione, malondialdehyde, catalase, and ischemia modified albumin assays.

3.4. Biochemical Assay

3.4.1. Preparation of blood sample

The blood sample was left for thawing then mixed thoroughly by a rotisserie shaker at room temperature.

3.4.2. Determination of reduced glutathione (GSH) activity

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

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

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

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

E1 = 1 in the calculations.13600 is the molar extinction coefficient of the yellow color that formed during the interaction of GSH and DTNB.

3.4.3. Determination of malondialdehyde (MDA) level

The reaction theory relies on the development of pink complex between malondialdehyde and thiobarbituric acid (TBA) with maximal absorption at 532 nm.

3.4.3.1. Assay procedure of MDA

The reaction of fatty acids with free radicals results in malondialdehyde, which is the final product of lipid peroxidation, being determined with a colored form of thibarbituric acid (Gutteridge, 1995). 200ml of lavender serum was taken and put into 1 tube.800ml phosphate buffer, 25ml BHT solution, and 500ml of %30 TCA were added.

The tubes were stirred with vortex and kept on ice for 2 hours. The tubes brought to room temperature Then centrifuged at 2000 rpm for 15 minutes, 1ml from supernatant (filtrate) was taken and transferred to other tubes. Then 75ml of EDTA and 250ml of TBA were added to 1 ml filtrates. Tubes were combined in the vortex and held for 15 minutes (70C) in a hot water pan. They were then taken to room temperature and absorbed in UV / V spectrometer at 532 nm.

Thiobarbituric acid reagent: 75 ml of thiobarbituric acid, 15 g Trichloroacetic acid, and 2.08 ml of 0.2 N HCl are prepared. Both were combined, and the amount of purified water (D / W) was up to 100 ml.

Calculation of malondialdehyde level:

$C=F\times 6.41\times A$

C: Concentration

F: Dilution factor

A: Absorbance

(moles/mL)

3.4.4. Sample measurement catalase activity CAT

In this research, catalase activity was calculated using Aebi method, which is used as a hydrogen peroxide substrate (Aebi, 1984).

3.4.4.1. Assay procedure of CAT

For measuring catalase activity one empty tube and one sample tube were obtained. In the empty tank, 2.8 ml of 30 mM hydrogen peroxide (H_2O_2) was put, and 0.2 ml of phosphate buffer was added. The blend was easily shook and assessed spectrophotometrically (Ati Unicam UV / VIS-UV2-100, England) twice at 240 nm with thirty-second intervals. Then again, 2.8 ml of 30 mM hydrogen peroxide (H_2O_2) was inserted into the sample tank, adding 0.2 ml of serum. The mixture was easily shook, and Hitachi U-2900 read absorbance at 240 nm (Aebi, 1984).

Activity = $(2.3 / \Delta x)$ x [(log A1 / log A2)]

Activity; Calculated as in U/L.

Δx = 30 seconds

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

3.4.5. Sample measurement ischemia-modified albumin (IMA)

Ischemia-modified albumin (IMA) is characterized by the use of muscle destruction in cardiac hypoxic tissue, reduced coronary blood flow channels and ischemically mediated reactive oxygen species (ROS) modifications (Chawla et al., 2006;Sbarouni et al., 2008). The reactive radicals of oxygen cause multiple losses. In this research, The IMA ascends soon after the start of ischemia, returns to normal within 24 hours of being extreme for 6–12 hours. In each other, IMA sensitivity has been reported as 80%, 31% specificity and 92% negative predictive value in ischemia diagnosis (Anwaruddin et al., 2005). In one research myocardial ischemia and ischemi are perfusion injury induced free oxygen radicals to be created by inducing oxidative stresses. In addition, oxidative alteration of certain molecules induces myocardial ischemia and ischemia-reperfusion injury which include lipids, DNA and proteins (Maneewong et al., 2011).

3.4.5.1. Assay procedure of IMA

Several studies have shown that free radical development decreases in stroke, in particular with ischemia and reperfusion (Zini et al., 1992). IMA is also correlated in another study with elevated oxidative stress in the myocardial and other ischemic fashions (Sbarouni et al., 2011). Disorders such as cardiac insufficiency, pulmonary embolism, cardiopulmonary resuscitation, renal diseases of the end degree, cerebrovascular ischemia, acute mesenteric ischemia, systemic sclerosis, arthroscopic knee surgery, submissive skelet al ischemia, diabetes mellitus are increased throughout the literature (Sbarouni et al., 2011).

Assessment of IMA levels by spectrophotometric IMA measurement process in serum has developed into spectrophotometrically calculated. For serum IMA assessment, 0.5 ml of cobalt chloride was applied to more than 200 μl of serum, which was detected 10 minutes later with incubation 50 µl dithiothreitol turned into a cuvette and matched in bulk. 1 ml of NaCl was applied after 2 min. The resulting colored complex transformed into spectrophotometrically measured at a wavelength of 470 nm (Bar-Or et al., 2000).

4. RESULTS AND DISCUSSION

4.1. Statistical analysis

The descriptive statistics for the features mentioned were expressed as mean and standard deviation. T-Test was used in the comparison of binary groups providing the condition of normal distribution. On the other hand, the statistics of Mann Whitney U test was used when the condition of normal distribution was not provided. The statistical significance level was taken as $p<0.01$ and the SPSS statistical software version 19.0 (SPSS Inc, Chicago, III, USA) pack has used for analyses.

4.2. Anthropometric Characteristics of Gout Patients and Control

The main anthropometric characteristics of a gout patients and healthy control subjects are summarized in Table (4-1).

Table 4. 1. Anthropometric Indices of gout patients and controls

Variables		Gout Patients (n=30)	Control (n=33)
Gender	Male	17 (57%)	16 (48%)
	Female	13 (43%)	17 (52%)
Age (Years)	(13-49)	12 (40%)	11 (33%)
	(50- >80)	18 (60%)	22 (67%)
Mean	Normal(18.5-24.9)	7 (24%)	13 (39%)
	Overweight (25-30)	20 (68%)	16 (49%)
	Obese (>30)	2 (8%)	4 (12%)

Table 4-1. reveals that the study included males and females in both gout patients and controls. In addition, two ranges of ages were collected (13-49 years) and (50->80 years). The frequency of the elderly gout patients is higher than that of the young gout patients. Also, it appears that the Overweight category is common among gout patients' group (68%) followed by the normal category (24%).

Since this study is intenteded to collect gout patients and control subjects with similar criteria, no major gaps between the two age groups, gender and in the prevalence of hypertension are observed except for BMI.

4.3. The Resaults

The results obtained in the present study were from a total number of 63 subjects out of which 33 were healthy controls and 30 gout cases. CAT, MDA, GSH, and IMA levels were a statistical significant, and that in the gout patients compared with the healthy controls. These results are an indication of the importance of what has been reached.

Catalase (CAT) enzyme activity was examined, we got values showing between control (0.2163 ±0.00799 U/L) and "the patient group levels" (0.0997 ± 0.12640 U/L) as in (Table 4.3), where these values were statistically-significant ($p<0.001$).

Furthermore, malondialdehyde (MDA) level was examined, we got values giving between control (0.8527 ± 0.05981 μmol/L) and "patient group levels" (1.7800 ± 0.13447 μmol/L) as in (Table 4.4) was statistically significant ($p<0.001$).

Also, reduced glutathione (GSH) level was examined, we got values showing between control (0.2082 ± 0.05525 μmol/L) and patient group levels (0.0564 ± 0.02333 μmol/L) as in (Table 4.5), where these values were statistically significant ($p<0.001$).

While (IMA) level was examined, we got values giving between control (0.4797 ± 0.02325 ABSU) and patient group levels (1.0573 ± 0.01202 ABSU) as in (Table 4.6), where were statistically significant ($p<0.001$).

Table 4.2.Contains descriptive statistics and comparative results for CAT, MDA, GSH, and IMA. When samples was examined, the difference between the patient and control group mean SOD, MDA, GSH and CAT was statistically significant ($P<0.001$).

Table 4.2. Descriptive statistics and Comparison results

Parameters	Patient (n=30) Mean±Std. Deviation	Control (n=33) Mean±Std. Deviation	P
Age	55.4333±17.50406	58.2121±17.06011	0.526
Length (m)	1.6863±0.06376	1.6970±0.06757	0.524
BMI	26.1817±2,37778	25.6366±3.39744	0.468
Weight (kg)	74.4000±7.15156	73.4848±7.78669	0.630
GSH (mmol/mL)	0.0564±0.02333	0.2082±0.05525	0.001
MDA (nmol/L)	1.7800±0.13447	0.8527±0.05981	0.001
CAT (U/L)	0.0997±0.12640	0.2163±0.00799	0.001
IMA (ABSU)	1.0573±0.01202	0.4797±0.02325	0.001

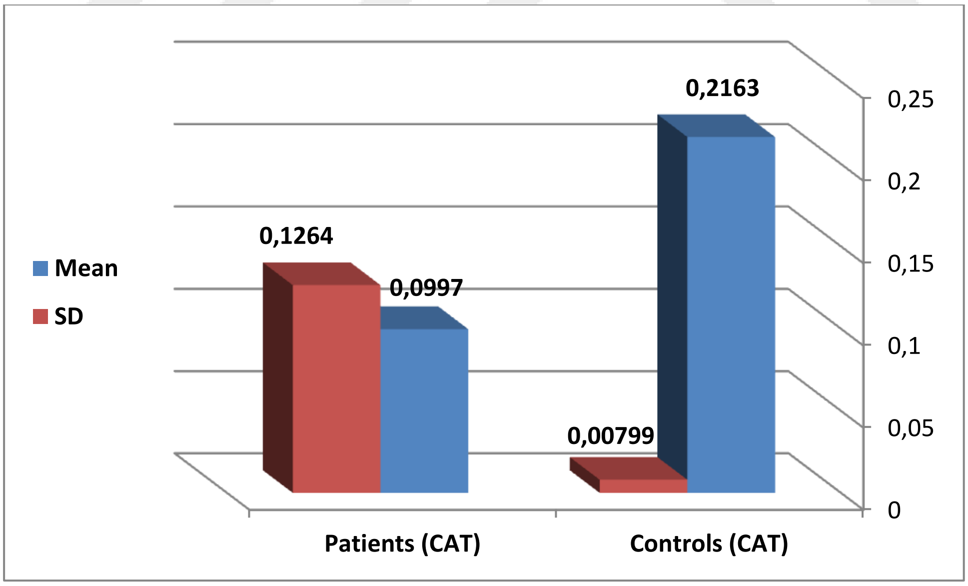

Figure 4.1. The activity of CAT enzyme compared between control and gout patients.

Catalase enzyme activity was examined. The difference between the means of the patient (0.0997±0.12640 U/L) and control groups (0.2163±0.00799 U/L) was statistically significant (P <0.001) (Figure 4.1).

Malondialdehyde (MDA) level was examined. The difference between the means of the patient(1.7800±0.13447µmol/L) and control groups (0.8527±0.05981µmol/L) was statistically significant (P <0.001) (Figure 4.2).

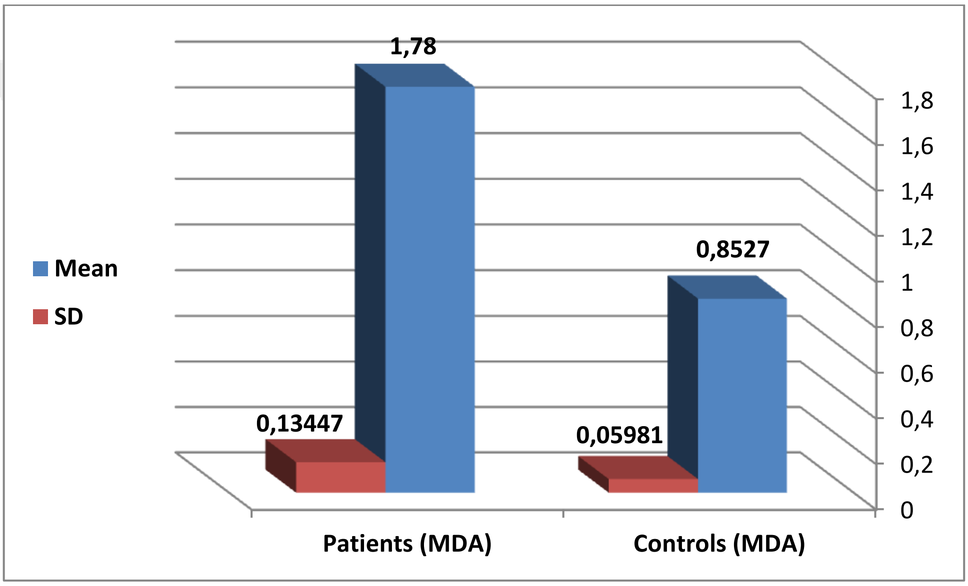

Figure 4.2. The level of MDA compared between control and gout patients.

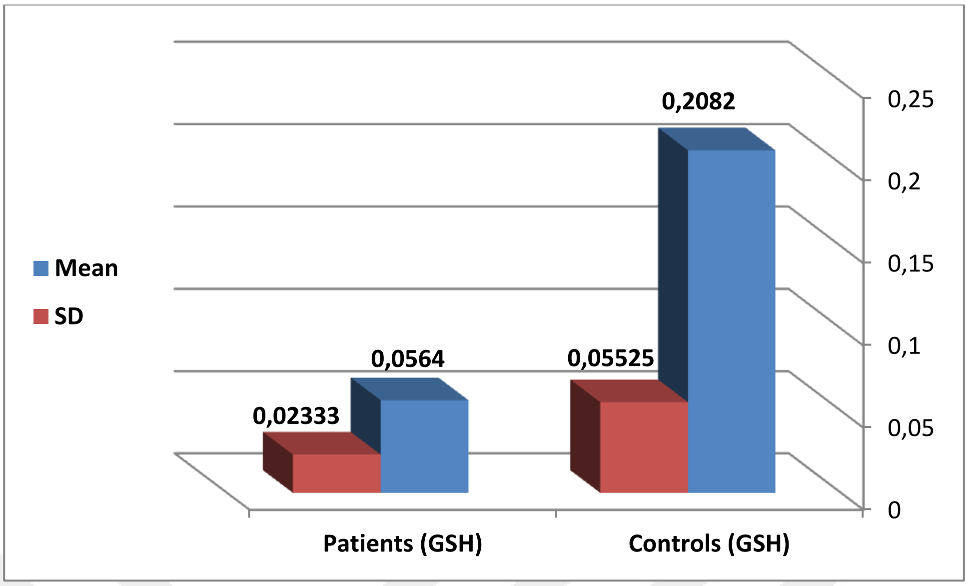

Figure 4.3. The level of GSH enzyme compared between control and Gout patients.

Reducted glutathione (GSH) level was examined. The difference between the means of the patient (0.0564±0.02333 µmol/L) and control groups (0.2082±0.05525µmol/L) was statistically significant (P <0.001) (Figure 4.3).

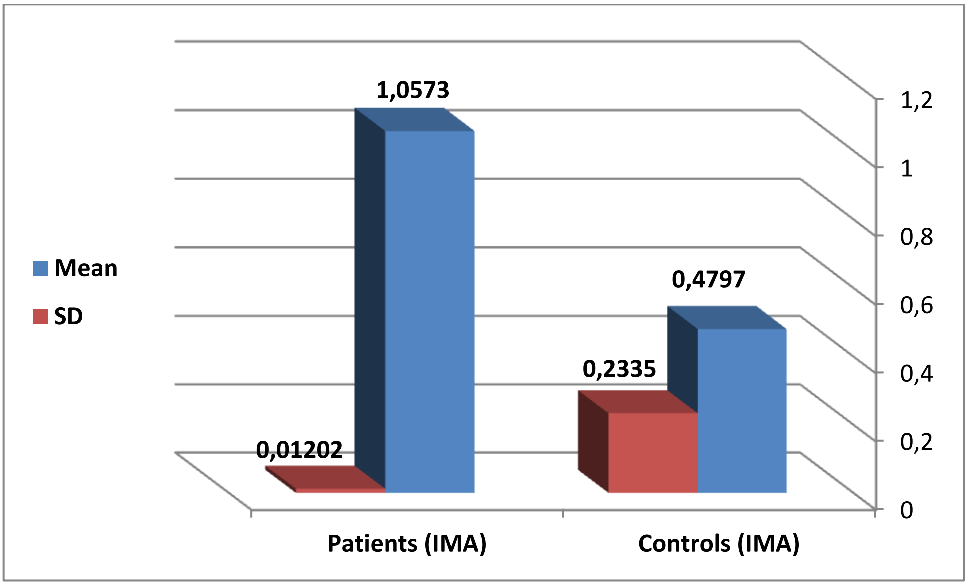

Figure 4.4. The level of IMA compared between control and gout patients.

(IMA) level was examined. The difference between the means of the patient (1.0573±0.01202 ABSU) and control groups (0.4797±0.02325 ABSU) was statistically significant (P <0.001) (Figure 4.4).

4.4. Discussion

The role of uric acid inside the body has been a hot topic. Some research say uric acid can play certain roles in physiological processes, such as shielding neurons from oxidative damage and regulating blood pressure (Hosomi et al., 2012; Watanabe et al., 2002). There was, however, an opposing opinion that the metabolite was considered simply a waste (Hyndman et al . , 2016; Gutman, 1965).

Epidemic evidence show that hyperuricemia and hypertension are strongly associated (Mortada, 2017). The association between uric acid and blood pressure was therefore more concomitant than causal. Both diseases depend on age, and unusual direct experimental data backed the hypothesis that hyperuricemia may cause hypertension, and vice versa. In fact, there were virtually no signs of uric acid suppressing drugs associated with increasing blood pressure (Cammalleri and Malaguarnera 2007). Uric acid has been reported to play an effective part in blood pressure regulation from the study either.

The end product of purines metabolism, uric acid, is produced by the liver and excreted by the kidney and intestines (de Oliveira and Burini, 2012). Overproduction or under exertion of uric acid will cause hyperuricemia, the major etiological factor of gout, as it may lead to crystal formation and inflammation response (Jin et al., 2012; Ruoff and Edwards, 2016). However, many studies suggested that gout patients have normal concentrations of serum uric acid at the acute attack of gout (Schlesinger, 2010; Roddy et al., 2013).

Other risk factors that predispose individuals to gout are gender, age, genetics, obesity, alcohol intake, diet, comorbidities and some drugs (McGill, 2011).

The results from the concentration of serum uric acid were significantly higher for males than for females. Because men are more likely to develop gout than women because of higher uric acid levels in the blood, however, women become more susceptible to gout after menopause due to increased uricemia in parallel with decreased levels of estrogen (Jin et al., 2012; Pasalic et al., 2012).

Serum uric acid concentration is also affected by (BMI). This was observed in the obese and overweight patients showed a significant elevation in serum uric acid (Wang et al.,2014). Demonstrated an association between body mass index (BMI) and uric acid levels. Obesity, and abdominal obesity in specific showed an association with uric acid levels may be due to increased production and reduced urate excretion in the renal system. Moreover, overweight and obesity are associated with insulin resistance which has been shown to be strongly associated with gout (Chan et al., 2014).

Uric acid unlikely to plays a major role in the defense of neurons or in the maintenance of uric acid in blood pressure. Uric acid was a purine metabolite while purines in the body were largely derived from endogenously degraded nucleosides and partially from food. Nucleosides are that originate from the used DNA or RNA. The cell in a robust metabolism state requires many mRNAs to synthesize-protein, and the mRNAs used are degraded. This will then be used to synthesize new mRNAs and a portion will be converted into uric acid. When a cell dies, however, the whole nucleus of abundant DNAs and cytoplasm RNA may be damaged and many of them turned into uric acid rather than recycled. Given that the nucleus contains much more nucleotides than the cytoplasm, a higher uric acid will lead to a dead cell. Cytoplasm nucleoside yield did not mean anything similar to nucleoside yield from the nucleus. Thus, there was no substantial relation between uric acid and carbon metabolism. RCD is the primary physiological mode of death in cells (Galluzzi et al . , 2018), and Casp3 is the leading member of RCD pathways.

The results obtained in the present study were from a total number of 61 subjects out of which 33 were healthy controls and 30 gout cases. CAT, MDA, GSH, and IMA levels were a statistical significant, and that in the gout patients compared with the healthy controls. These results are an indication of the importance of what has been reached.

It is known that inside of the kidney there are aerobic cells in which they contain high amount of catalase. CAT has an important role in reducing ROS as well as it leads to inhibition of lipid peroxidation. The failure of mitochondrial function comes from catalase defection, which leads to accumulation of ROS inside of mitochondria and results of mitochondrial disfunction (Ratliff et al., 2016). The two important groups of antioxidant enzymes which they are glutathione peroxidase and catalase contributes in oxidation of H_2O_2 and results in water molecule and oxygen. In one study, CAT activity has found to be high in patients with acute gout disease (JNkeck et al., 2017). In the literature, it has been found that CAT level is high in gout (Acharya et al., 2014). Catalase enzyme activity was examined. The difference between the means of the patient (0.0997±0.12640 U/L) and control groups (0.2163±0.00799 U/L) was statistically significant (P <0.001) (Table 3.2). The presence of low catalase activity in gout can be said that the cells are exposed to oxidative stress.

Lipid peroxidation causes many pathological conditions that damage living cells. Species of reactive oxygen contain radicals including hydroxyl radical and hydrogen peroxide (Mylonas and Kouretas, 1999). In one study, MDA rates in patients with acute gout disease have been shown to be elevated (JNkeck et al., 2017). In the literature, it has been found that MDA level is high in gout (Acharya et al., 2014). Malondialdehyde (MDA) level was examined. The difference between the means of the patient (1.7800±0.13447μmol/L) and control groups (0.8527±0.05981μmol/L) was statistically significant (P <0.001) (Table 3.2). The presence of high MDA in gout can be said that the cells are exposed to oxidative stress.

GSH plays a key role in cellular resistance against oxidative damage. GSH is an important antioxidant found in cells. GSH / GSSG ratio is the main determinant of oxidative stress. GSH shows its antioxidant effects in different ways. It detoxifies hydrogen peroxide and lipid peroxides with the effect of GSH-Px. Reducted glutathione (GSH) level was examined. The difference between the means of the patient (0.0564±0.02333 μmol/L) and control groups (0.2082±0.05525μmol/L) was statistically significant (P <0.001) (Table 3.2). The presence of low reduced glutathione (GSH) level in gout can be said that the cells are exposed to oxidative stress.

Ischemic modified albumin is a form of albumin that has the ability to bind metals such as cobalt, copper and nickel under normal conditions after the N-terminal end of albumin loses its ability to bind these metals by changing in conditions such as ischemia (Bulanık et al., 2019). Its findings also showed a significant elevated level of IMA and IMA/albumin in the PKU patients compared to non-PKU control subjects (Keshavarzi et al., 2018). (IMA) level was examined. The difference between the means of the patient (1.0573±0.01202 ABSU) and control groups (0.4797±0.02325 ABSU) was statistically significant (P <0.001) (Table 3.2).

In short, dead cells were the primary source of damaged nucleosides, so they needed to transform the nucleoside purines locally into uric acid which is applied to the blood, and then excreted or ingested internally by the kidney or intestinal tract to cause damage to the tissue.“The amount of uric-acid is possible to be employed as an accurate, but sometimes it isn’t sensitive, marker for all cell death assessment.

5. CONCLUSION

Oxygen-free radicals and nitrogen-free technologies are a natural consequence of human activity. Sources of loose radicals are significant, including electron leakage from the mitochondrial electron shipping chain, xenobiotic metabolism using the cytochrome p450 enzyme system in the endoplasmic reticulum, and NADPH oxidase activation in inflammatory cells. Unpaired electron is answerable for the extraordinarily excessive biological reactivity of unfastened radicals. Oxygen and nitrogen free radicals represent a constant source of attacks upon cellular lipids, proteins, nucleic acids, and other biomolecules. Yet, it would be inaccurate to consider entirely the dangerous biological consequences of ROS. At low or moderate concentrations, free radicals are important to body fitness. they're crucial for host defense mechanisms, and play a key role inside regulation of intracellular signaling cascades in many nonphagocytic cell types. Publicity of cells to increased concentrations of reactive species via either endogenous or exogenous insults gives rise to oxidative strain. The harmful impact of oxidative stress is counteracted via the motion of both antioxidant enzymes and non enzymatic antioxidants. Oxidative pressure has been diagnosed as a contributor to some of pathophysiological conditions, particularly cardiovascular damage. In this context, antioxidant compounds of diverse structures were demonstrated to be protective in lots of sickness strategies in numerous animal fashions. But, for maximum, if now not all, of the antioxidant-primarily based modalities shown to be effective in animal fashions, their medical efficacy in human suffers remains to be set up. As new information on free radicals in health and sickness will increase, new possibilities could end up available to assist expand modern mechanistically primarily based modalities for the intervention of unfastened radical disorder without compromising the physiological function of those reactive species.

In conclusion, this study, oxidative stress, antioxidant activity, and IMA may play an important role in the development of gout disease.

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GENİŞLETİLMİŞ TÜRKÇE ÖZET
(EXTENDED TURKISH SUMMARY)

GUT HASTALARINDA OKSİDATİF STRES DÜZEYİ (MALONDİALDEHİT), BAZI ANTİOKSİDAN AKTİVİTELERİN (KATALAZ VE REDÜKTE GLUTATYON) VE İMA (İSKEMİ MODİFİYE ALBUMİN) DÜZEYİNİN BELİRLENMESİ

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1. ÖZ

Gut, herkesi etkileyebilecek yaygın ve kompleks bir artrit şeklidir. Gut, eklemlerde ani ve şiddetli ağrı, şişme ve kızarıklık hissi ile ortaya çıkmaktadır, genellikle ayak başparmağının dibinde eklemde ürik asit kristalleri birikerek iltihaplanma ve akut ağrıya neden olan bir hastalıktır. Ürik asit seviyesi yüksek olan kişilerde ürik asit kristalleri oluşur. Vücutta ve belirli yiyecek türlerinde doğal olarak bulunan bir madde olan pürin işleminin bir parçası olarak ürik asit üretir. Bu çalışmada, Van Yüzüncü Yıl Üniversitesi Hastanesi Ortopedi ve Travmatoloji Kliniğinden gut hastalarından kan serumu örnekleri alındı. Bu çalışmanın amacı, gut hastalarının kan serumunda İskemi modifiye albümin (İMA) ile lipid peroksidasyonu olan malondialdehit (MDA) ve indirgenmiş glutatyon (GSH), katalaz (CAT) gibi antioksidanların aktivitelerini belirlemekti. Hasta grupları sağlıklı kontrol grubuna göre CAT ve GSH aktiviteleri anlamlı olarak azaldığı tesbit edildi ($p < 0.001$). Hasta grubu sağlıklı kontrol grubuna göre MDA düzeyleri ise anlamlı olarak yüksek bulundu ($p < 0.001$). Sonuç olarak, oksidatif stres, gut hastalığının gelişiminde önemli bir rol oynayabilir.

Anahtar kelimeler: GSH, CAT, MDA,IMA,UA.

2. GİRİŞ

Gut, herkesi etkileyebilecek yaygın ve kompleks bir artrit şeklidir. Eklemlerde ani ve şiddetli ağrı, şişme ve kızarıklık hissi ile ortaya çıkmaktadır, genellikle ayak başparmağının dibinde eklemde ürik asit kristalleri birikerek iltihaplanma ve akut ağrıya neden olan gut krizidir. Ürik asit seviyesi yüksek olan kişilerde ürik asit kristalleri oluşur. Vücutta ve belirli yiyecek türlerinde doğal olarak bulunan bir madde olan pürin işleminin bir parçası olarak ürik asit üretir.

Bu çalışmanın amacı, gut hastalarının kan serumunda iskemi modifiye albümin (İMA) ile lipid peroksidasyonu ürünü olan malondialdehit (MDA), indirgenmiş glutatyon (GSH) ve katalaz (CAT) gibi bazı antioksidan aktiviteleri belirlemekti.

3. MATERYAL VE YÖNTEM

Bu çalışmada, Van Yüzüncü Yıl Üniversitesi Hastanesi Ortopedi ve Travmatoloji Kliniğinden gut hastalarından kan serumu örnekleri alındı. Gut hastaların yaş aralığı 13 ile 87 arasında alındı ve toplam 30 bireyden ouşturuldu. Çalışma için ayrıca yaşları 14 ila 88 arasında değişen 33 sağlıklı kontrolü bireylerden ouşturuldu. Her hasta için isim, yaş, kilo, boy, ilaçlar, kronik hastalıklar dahil olmak üzere kişisel bilgiler alındı. Gut hastalarının kan serumunda İskemi modifiye albümin (İMA) ile lipid peroksidasyonuolan malondialdehit (MDA), indirgenmiş glutatyon (GSH) ve katalaz (CAT) gibi bazı antioksidan aktiviteleri spektrofotometrik olarak tayin edildi.

3.1. Katalaz (CAT) aktivitesi tayini

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

Kullanılan çözeltiler:

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

Aktivite Hesabı:

E.Ü.= $(2{,}3 / \Delta x) \times [(\log A_1 / \log A_2)]$ Aktivite; U/L cinsinden hesaplandı.

Δx= 30 saniye

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

3.2 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.

3.3 Redükte glutatyon (GSH) tayini

Kullanılan çözeltiler:

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

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

GSH tayin yöntemi:

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

Hesaplama:

Glutatyon derişimi mmol/g protein biriminden hesaplandı.

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

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

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

1000: mmol'e dönüşüm katsayısı.

C: mmol / glutatyon (mg/dl)

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

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

3.4. 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.

4. BULGULAR:

Table 3.2. Biyokimyasal parametrelerin istatistiksel sonuçları

Parameters	Hasta(n=30) Mean±Std. Deviation	Kontrol (n=33) Mean±Std. Deviation	p
Yaş	55.4333±17.50406	58.2121±17.06011	0.526
Boy (m)	1.6863±0.06376	1.6970±0.06757	0.524
BMI	26.1817±2,37778	25.6366±3.39744	0.468
Ağırlık (kg)	74.4000±7.15156	73.4848±7.78669	0.630
GSH (mmol/mL)	0.0564±0.02333	0.2082±0.05525	0.001
MDA (nmol/L)	1.7800±0.13447	0.8527±0.05981	0.001
CAT (U/L)	0.0997±0.12640	0.2163±0.00799	0.001
IMA (ABSU)	1.0573±0.01202	0.4797±0.02325	0.001

Hasta grupları sağlıklı kontrol grubuna göre CAT ve GSH aktiviteleri anlamlı olarak azaldığı saptandı ($p<0.001$). Hasta grubu sağlıklı kontrol grubuna göre MDA düzeyleri anlamlı olarak yüksek bulundu ($p<0.001$).

5. TARTIŞMA :

Hücreler, üç nedenden dolayı oksidatif strese maruz kalırlar: Birincisi, oksidanların yaşının artması, ikincisi, antioksidanların üretiminde bir azalma ve üçüncüsü ise, oksidan hasarının düzeltememe durumudur. Hücrenin hasarı, Reaktif Oksijen Türlerine (ROS) etki eder. *In vivo* reaktif oksijen türü kaynağı, yağ asitlerinin

(ROS) aynı zamanda peroksizomal β-oksidasyon, (mikrozomal-sitokromP450), ksenobiyotik bileşiklerin sindirimi, patojenler tarafından fagositozun tetiklenmesi ile oluşması sonucu meydan gelir (Fies ve diğerleri, 1999: Schafer & Buettner, 2001). Süperoksit dismutaz (SOD), katalaz CAT veya glutatyon-peroksidaz (GSH) (Hayes ve McLELLAN, 1999) aktiviteleri reaktif oksijen türlerini (ROS) normal durumdaki hücrelerden temizler. Reaktif oksijen türlerinden kaynaklanan hücrelere verilen birincil hasar, makromoleküllerin, örneğin membran lipidi çoklu doymamış yağlar, temel protein ve DNA'nın oluşmasına neden olur, oksidatif stress ve reaktif oksijen türleri, inflamasyon (Rheumatoid Artritis RA) (Hitchon CA, El-Gabalawy HS) Alzheimer hastalığı ve diyabetin neden olduğu bildirilmiştir (Christen, 2000).

Gut hastalarında CAT ve GSH aktiviteleri sağlıklı kontrol grubuna göre anlamlı olarak azaldığı saptandı ($p<0.001$). Gut hastalarında MDA düzeyleri sağlıklı kontrol grubuna göre anlamlı olarak yüksek bulundu ($p<0.001$).

İskemik modifiye albümin, albüminin N-terminal ucu iskemi gibi koşullarda değişerek bu metalleri bağlama kabiliyetini kaybettikten sonra normal şartlarda kobalt, bakır ve nikel gibi metalleri bağlama kabiliyetine sahip olan bir albümin formudur (Bulanık et al., 2019). Yapılan çalışmalarda ayrıca, PTU hastalarında kontrol grubuna kıyasla önemli bir yüksek IMA seviyesi bulunmuştur (Keshavarzi ve diğerleri, 2018). Gut hastalarında İMA düzeyleri sağlıklı kontrol grubuna göre anlamlı olarak yüksek bulundu ($p<0.001$).

6. SONUÇ :

GSH düzeyi ve CAT aktivitesi gut hastalarında düşük bulunurken MDA düzeyi ise yüksek bulundu. Sonuç olarak, oksidatif stres, gut hastalığının gelişiminde önemli bir rol oynayabilir.

CURRICULUM VITAE

Nawar Yaseen MOHSIN was born in 1994, in Wasit province, Iraq. He was completed in medical laboratory diploma in 2005 and he was worked in the field of medical laboratories from 2005 until 2011, He was joined Wasit University College of Science and got a bachelor’s degree in chemistry. He Has completed his MSc in The Department in of biochemistry, The Institute of Natural and Applied Sciences, Van Yuzuncu Yil University in Van -Turkey on October. 2020. He was obtained training certificates in the field of laboratory medicine and participated in the number of medical conferences inside and outside Iraq.

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THESIS ORIGINALITY REPORT

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