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Study the Effect of Oxidative Stress on Patients with Vitiligo in Anbar Governorate

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Dedication:

To my dear mother & father

To my dear sisters & brothers

To everybody supported me

To my country…. IRAQ

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Summary

Oxidative stress is an imbalance between oxidants and antioxidants, Which play an important role in initiation and progression of vitiligo. Vitiligo is an acquired, chronic depigmenting disorder of skin. The disease results from the selective loss of melanocytes. The aim of study is to evaluate effect of oxidative stress, heat and light on treatment of patients with vitiligo in AL-Anbar governorate.

The study has been included 80 blood samples of vitiligo patients (30 males and 50 females) and 40 blood samples from healthy individuals (8 males and 32 females), the date of sampling persists from July 2018 up to January 2019. Many biochemical parameters are performed for all patients and healthy individuals to know the pathogenesis of *Vitiligo* in addition to questioner list which has been filled for each patient .

Their ages are divided into different groups: group (A) ranges between (10-20) years, group (B) ranges between (21-40) years, group (C) ranges between (41-70) years. Family history of vitiligo was positive in a percentage of (30%) of the patients. The duration of disease ranged between 1 month to 20 years . History of seasonal variation of disease was positive in(60%) patients. Out of these (10%) noticed exacerbation of disease in winter while (50%) in summer season. Bad emotional state exacerbates of about (70%) of vitiligo patients, while the other (30%) does not affect.

This study shows that there is no relationship between the occupation, accommodation, most common diet, most common drinks and spiritual side and vitiligo.

Hemoglobin Concentration and Packed cells Volume determination of patient's group were significantly lower than those of control group with ($P \leq 0.05$).

The results obtained from this study showed a significant increase in Malondialdehyde (MDA) concentration with ($P \leq 0.05$) in vitiligo patients at comparison with control group and showed that there are no significant differences with ($P \geq 0.05$) in the concentrations of total bilirubin in vitiligo patients at comparison with control group and showed significant increase in the concentrations of uric acid with ($P \leq 0.05$) in vitiligo patients at comparison with control and showed significantly increase in glutathion concentration with ($P \leq 0.05$) in vitiligo patients at comparison with control group.

The statistical analysis for the determination some of vitamins showed that there are no significant differences in the concentrations of vitamin E and C with ($P \geq 0.05$) in vitiligo patients at comparison with control group.

The statistical analysis for the determination some of trace elements showed a significant increase in the concentrations of Zn and Cu with ($P \leq 0.05$) in vitiligo patients at comparison with control group.

This study shows that there is no correlation relationship between oxidants MDA and antioxidants (vitamin C, vitamin E, Zn). But it shows a negative correlation between MDA and Glutathione ($r=-0.447$) at significant ($p\leq0.01$) and a positive correlation between MDA and Cu ($r=0.409$) at significant ($p\leq0.01$).

Lipids profile is determined and the statistical analysis results show that serum cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol) and very low density lipoprotein cholesterol (VLDL-cholesterol) were no significant differences than those in control group with ($P \geq 0.05$).

The results of thyroid hormones determination were in the patient's group T3 and T4 were no significant differences than those in control group ($P \geq 0.05$).while TSH was significantly higher than those in control group ($P \leq 0.05$).

This study evaluated the effect of Narrow-band ultraviolet B (NB-UVB) on the 20 patients (12 women and 8 men) with vitiligo. The response of 80-90%was given grade excellent and response of 70-80% was given grade good and response of 60-70% was given grade moderate, while response of 50-60% was given grade mild and response of 40-50% was given grade poor.

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Abbreviation	Description
ATP	Adenosine triphosphate
ADH	Alcohol dehydrogenase
BR·	Bilirubin radical
CV	Contact vitiligo
CAT	Catalase
DCT	Dopachrome tatomerase
DNA	Deoxyribonucleic acid
GPX	Glutathione peroxidase
GRX	Glutathione reductase
GSH	Glutathione reduced
GSSH	Glutathione oxidized
Hb	Hemoglobin
·OH	Hydroxyl radical
H_2O_2	Hydrogen peroxide
LOO.	Lipid peroxyl radical
LOOH	Lipid hydroperoxide
LDL	Low density lipoprotine

MDA	Malondialdehyde
MEL	Monochromic excimer laser
µmol	Micromole
NSV	Non-segmental vitiligo
$NO^{\cdot}$	Nitric oxide radical
$NO_2^{\cdot}$	Nitrogen dioxide radical
NADPH	Nicotinamide adenine dinucleotide phosphate
NB-UVB	Narrow band- ultraviolet B
PBMC	Peripheral blood mononuclear cells
ONO_2	Peroxynitrite
PUFA	Polyunsaturated fatty acid
PUVA	Psoralen plus ultraviolet A
PCV	Packed cells volume
ROS	Reactive oxygen species
$O_2^{\cdot -}$	Superoxide anion
RNS	Reactive nitrogen species
SV	Segmental vitiligo
SOD	Superoxide dismutase
S.T.B	Serum total bilirubin
TG	Triglycerides
T4	Thyroxin
T3	Triiodothyronin
TSH	Thyroid stimulating hormone
TYR	Tyrosinase
TYRP1	Tyrosine related proteine-1
TC	Total cholesterol
UV	Ultraviolet
UVB	Ultraviolet-B
USA	United States of America
Vit.C	Ascorbic acid
VASI	Vitiligo area scoring index
XeCl	Xenon chloride

Chapter One

Introduction and Literature Review

Chapter One

1. Introduction and Literature Review:-

1.1. Vitiligo

Vitiligo is an acquired, chronic depigmenting disorder of the skin. Although the exact cause is still under debate, the disease results from the selective loss of melanocytes, which in turn causes pigment dilution in the affected areas of the skin and mucosa. Melanocyte precursors can be found in the hair follicle bulge; differentiated, pigment-producing melanocytes reside in the basal layers of the epidermis and the hair matrix (FIG.1.1). Depending on the disease course, skin and hair are affected to different degrees. Clinically, skin lesions present as milky white, non-scaly patches with distinct margins[1,2].

Prevalence of vitiligo is about 0.5-2% in most populations worldwide [3]. On the basis of population surveys and clinical records, the prevalence of vitiligo disease has been estimated to be 0.38% in Denmark [4] and 1% in United States [5]. In China, 0.19% of the population is affected with vitiligo [6]. From Indian subcontinent, the highest disease incidence was reported in India between 1-2% [7].

The only symptom of vitiligo is the presence of pale patchy areas of depigmented skin. The patches are initially small, but often grow and change shape[8]. When skin lesions occur, they are most prominent on the face, hands and wrists. The loss of skin pigmentation is particularly noticeable around body orifices, such as the mouth, eyes, nostrils, genitalia and umbilicus. Some lesions have increased skin pigment around the edges[9].

Although its pathophysiology is still unknown, divers theories have been proposed, including autoimmune, neural, oxidative stress, apoptosis, and genetic factors[10].

Previous studies have suggested that oxidative stress might play a prominent role in the pathogenesis of vitiligo [11]. Oxidative stress is defined

as a disruption of delicate balance between the formation of reactive oxygen species (ROS) and the antioxidant defense system[12]. As patients with vitiligo have an imbalanced redox state of the skin, resulting in the excess production of ROS. These disturbances and ROS accumulation can have toxic effects on all components of the cell (e.g., proteins, lipids), and could potentially result in the destruction of melanocytes[13].

Both sexes are equally affected[14]. A recorded predominance of women may reflect their greater willingness to express concern about cosmetically relevant issues [15].

Family history appears to lie between 15 and 20 % in most studies. Consanguinity can account for elevated incidence in certain regions of the world .Consanguinity cases had an earlier age of onset[16].

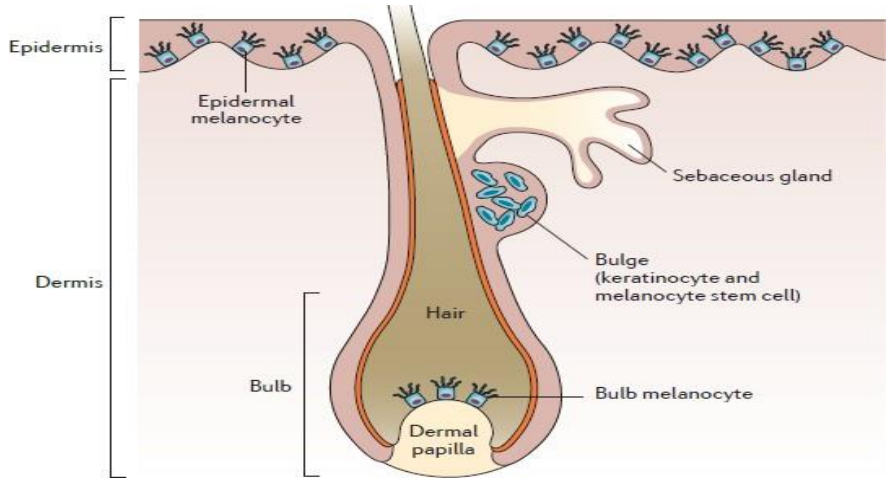


Figure (1.1) The hair follicle unit[1].

1.2. Types of vitiligo

Vitiligo has been classified based on clinical basis into two major forms, namely, segmental vitiligo (SV) and non-segmental vitiligo (NSV), the latter including different variants (acrofacial vitiligo, generalized vitiligo, universal vitiligo) [17][18] (Figure 1. 2).

1. **Non-segmental vitiligo (NSV):** Clinically, NSV is characterized by depigmented macules that differ in size from a few to several cm in diameter, often involving both sides of the body with tendency toward symmetrical distribution. In NSV, body hairs are usually spared and

remain pigmented, although hair depigmentation may occur with disease progression[18].

- **Focal vitiligo:** Focal vitiligo refers to an acquired, small, isolated hypopigmented patch that does not fit a typical segmental distribution, and which has not evolved into NSV after a period of 1-2 years[18].
- **Mucosal vitiligo:** Mucosal vitiligo classically refers to the involvement of the oral and/or genital mucosae[18] [19].
- **Universal vitiligo:** Universal vitiligo corresponds to complete or nearly complete depigmentation of the skin. This term is commonly used when NSV progressively leads to complete depigmentation of the skin and body hair, and sometimes oral/genital mucosae[19].

2. **Segmental vitiligo (SV):** Segmental vitiligo typically has a rapidly progressive but limited course, depigmentation spreads within the segment during a period of 6–24 months and then stops; further extension is rare[20][21].

- **Mixed vitiligo:** The coexistence of SV and NSV or NSV patient with preexisting segmental lesions is termed as mixed vitiligo [22][23][24].
- **Occupational/ contact vitiligo:** The terms contact or occupational vitiligo (CV) have been used to describe a distinct form of vitiligo induced by exposure to certain chemicals in the workplace or at home, principally aliphatic or aromatic derivatives of catechols and phenols [25][26].

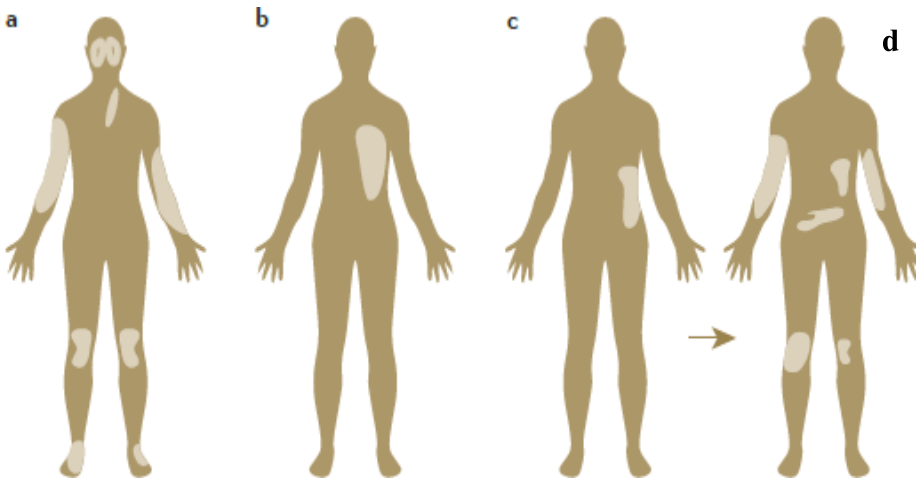


Figure (1.2): Classification of vitiligo: a) Non-segmental Vitiligo, b&c) Segmental Vitiligo, (d) Mixed Vitiligo [27]

1.3. Melanin pigment and its function

Melanin pigment is responsible for the skin and hair colour. It is responsible for the most striking polymorphic traits of human race and for the skin color. Melanin is synthesized in specialized intracellular membrane coated organelles present in the cytoplasm of melanocytes called melanosomes. Melanin pigment can be classified into two major types: Eumelanin (dark brown and black) and Pheomelanin (red, yellow and light brown) [28][29][30]. Eumelanin is the pigment present in the humans with dark skin and hair, while the red hairs and the skin types I and II predominately have phaeomelanin [31]. Tyrosinase is a major enzyme responsible for the biosynthesis of melanin pigment, converting tyrosine to dopa products for polymerization to melanin [32] (Figure 1.3). Other enzymes involved in the synthesis of melanin are TYRP1 and DCT [33]. Melanin protects the skin from harmful effects of solar UV rays by absorbing their radiant energy. Melanin is active scavenger of free radicals [34]. It has antioxidant, photo-protective and radical scavenging properties [35]. Epidemiological and clinical evidence states the role of melanin pigment in prevention of skin cancers induced by harmful sun rays [36]. Melanin pigment is able to dissipate over 99.9% of absorbed UV radiation [37]. It contains cations, anions and chemicals which absorb the harmful UVB radiations and plays an important protective role within melanocytes [38]. Lack of melanin in epidermis increases the susceptibility to skin cancers [39][40] and is an indicator of skin aging [41].

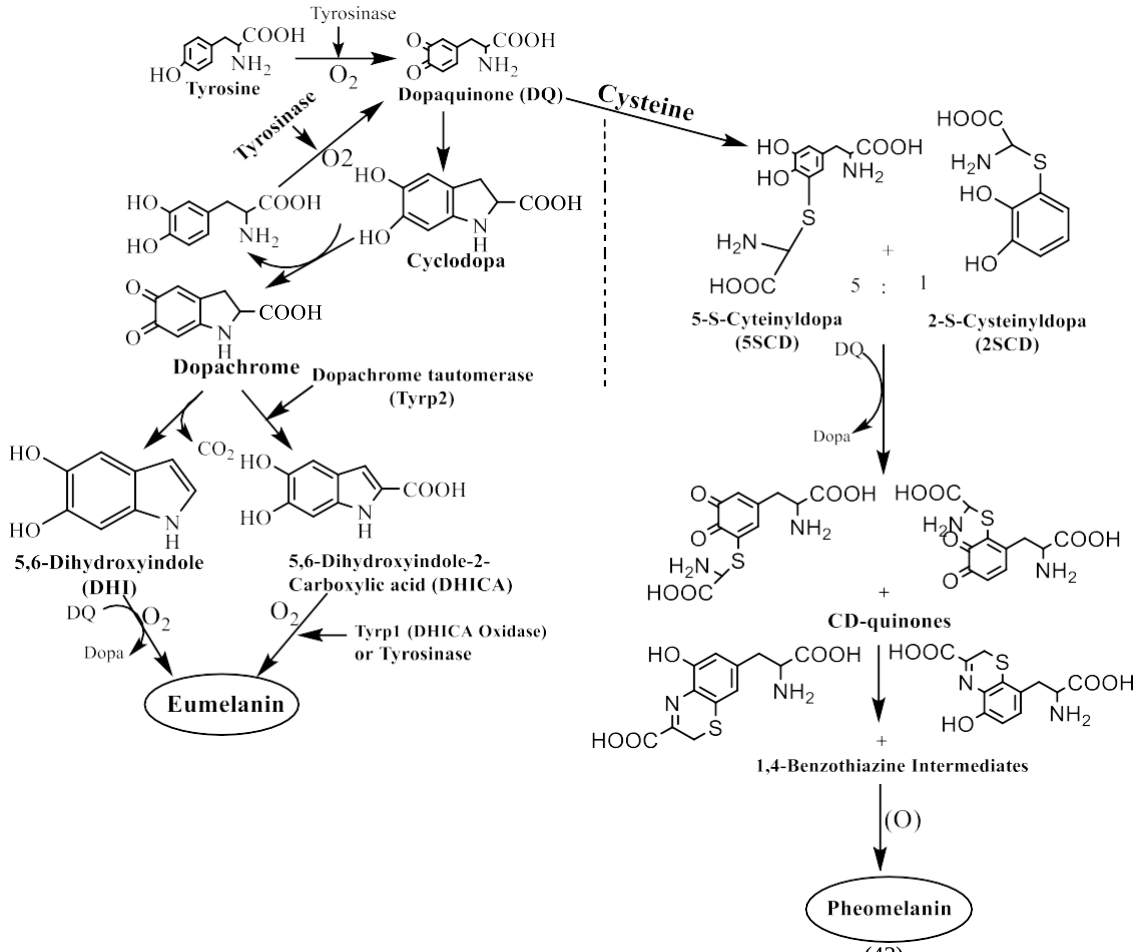


Figure 1.3: Melanin biosynthetic pathway [42].

1.4. **Vitiligo and Oxidative Stress**

Oxidative stress is a consequence of imbalance between pro- and anti-oxidant activities in cells. The occurrence of oxidative stress affecting the overall epidermis does appear to be involved. *In vivo*, increased H_2O_2 production, together with a decrease in the expression and activity of catalase has been described in the epidermis of vitiligo patients [11][43]. In cultured melanocytes, an altered redox status is associated with an increased susceptibility of the melanocytes to pro-oxidant agents [26][44]. In addition to the involvement of cells of the epidermis, clinical and experimental evidence suggests the occurrence of oxidative stress in non-epidermal cells [45]. Various studies have reported increased ROS production with subsequent alterations in the redox status of cells from vitiligo patients, even in erythrocytes, peripheral blood mononuclear cells (PBMC), serum, plasma or whole blood.

Skin serves as an important environmental interface providing a protective envelope that is critical for homeostasis. Also, it is a major target for toxic insults by a broad spectrum of chemical (xenobiotic) and physical (UV radiation) agents that are capable of altering its structure and function. Many environmental pollutants act as oxidants or catalyze the production of reactive oxygen species (ROS) directly or indirectly. ROS act largely by driving several important molecular pathways that play important roles in various pathologic conditions including ischemia–reperfusion injury, atherosclerosis, and inflammatory responses. The skin holds a range of defense mechanisms that interact with toxicants to obviate their deleterious effect. These include nonenzymatic and enzymatic molecules that function as potent antioxidants or oxidant-degrading systems. Unfortunately, these homeostatic defense mechanisms, though highly effective, have limited capacity and can be overwhelmed thereby leading to augmented ROS in the skin that can promote the development of dermatological diseases. One approach to treating or preventing these ROS-mediated diseases is based on the administration of various antioxidants in an effort to restore homeostasis. Even though many antioxidants have revealed considerable efficiency in cell culture systems and in animal models of oxidant damage, definite confirmation of their beneficial effects in human populations remains elusive [46].

1.5. Oxidants

The oxidants are classified into two groups free radical and non-free radical species. A free radical is an atom or fragment of molecule having one or more unpaired electron [47]. Oxidants have features are short-lived, highly reactive, and unstable [48]. So it captures electrons from other compounds to stabilize itself. [49] Oxidants species are termed as reactive oxygen species (ROS) and reactive nitrogen species (RNS) [47].The major ROS are Hydroxyl radicals ($^{\bullet}OH$), superoxide anion ($^{\bullet}O_2^-$), and Hydrogen peroxide (H_2O_2), RNS as Nitric oxide ($NO^{\bullet}$), Nitrogen dioxide ($NO_2^{\bullet}$), and Peroxynitrite ($ONO_2^{\bullet}$), as shown in the Table (1.1) [47]

Superoxide radical has a long half-life and it's highly reactive species which produced in the mitochondrial membrane. The enzymes could produce superoxide which includes xanthine oxidase, lipoxygenase, cyclooxygenase, and NADPH dependent oxidase. Toxicity of Superoxide radical is not necessarily correlated with reactivity [50][51][52] .

$$O_2 + e^- \rightarrow O_2^{\cdot -}$$

$$O_2 + Fe^{+2} \rightarrow Fe^{+3} + O^{\cdot -}_2 \text{ (auto-oxidation)}$$

Hypochlorous Acid (HoCl) is non-free radical molecule generated by activation of neutrophils at the site of inflammation. As a result of reaction of hydrogen peroxide with chloride ion by catalyzed the enzyme myeloperoxidase, that produces HOCl[53][49].

$$H_2O_2 + Cl^- \rightarrow HOCl + OH^{\cdot}$$

Hypochlorous Acid reacts with hydrogen peroxide to produce singlet oxygen which is non- free radical and damages the cells. [52]

$$HOCl + H_2O_2 \rightarrow {}^1O_2 + H_2O + Cl^-$$

Table (1.1) Types of oxidant according to free radical and non-free radical. [47, 50]

Types	Free Radical	Non-Free Radical
Reactive Oxygen Species (ROS)	Superoxide $^{\cdot}O_2^-$ Hydroxyl $^{\cdot}OH$ Peroxyl $RO_2^{\cdot}$ Aloxyl $RO^{\cdot}$ Hydroperoxyl $HO_2^{\cdot}$	Hydrogen Peroxide H_2O_2 Hypochlorus HOCl Ozone O_3 Singlet oxygen $^1O_2$ Hypobromous HOBr

Reactive Nitrogen Species (RNS)	Nitric oxide $NO^{\cdot}$ Nitrogen dioxide $NO_2^{\cdot}$	Nitrous acid HNO_2 Nitrosyl cation NO^+ Nitroxyl anion NO^- Peroxynitrite ONO_2 Nitronuim cation $^+NO_2$ Nitryl chloride NO_2Cl

ROS has two sources: Endogenous (mitochondria, peroxisomes, phagocytic cells etc.) and exogenous (pathogens, bacteria, pollutants, and viruses). Endogenous sources are more important and extensive than exogenous sources because these sources are a continuous process during the lifespan of every cell in the organism. (53)(54)

In physiological condition, ROS are, in physiological condition, the energy needed for various cell processes when synthesis adenosine triphosphate (ATP). Reduction of oxygen in mitochondria to produce ATP by the donation of 4 electrons to oxygen to produce water [55]. This process is rarely 100% efficient, oxidase often generate incompletely reduce oxygen species [55], as shown in the figure (1.4).

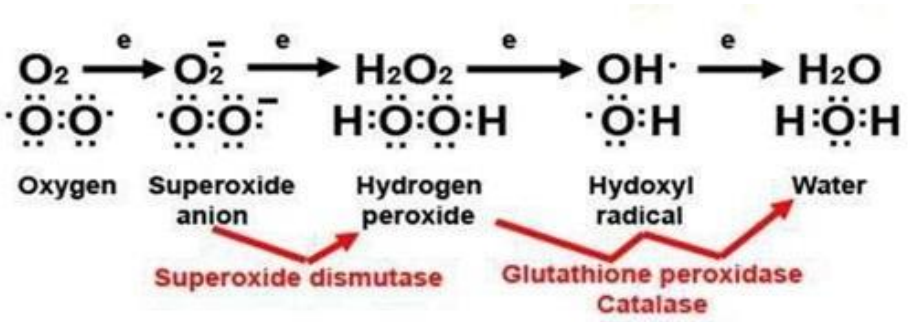


Figure (1.4) formation of reactive oxygen species.

In pathological condition, overproduction of ROS leads to damage in cellular components, such as lipids in the cell membranes, proteins, and DNA. [56]

ROS are molecules that play a crucial role in the progression of inflammatory diseases [53]. They generally function as modifying agents of cellular components or as signaling molecules in an immune response [57].Many inflammatory cells can produce significant amounts of superoxide in an effort to protect against invading organisms. [51]

The importance of ROS generation processes; it's occurring as part of the cell-mediated immunity. (i.e.) activate neutrophils and in general, phagocytes, give origin to the so-called "respiratory burst"[57].

1.6. Antioxidants Defense System

Antioxidants are molecules have the ability to neutralize oxidants and to prevent damage of lipid, protein, carbohydrate, and other components in the cell [58]. The Antioxidants can be classified into two groups: Enzymatic and non-enzymatic antioxidants defense systems:

1. Enzymatic antioxidant regard as endogenous antioxidants like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GRx). [59]

2. Non-enzymatic antioxidants subdivided into metabolic antioxidants and nutrient antioxidants:

A. Metabolic antioxidants is regard endogenous antioxidants that produced by metabolism in the body to protect it from different disease leads to damage the tissue. Metabolic antioxidants involve glutathione, coenzyme Q10, melatonin, uric acid, bilirubin, albumin, ceruloplasmin, and transferrin[60][61][62].

B. Nutrient antioxidants is regard exogenous antioxidants which cannot be produced in the body and must be provided from foods or supplements, such as tocopherols (vitamin E), Ascorbic acid (vitamin C), Carotenoids, trace elements (selenium, manganese, and zinc), and flavonoids[61][62].

1.7. Oxidative Stress Biomarkers:

Oxidative stress biomarkers include oxidants and antioxidants. The Oxidative stress of vitiligo results from accumulation of ROS that which can have several toxic effects on all the cell components it could result in melanocytes destruction and causing depigmented lesions as observed in vitiligo patients [13].

It can measure the oxidative stress in vitiligo patients by oxidative stress biomarker like Malondialdehyde as oxidants biomarkers and (total bilirubin, uric acid, and Glutathione) as the endogenous antioxidants biomarkers and (vitamin E), Ascorbic acid (vitamin C), trace elements (cu and zinc), as the exogenous antioxidants biomarkers.

1.7.1. Malondialdehyde (MDA) as a marker for lipid peroxidation

MDA is a three-carbondialdehyde [63], as shown in the Figure (1.5) has high reactivity, produced during polyunsaturated fatty acid peroxidation and arachidonic acid metabolism. MDA consider byproduct of lipid peroxidation[63][64].

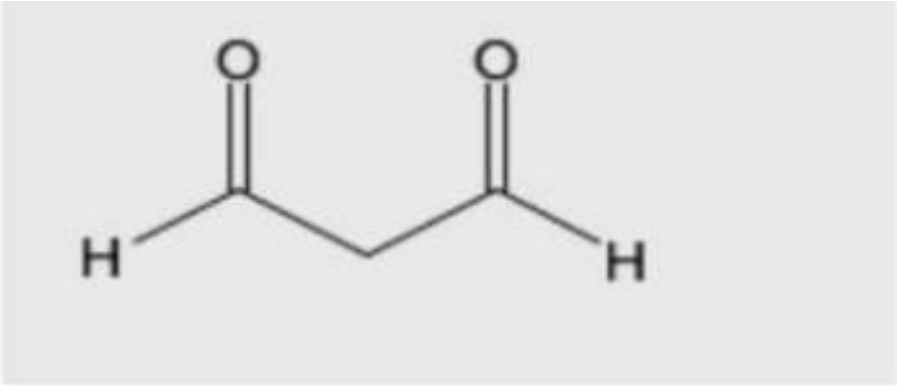


Figure (1.5) Chemical structure of Malondialdehyde[65]

MDA has low chemical reactivity at physiological state, but pH decrease lead to increase chemical reactivity of MDA. However, this molecule is able to interact with nucleic acid bases to form several different adducts [65], which possesses a blocked Watson-Crick base pairing region that has been shown to be mutagenic [66].The Product of MDA can cause the cross-linkage of membrane elements by affecting the ion exchange from cell membranes, which gives way to aftermaths including a change in ion permeability and enzyme activity [67]. Therefore, MDA is able to impair several physiological mechanisms of human body through its ability to react with molecules such as DNA and proteins; therefore it's useful to consider this molecule as something more than a lipid peroxidation product [65]. Thiobarbituric acid reacts with malondialdehyde to yield a pink product to easy measurement as shown in the figure (1.6)

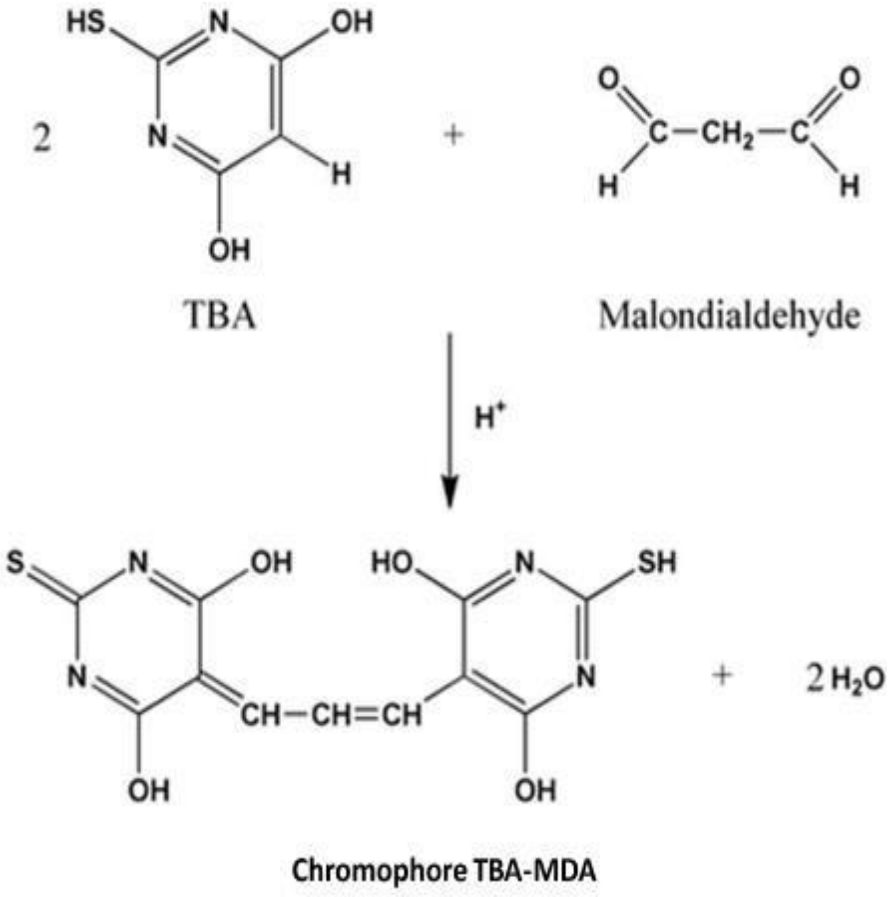


Figure (1.6) Reaction MDA with TBA to produce compound TBA-MDA[63].

1.7.2. Bilirubin

Bilirubin is an orange-yellow pigment in the bile that forms as a byproduct of heme degradation. It is the major dye component in both jaundice and contusions. Bilirubin was found to be in two forms direct bilirubin present in the bile, and indirect present in the blood [(68)]. Bilirubin metabolism is used as a marker to localize the site of liver disease [(69)].

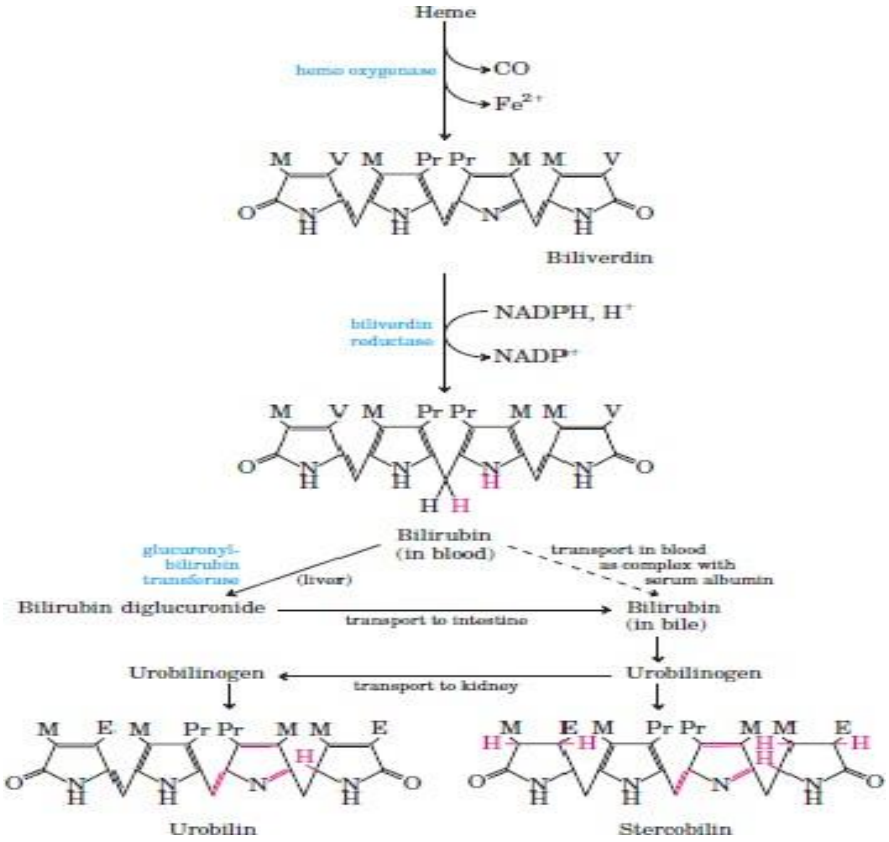


Figure (1.7) Structure of Bilirubin[(69)]

Bilirubin is a potent endogenous antioxidant could inhibit lipid peroxidation and other types of oxidation [(70)]. Bilirubin has the capability of scavenging newly formed ROS or other radicals before they can initiate a chain reaction [(71)(72)].

Bilirubin included an extended system of conjugated double bonds and a pair of reactive hydrogen atoms, and lead to bilirubin possess an antioxidant activity. Lipid peroxyl radical ($LOO^{\cdot}$) reacts with bilirubin by an initial donation of a hydrogen atom to forms lipid hydroperoxide (LOOH) and bilirubin radical [(73)(74)].

$$LOO^{\cdot} + BR \rightarrow LOOH + BR^{\cdot}$$

$BR^{\cdot}$ maybe react or with another peroxyl radical to give a non-radical product, as shown in equation below:

$$BR^{\cdot} + LOO^{\cdot} \rightarrow BR\text{-}OOL$$

1.7.3. Uric acid

Uric acid is the last enzymatic product of the degradation of purine nucleosides [75] the structure is shown in the figure (1.8).

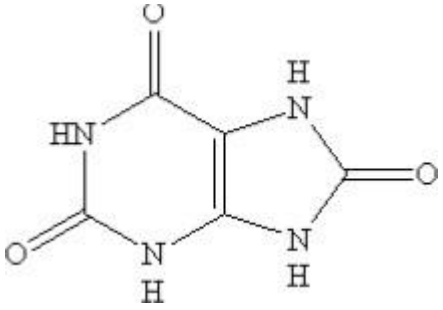


Figure (1.8) uric acid structure[76]

Uric acid generates by enzymatic conversion of hypoxanthine to xanthine which converts to uric acid at presence of xanthine oxidase, as shown in the figure (1.9)

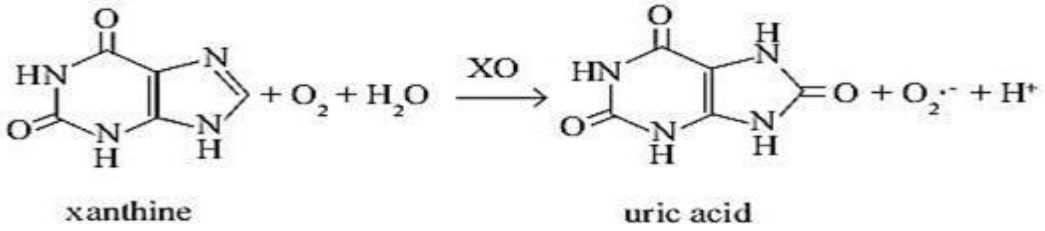


Figure (1.9) conversion xanthine to uric acid[77]

Uric acid considers an endogenous antioxidant in plasma, which produces about half of the total antioxidant capacity of human plasma with low molecular mass in the body fluids [78] [79].Uric acid able to scavenge oxidants such as singlet oxygen and oxygen radical's. It considers a major protective antioxidant against NO_2 and HOCI. Uric acid has the ability to chelate iron

[80] and copper and converting them to poorly reactive forms unable to catalyze free radical reactions [81].When uric acid reacts with free radical which produce relatively stable urate radical[82].

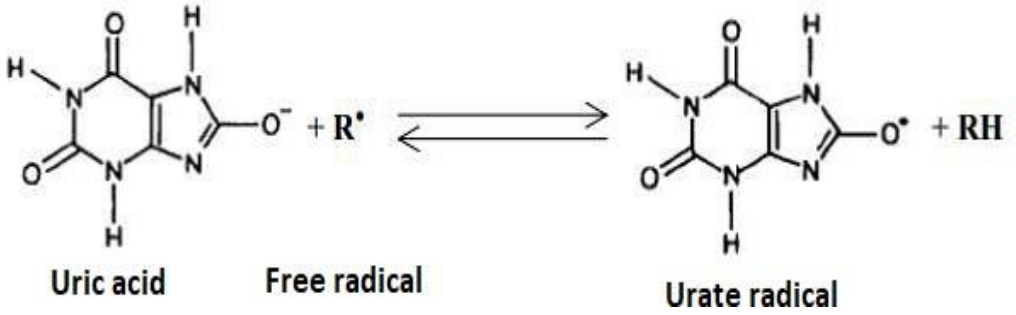


Figure (1.10) uric acid conversion to urate radical.

Uric acid has the ability to react with non-free radical like HOCl to form Allantoin. Allantoin considers most abundant and most stable oxidation product of uric acid. It can be measured in serum and synovial fluid, so it considers a biomarker of oxidative stress in Rheumatoid arthritis [83].

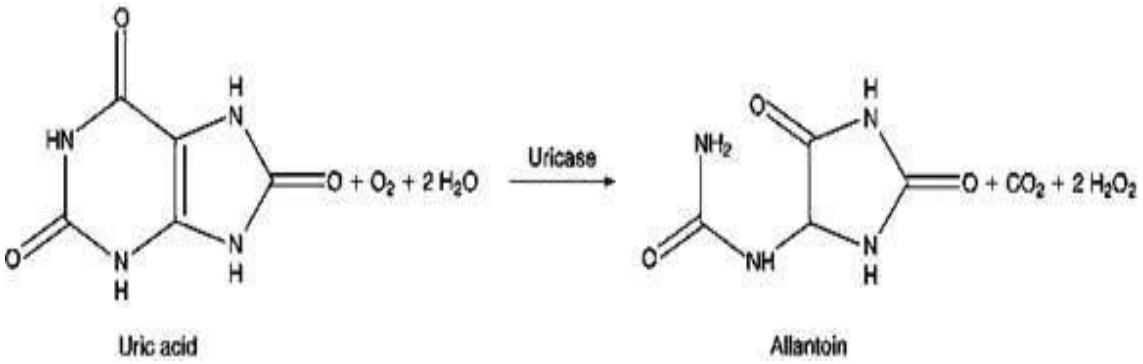


Figure (1.11) production of allantoin[84]

1.7.4. Glutathione (GSH)

It is also called γ-glutamyl, cysteinyl glycine. Glutathione is a tripeptide formed of three amino acids : (L-glutamyl, L-cysteninyl, glycine)[85][86]. GSH is an antioxidant found in plants, animals, fungi, and some bacteria and preventing damage to important cellular components caused by reactive oxygen species such as free radical ,peroxides, lipid peroxide, and heavy

metals[87].It is synthesized in two steps catalyzed by γ-glutamyl cysteine synthetase and glutathione synthetase[88].

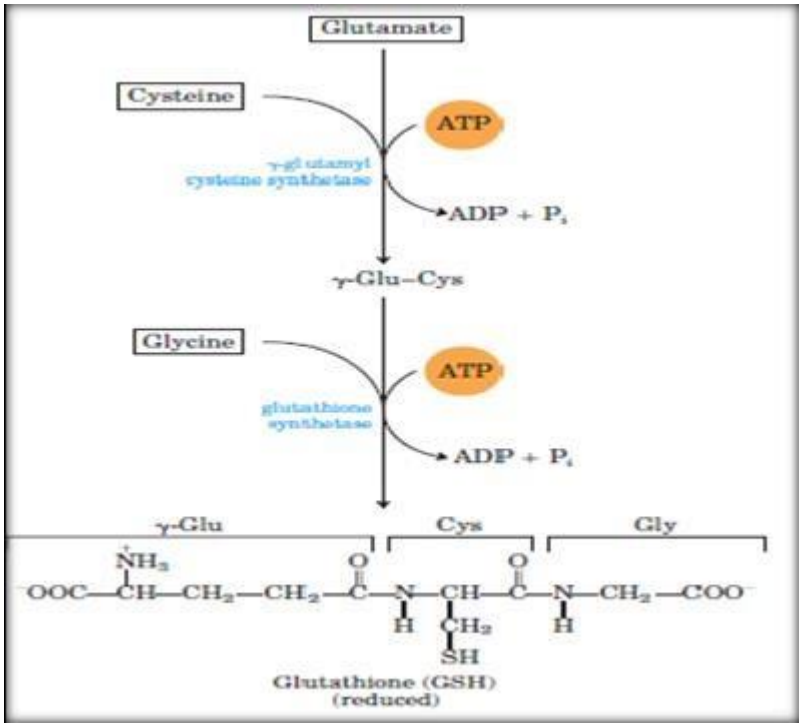


Figure (1.12) glutathione biosynthesis[85]

Glutathione have two forms which are present :reduced (G-SH) and oxidized (GSSG) . The -SH group indicates the sulfhydryl group of the cysteine and it is the most active part of the molecule[89].(GSSG) is converted to(G-SH) by the enzyme glutathione reductase (GR) and $NADPH^{+} + H^{+}$ [90].

Glutathione has several important functions, including defense mechanism against certain toxic compounds (T) as some drugs and carcinogens i.e. substances cause cancer. It combines with them to produce nontoxic compounds[91]:

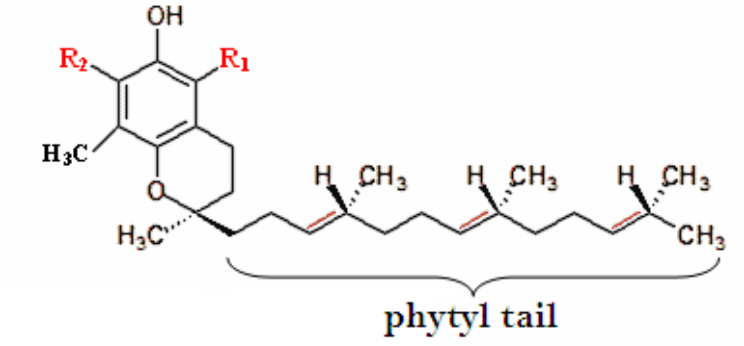
$$T\ (toxic) + 2G\text{-}SH \xrightarrow{\text{Glutathione S-transferase}} T\text{-}GS\ (nontoxic)$$

GSH breakdown the toxic hydrogen peroxide (H_2O_2), which causes damage to cell walls , e.g. protect RBC_S from hemolysis [92].

$$2GSH + H_2O_2 \longrightarrow GSSH + 2H_2O$$

1.7.5. α- Tocopherol (Vitamin E):

The fat soluble vitamin E refers to a group of antioxidants, which consists of four tocopherols and tocotrienols (α, β, γ, δ) , in which α-tocopherol has the highest biological activity[93].



	R1	R2
α-tocophérol	CH3	CH3
β-tocophérol	CH3	H
γ-tocotriénol	H	CH3
δ-tocopherol	H	H

Figure (1.13) the chemical structure of different forms of tocopherols[93]

Vitamin E is membrane-bound. Alpha tocopherol is the major lipid soluble, chain breaking antioxidant, which protects mammalian membranes and lipoproteins from damage. Vitamin E is mainly found on membranes where they either interrupt the propagation step of lipid peroxidation by destroying peroxyl radicals ($ROO^{\cdot}$) or block the formation of hydroperoxides from singlet oxygen [94][95]. Alpha tocopherols are efficient scavengers of peroxyl radicals in phospholipid bilayers. It scavenges lipid peroxyl radicals ($LOO^{\cdot}$) through hydrogen atom transfer. The α-tocopherol radical might also react with a further peroxyl radical to give a non radical product i.e. one molecule of α-tocopherol is capable of terminating two peroxidation chains [96].

$$\alpha\text{-TOH} + LOO^{\cdot} \longrightarrow \alpha\text{-TO} + LOOH$$

$$LOO^{\cdot} + \alpha\text{-TO}^{\cdot} \longrightarrow \alpha\text{-TocOOL}$$

1.7.6. L-Ascorbic acid (Vitamin -C)

Vitamin C is the major water-soluble antioxidant. It was found in vegetables, fruits (citrus) .At later time it was named Ascorbic acid [(97)].Vitamin C is very important factor to human health . This vitamin cannot be created via humans, so, it has been extracted from our diet [(98)]. There are many causes why vitamin C is crucial to our health. But several including its part as fundamental component in the synthesis of the collagen, norepinephrine and carnitine [(99)].

It was first suggested as an agent for cancer treatment in the 1950, its role in production and protection of collagen led scientists to assumption that ascorbate renewal would protect normal tissue from tumor invasiveness metastasis [(100)] and oxidative stress can also be assessed by measuring the serum antioxidant vitamins. Vitamin C is an excellent serum antioxidant because it dissolves in water and vitamin E dissolves in lipids. Both of them are naturally occurring free radical scavengers [(101)]. As concerns vitamin C, it has a potential role in the chemoprevention of cancer due to its function as a scavenger of free radicals, as well as the role it plays in vitamin E recycling [(102)], So its role in protect polyunsaturated fatty acid PUFA from oxidative damage [(103)], and in decreasing oxidative DNA damage [(102)(104)]. Studies from numerous laboratories in a variety animal models, using hepatoma, prostate cancer, pancreatic cancer, colon cancer, leukemia, sarcoma, and mesothelioma confirm that ascorbate concentrations which are enough for its cytotoxicity can be attained in vivo, and that treatments can minimize growth of tumor [(105)].

1.7.7. Trace elements

Trace elements like zinc, copper, selenium, magnesium, and manganese is involved in the antioxidant protection as cofactors for enzymes[(106)]. Superoxide dismutase is an antioxidant enzyme that

contains the trace elements zinc and copper [107]. Any changes in balance of the optimum levels of trace elements such as Zn, copper (Cu) and selenium (Se) may affect the biological pathways and they may associate with numerous diseases including cancer and chronic disorders [108].

1.7.7.1. Zinc

zinc element is found in the periodic table in group IIb, together with the two toxic metals cadmium and mercury, nevertheless zinc is considered to be comparatively nontoxic to humans [109]. Zn as a trace element has indispensable role in human health and diseases [110]. The wide distribution of zinc in all body tissues and fluids mirrors its essential role in metabolic activity as a component of key cell enzymes [111]. Zn is a constituent of more than three hundred enzymes which are involved in a broad series of activities, including anti-inflammatory and antioxidant effects [112] and genetic transcription regulation [113]. Zinc is a component of three hundred enzymes metalloenzymes and is important for cell growth and replication. Also Zn is important in conservation of suitable immune response [114].

1.7.7.2. Copper

Copper is a transitional element in periodic table. The copper II forms complexes with proteins, most of which are enzymes. A group of these constitutes copper metallic enzymes with oxidized activity [114], such as cytochrome oxidase, tyrosinase, ceruloplasmine, and monoamine oxidase [115]. Copper and zinc are important trace elements related to health and disease. Abnormalities of Cu and Zn are involved in the etiology and pathogenesis of vitiligo. First, Cu and Zn are integral parts of many metalloenzymes necessary in the process of melanogenesis. During the final stage of eumelanin formation in melanogenesis, those metalloenzymes catalyze the rearrangement of dopachrome to form 5,6-dihydroxy indole-2 carboxylic acid and the enhancement of eumelanin polymer formation from monomers[116][117]. Second, Cu and zinc are

considered as antioxidants, being constituents of superoxide dismutase, protecting the body against cytotoxicity of melanocytes and inhibition of tyrosinase in oxidative stress[118].

1.8. Lipid profile

1.8.1. Cholesterol

Cholesterol is a soft, waxy substance found in the blood stream and the body's cells, it consists of four linked hydrocarbon rings forming the bulky steroid structure. There is a hydrocarbon tail linked to one end of the steroid and a hydroxyl group linked to the other end. Cholesterol is known as a "sterol" because it is made out of alcohol and steroid [119]. It is carried by two types of lipoprotein; low-density lipoprotein (LDL) carried bad cholesterol and high-density lipoprotein (HDL) carried good cholesterol. Cholesterol is an extremely important biological molecule that has roles in contributes to the structure of cell walls, makes up digestive bile acids in the intestine, allows the body to produce vitamin D and enables the body to make certain hormones[120].Synthesis of cholesterol, like that of most biological lipids, begins from the two-carbon acetate group of acetyl-CoA. The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (e.g., fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that described for fatty acid synthesis. Acetyl-CoA can also be synthesized from cytosolic acetate derived from cytoplasmic oxidation of ethanol which is initiated by cytoplasmic alcohol dehydrogenase (ADH). All the reduction reactions of cholesterol biosynthesis use NADPH as a cofactor.

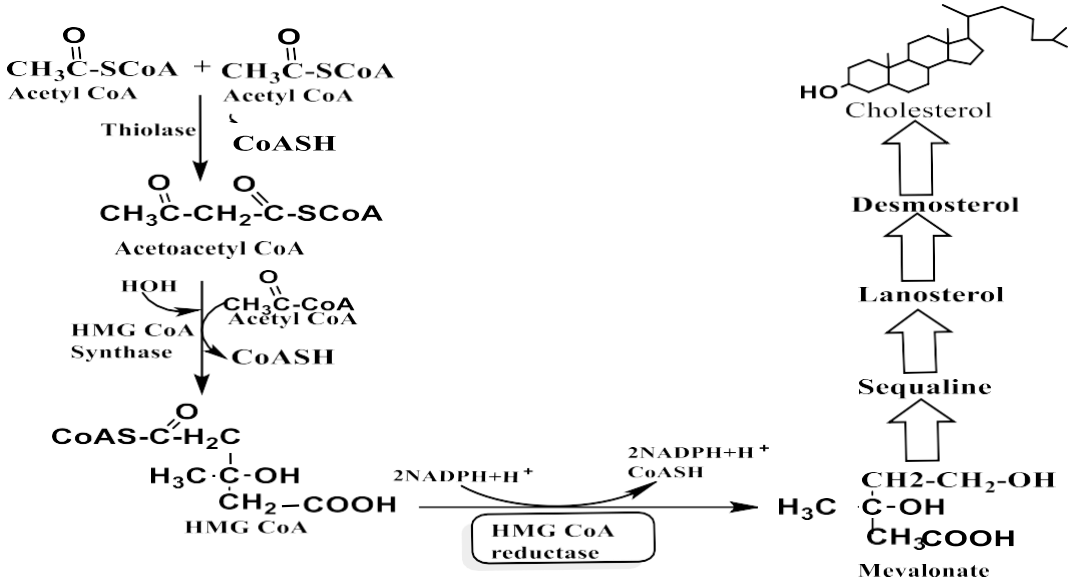


Figure (1.14) Cholesterol biosynthesis[76].

Hypercholesterolemia is a condition when there is an extremely high level of cholesterol in the body. Usually this means that there is a high concentration of LDL and low concentration of HDL. When too much LDL circulates the blood cell, it can built up the inner walls of arteries that feed the heart and brain, therefore, cause the clogging of the arteries. The health significance is that they are prone to cardiovascular diseases. If clot forms and blocks the narrowed artery, a series of cardiovascular diseases such as hypertension, arteriosclerosis, heart attack or stroke can result. High levels of cholesterol are also closely associated to diabetes [121].

1.8.2. Triglycerides (TG)

A triglyceride is an ester derived from glycerol and three fatty acids. Triglycerides are the main constituent of body fat in humans and animals. There are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils [122]. There are many different types of triglyceride, with the main division being between saturated and unsaturated types. Saturated fat are "saturated" with hydrogen all available places where hydrogen atoms could be bonded to carbon atoms are occupied. These are a higher melting point and are more likely to be

solid at room temperature. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These are a lower melting point and are more likely to be liquid at room temperature [123]. The overall process of triglyceride biosynthesis consists of four biochemical pathways: fatty acyl-CoA biosynthesis, conversion of fatty acyl-CoA to phosphatidic acid, conversion of phosphatidic acid to diacylglycerol, finally conversion of diacylglycerol to triglycerol [124].

1.8.3. High density lipoprotein (HDL)

High density lipoprotein is the smallest of the lipoprotein particles . It is composed of 80-100 proteins particle which transport all fat molecule (lipids) around the body within the water outside cells. The fat carried include cholesterol, phospholipids, and triglycerides [125]. The liver synthesizes lipoproteins as complex of apolipoproteins and phospholipid, which resemble cholesterol-free flattened spherical lipoprotein particles, the complexes are capable of picking up cholesterol carried internally from cells by interaction with the ATP-binding cassette transporter A1(ABC A1). A plasma enzyme called lecithin-cholesterol acyl transferase (LCAT) converts the free cholesterol into cholesterol ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of the lipoprotein particle, eventually causing the newly synthesized HDL to assume a spherical shape. HDL particles increase in size as they circulate through the blood stream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins [126].

1.8.4. Low density lipoprotein (LDL)

Low density lipoprotein is one of the five major groups of lipoprotein, LDL has a highly hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and hundreds to thousands esterified and unesterified cholesterol molecules. This core carries varying numbers of triglycerides and other fats and is surrounded by a shell of phospholipids

and unesterified cholesterol [127]. LDL particles are sometimes referred to as bad cholesterol because they can transport their content of fat molecules into artery walls, attract macrophages and thus atherosclerosis [128].

1.9. Thyroid hormones

The thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are tyrosine-based hormones produced by the thyroid gland that are primarily responsible for regulation of metabolism. Iodine is necessary for the production of T3 and T4. A deficiency of iodine leads to decreased production of T3and T4, enlarges the thyroid tissue and will cause the disease known as goitre. The major form of thyroid hormone in the blood is thyroxine (T4), which has a longer half-life than T3. The thyronines act on nearly every cell in the body. They act to increase the basal metabolic rate, affect protein synthesis, help regulate long bone growth (synergy with growth hormone) and neuronal maturation, and increase the body's sensitivity to catecholamines (such as adrenaline) by permissiveness. The thyroid hormones are essential to proper development and differentiation of all cells of the human body. These hormones also regulate protein, fat, and carbohydrate metabolism, affecting how human cells use energetic compounds. They also stimulate vitamin metabolism. Numerous physiological and pathological stimuli influence thyroid hormone synthesis. Thyroid hormones (T4 and T3) are produced by the follicular cells of the thyroid gland and are regulated by TSH made by the thyrotropes of the anterior pituitary gland[129].

1.9.1. Effect of iodine deficiency on thyroid hormones synthesis.

If there is a deficiency of dietary iodine, the thyroid will not be able to make thyroid hormone. The lack of thyroid hormone will lead to decreased negative feedback on the pituitary, leading to increased production of thyroid-stimulating hormone, which causes the thyroid to enlarge[130][131].

Hyperthyroidism (an example is Graves Disease) is the clinical syndrome caused by an excess of circulating free thyroxine, free

triiodothyronine, or both. It is a common disorder that affects approximately 2% of women and 0.2% of men.

Hypothyroidism (an example is Hashimoto's thyroiditis) is the case where there is a deficiency of thyroxine, triiodiothyronine, or both. Clinical depression can sometimes be caused by hypothyroidism[132][133].

1.9.2. Thyroid-stimulating hormone

Thyroid-stimulating hormone (also known as TSH or thyrotropin) is a hormone that stimulates the thyroid gland to produce thyroxine (T4), and then triiodothyronine (T3) which stimulates the metabolism of almost every tissue in the body. It is a glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, which regulates the endocrine function of thethyroid gland[134]. The hypothalamus, in the base of the brain, produces thyrotropin-releasing hormone (TRH). TRH stimulates the pituitary gland to produce TSH. Somatostatin is also produced by the hypothalamus, and has an opposite effect on the pituitary production of TSH, decreasing or inhibiting its release. The concentration of thyroid hormones (T3 and T4) in the blood regulates the pituitary release of TSH; when T3 and T4 concentrations are low, the production of TSH is increased, and, conversely, when T3 and T4 concentrations are high, TSH production is decreased. This is an example of a negative feedback loop[135][136]. TSH concentrations are measured as part of a thyroid function test in patients suspected of having an excess (hyperthyroidism) or deficiency (hypothyroidism) of thyroid hormones[137].

1.10. Phototherapy of vitiligo

The first report of the use of phototherapy in the treatment of skin disorders in India goes back to 1400 BC when the vitiligo patients were given certain plant extracts such as *Ammi majus* and *Psoralea corylifolia* (whose active ingredients included psoralen) and then provided with sun exposure [138]. The modern day phototherapy came into existence in 1903 when Niels Finsen used UV irradiation

for treating lupus vulgris [139]. Systemic phototherapy gives very satisfactory repigmentation in up to 70% of patients having early or localized depigmented macules [140]. The phototherapy is widely used for the treatment of vitiligo and it is very efficient method in the treatment of vitiligo. The ultimate aim of phototherapy is to stimulate the melanocytes residing in the hair follicles, to migrate and repopulate the lesional areas of vitiligo patients in which the melanocytes are lost.

1.10.1. Psoralen plus ultraviolet A (PUVA) phototherapy

It involves the use of psoralen along with UVA radiations. In this case, the treatment contained dosage of 5-methoxypsoralen, 8-methoxypsoralen and trimethylpsoralen with UVA (PUVA). It is specially given to the patients having widespread vitiligo. Psoralens can be administered either orally or topically, followed by exposure with natural sunlight or artificial UV light. This therapy gives best results on the face, proximal parts of the extremities and trunk region. After long-term treatment with PUVA approximately 50% of patients shows some repigmentation [141].

1.10.2. Narrow-band UV-B (NB-UVB) phototherapy

Phototherapy is widely used that gives good results. A narrow-band fluorescent tube with an emission spectrum of wavelength ranging from 310-315 nm is used in vitiligo treatment. This phototherapy treatment can also be used safely in pregnant ladies, children and lactating women[142]. NB-UVB radiations also impart some short-term adverse effects including xerosis and pruritis. The advantages of NB-UVB over PUVA include no drug costs, no time wastage, short duration, no adverse gastro-intestinal effects (e.g. nausea) and no need for subsequent photoprotection.

1.10.3. UV-B narrow-band microphototherapy

This therapy targets small and specific depigmented macules. In this case, selective NB-UVB with specific wavelength of 311 nm is used with a fibre optic system to direct the radiation to specific affected areas of skin. Because of fewer side effects, it has become the first choice of therapy used for children and adults with generalized vitiligo [(143)(144)].

1.10.4. Excimer laser treatment

Targeted phototherapy involves the administration of light to localized diseased skin areas. Since only the specific affected area is exposed to the radiations, higher doses of radiations can be used to achieve faster and better results with lower hazards of phototoxicity. It can be used to treat those areas of skin which are not easily reachable like skin folds. The treatment using ultraviolet B excimer laser also known as Xenon Chloride laser (XeCl) and monochromatic excimer light utilize UV light of wavelength 308 nm and are almost similar to the NBUV-B treatment. The difference between the two is that the excimer lasers are more selective and specific in vitiligo treatment with fewer side effects. The use of MEL i.e. monochromatic excimer was first described in 1997 [(145)]. This laser offers the advantage of delivering high doses of light to localized areas [(146)(147)]. These methods seem to be helpful in especially young patients, particularly with localised and SV [(143)].

1.11. Aims of the study

This study aims to:

1- Study the oxidative stress in serum of vitiligo patients by evaluating some of its parameters levels (oxidants and antioxidants) in serum of patients with the disease as a whole and along disease activity.

2- The prognosis of drugs and the response of body to that drug by measuring the biochemical testes.
3- Find the pathogenesis of vitiligo.
4- Find a relationship between the disease and the biochemical testes.
5- This study is designed to evaluate the efficacy and safety of narrow band UVB (NB UVB) on the improvement of the drugs that used in the treatment of vitiligo.

Chapter Two

Materials & Methods

Chapter Two
Materials and Methods

2.1. Specimen Collections:

Specimen for 80 patients (30 males and 50 females) and 40 healthy individuals (8 males and 32 females) were collected from the specific dermatological clinic by using sterile disposable syringe (10 ml) and then centrifuged to separate the serum. The sampling persists from july 2018 to january 2019. Then the sera are stored by 10 tubes in deep freeze -20˚C until use.

2.2. Chemicals and instruments

The specific chemicals and instruments used in this study are listed in tables (2.1) and (2.2) respectively with the its companies produced.

Table (2.1): Chemicals and reagents.

No	Chemicals & Reagents	Company
1	5,5- Di-thio bis (2-nitro benzoic acid) (DTNB) (C14H8S2O8N2)	BDH chemical Ltd., England
2	Cholesterol kit	Linear (Spain)
3	HDL-cholesterol kit	Linear (Spain)
4	LDL-cholesterol kit	Linear (Spain)
5	MDA	Biodiagnostic (Egypt)
6	Total bilirubin	Randox (England)
7	Triglyceride kit	Linear (Spain)
8	T3 kit	Monobind (USA)
9	T4 kit	Monobind (USA)
10	TSH kit	Monobind (USA)
11	Uric acid	Linear (Spain)
12	Vitamin C standard	Fluka (Germany)
13	Vitamin E standard	Fluka (Germany)

Table (2.2): Instruments and their manufacture company

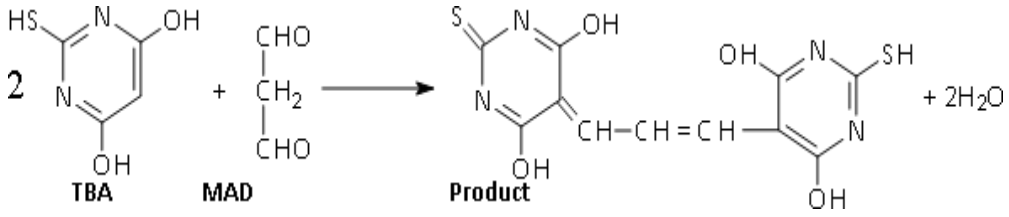
No	Instruments	Company
1	Atomic absorption spectrophotometer	Shimadzu AA-7000
2	Centrifuge	KUBUTA-Tokyo
3	CBC Hematology Analyzer	Swelab-Switerland
4	Deep Freeze	Germany
5	ELISA Reader &Washer	Biotic-USA
6	Incubator	Memmert-Germany
7	pH- meter	Oakton-Singapore
8	Sensitive balance	Metter Toledo-Switzerland
9	UV-visible spectrophotometer	Aquarius-England

2.3. Oxidative Stress Biomarkers

2.3.1. Estimation of Serum Lipid Peroxide Malondialdehyde (MDA)

Basic Principle:

Lipid peroxidation end products, particularly malondialdehyde (MDA) react with thiobarbituric acid under acidic conditions and heating (95^{o}C (for 30 min to give a pink color that is measured spectrophotometrically at 534 nm [148][149] by using kit biodiagnostic (Egypt).



Using Reagents:

	Reagent	Concentration
1.	Standard MDA	10 nmol/ml
2.	Chromogen Thiobarbituric acid Detergent Stabilizer	25 nmol/ml

Procedure:

TUBES	**Sample** (ml)	**Standard** (ml)	**Blank** (ml)
Sample (Serum)	0.2	--	--
Standard	--	0.2	--
Chromogen	1.0	1.0	1.0
Mix well; incubate in a boiling water bath 95^{o}C for 30 minutes, cooling ,added:-			
Sample (Serum)	--	--	0.2

Calculation:

$$\textbf{Malondialdehyde} = \frac{A_{Sample}}{A_{Standard}} \times 10 \text{ (nmol/ml)}$$

2.3.2. Estimation of total serum bilirubin

Principle

Sulfanilic acid reacts with sodium nitrite to form diazotized sulfanilic acid. In the presence of caffeine, total bilirubin reacts with diazotized sulfanitic acid to form azobilirubin [(150)] by using kit Randox (England).

Solutions

	TUBES	Initial concentration of solution
1.	**Sulphanilic acid** **Hydrochloric acid**	**29 mmol/l** **0.17 N**
2.	**Sodium nitrite**	**25mmol/l**
3.	**Caffeine** **Sodium benzoate**	**0.26 mol/l** **0.52 mol/l**
4.	**Tartrate** **Sodium hydroxide**	**0.93 mol/l** **1.9 N**

Procedure

TUBES	**Blank** (ml)	**Sample** (ml)
Reagent 1	0.2	0.2
Reagent 2	-	0.05
Reagent 3	1.0	1.0
Sample	0.2	0.2

Tubes were mixed well and incubate for 10 minutes at (20- 25°C) and added 1(ml) reagent 4.Mixed well and incubate for 15 minutes at (20-25°C). Read the absorbance of A_{TB} at 560 nm.

Calculation

S. T.B (mg/dl) = ATB x 10.8

2.3.3. Estimation of Serum Uric acid [151].

Princple :

Uric acid is oxidized by uricase to allantoin with the formation of hydrogen peroxide. In the presence of peroxidase (POD), a mixture of dichlorophenol sulphonate (DCPS) and 4-aminoantipyrine (4-AA) is oxidized by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of uric acid in the sample.

$$\text{Uric acid} + O_2 + 2H_2O \xrightarrow{\text{Uricase}} \text{Allantoin} + H_2O_2$$

$$\text{4-AA} + \text{DCPS} \xrightarrow[\text{POD}]{H_2O_2} \text{Quinoneimine} + 4\ H2O$$

Reagents: -

R_1 Mono reagent	Concentration
Phosphate Buffer pH = 7.8	100 mmol/l
Uricase	> 50 U/L
Peroxidase	> 0.1kU/L
4-Aminophenazone	0.32 mmol/l
ascorbate oxidase	> 0.1 KU/l
DCPS	2 mmol/l
non-ionic tensioactives	2 g/L (w/v)
Biocides	
CAL. Standard	Concentration
Uric acid standard	6 mg/dL

Procedure

1. I bring reagents and samples to room temperature.
2. I pipette into labeled tubes:

Tubes	Blank (µl)	Sample (µl)	CAL.Standard (µl)
R1.Monoreagent	1000	1000	1000
Sample	-	25	-
CAL.Standard	-	-	25

3. I mix and let the tubes stand 10 minutes at room temperature or 5 minutes at 37ºC.

4. Finally I record the absorbance (A) at 520 nm for the samples and the standard against the blank solution.

Calculations

$$\textbf{Uric acid mg/dl} = \frac{A_{Sample}}{A_{Standard}} \times C_{Standard}$$

2.3.4. Estimation of Glutathion Concentration in Blood Serum

The level of glutathion in blood serum is measured by using Ellman's reagent method[152][153].

Basic Principle

Total glutathion was determined by using Ellman's reagent it is (5,5 di thio bis (2-Nitrobenzoic acid) (DTNB),it reacts quickly with gluthathion and reduced (SH group) of glutathion to give a colored product that is measured spectrophotometrically at 412 nm .

Preparation of Reagents

1- Sulfosalicylic acid (S.S.A)

   Prepare by dissolve (4gm) sulfosalicylic acid in (100ml) of distilled water and keep in refrigerator.

2- Phosphate buffer solution

   Prepare by mixing (0.6M KH_2PO_4) and (0.08M Na_2HPO_4) at (pH8)

3- Ellman's reagent(0.1mM)

   Prepare by dissolve (0.00396gm) DTNB in (100ml) of buffer solution Na_2HPO_4 (pH8) and keep in refrigerator.

Procedure

TUBES	**Sample** (ml)	**Blank** (ml)
Serum	0.150	--
Distilled water	--	0.150
Sulfosalicylic acid 4%	0.150	0.150
For 5min Mix and put in centrifuge at 2000 rpm		
Supernatant	0.150	0.150
Ellman's reagent 0.1 mmol	4.50	4.50

Calculations

The concentration of GSH µmol/L = $\frac{A_{Samble}}{{}^{*}E_o \times {}^{*}L} x\ 10^6$

*E = Extinction coefficient 13600 M^{-1} cm^{-1}

*L = light bath 1 cm

2.3.5. Estimation of Vitamin E (α-Tocopherol) in blood serum

Basic Principle

Tocopherol can be estimated using Emmeric-Engle reaction which based on the oxidation-reduction reactions, The principle of determination includes the oxidation of Tocopherol to Tocopherol Quinon by ($FeCl_2$ Ferous Chloride), reducing Ferric to Ferrous Fe(II) ion, which then forms complex a red colour with α-α-dipyridyl .Tocopherol and Carotenes are first extracted with Xylene and the extraction read at 460 nm to measure Carotenes. A correlation is made for these after adding ferric chloride and reading at 520 nm to measure vitamin E[(154)].

Preparation of Reagents

1- Absolute ethanol

2- Xylene

3- α-α-dipyridyl

Prepare by dissolve (1.2gm) α-α-dipyridyl in (100ml) of propanol.

4- Ferric chloride solution

Prepare by dissolve (1.2gm) ($FeCl_3.6H_2O$) in (100ml) of ethanol.

5- Standard solution of vitamin E

Prepare by dissolve (10 gm) of vitamin E in (100ml) of ethanol.

Procedure

TUBES	**Blank** (ml)	**Standard** (ml)	**Test** (ml)
Distilled Water	1.5	-	-
Standard Solution	-	1.5	-
Serum	-	-	1.5
Ethanol	1.5	-	1.5
Distilled Water	-	1.5	-
Xylene	1.5	1.5	1.5
TUBES	**Blank** (ml)	**Standard** (ml)	**Test** (ml)
Xylene layer	1.00	1.00	1.00
α–α-Dipyridyl	1.00	1.00	1.00

Tubes were mixed well and read the absorbance at 460 nm and added 0.33 ml ferric chloride solution and read absorbance at 520 nm.

Calculation

$$\text{Serum Tocopherol (mg/l)} = \frac{A_{\text{test at 520 nm}} - (A_{\text{test at 460 mn}} \times 0.29)}{A_{\text{standard at 520 nm}}} \times (10 \text{ mg/l})$$

2.3.6. Assay of Serum ascorbic acid (Vitamin-C) [(155)].

Principle: -

Vitamin C was evaluated by the method of Omaye et al., (1962). Ascorbic acid was oxidized by copper to form dehydroascorbic acid and di-keto glutaric acid. These products when treated with 2,4-

dinitrophenylhydrazine (DNPH) formed the derivative bis-2-4-dinitrophenylhydrazone, which underwent rearrangement to form a product with maximum absorption at 520 nm (fig. 2-1). Thiourea provided a mild reducing medium that helped to prevent interference from non-ascorbic acid chromogens.

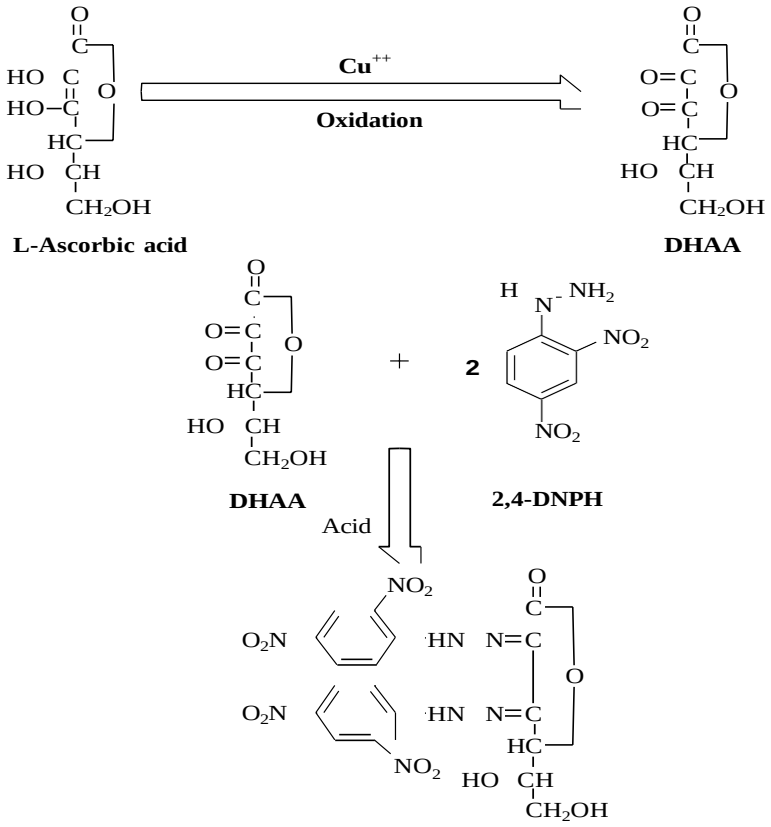


Figure (2.1): Formation of bis-2-4-dinitrophenylhydrazone derivative from reaction vitamin-C

Reagents:-

No	Reagents	Amounts
1.	**DTC Reagent:** 0.4 gm thiourea, 0.05 gm copper sulphate and 3.0 gm DNPH were dissolved in 100.0 ml of 9N H_2SO_4.	100 ml
2.	TCA 5%	500 ml
3	TCA 10%	100 ml
4	H_2SO_4 9N	100 ml
5	H_2SO_4 65 %	150 ml

Preparation of Standard Vitamin-C solution:

I dissolve 2 mg of powder standard vitamin C in 100 ml of 5 % TCA, and then make several concentrations that range between (0-20) mg/l.

Procedure:

1. Two sets of tubes were prepared as follow:

Reagent	Sample µl	Blank µl
Serum	500	-
TCA 10%	500	-
Sample test tube was mixed, and centrifuged at 3500 rpm at 4°C for (20) minutes, then:		
Supernatant	500	-
d. H_2O	-	500
DTC	100	100
The tubes were incubated at 37°C for three hours		
Ice cold 65 % H_2SO_4	750	750

1. I mix tubes then let them in room temperature for 30 mints.
2. Finally I read the absorbance for each standards and samples at 450 nm.

Calculation:-

1. I draw a standard curve by plotting the absorbance obtained for each standard against its concentration in mg/l, I make the values of absorbance on the vertical or Y axis, and concentrations on the horizontal or X axis.
2. To determine the concentration of vitamin-C for an unknown, I locate absorbance of the unknown on the vertical axis of the diagram, I find the intersecting point on the curve, and I read the concentration in (mg/dl) from the horizontal axis of the diagram.

2.3.7. Determination of copper and zinc [156].

The determination of concentration Cu and Zn in serum by atomic absorption spectrophotometer (AAS) is the most reliable test analytically . In AAS, the element is merely dissociated from its chemical bonds and it is place in a ground state (neutral atom). Thus, the neutral atom is at a low

energy level in which is capable of absorbing radiation at a very narrow band width conformable to its own line spectrum.

Reagents

Standard solution of copper and zinc (1000µg/ml).

Parameter condition

- Wave length for Cu hollow cathode lamp used was 324 nm.
- Wave length from Zn hollow cathode lamp used was 213.9 nm.

Procedure

The serum samples were diluted by using double distil water in ratio 1:3 before the determination of the ions of the elements by the atomic absorption spectrophotometer.

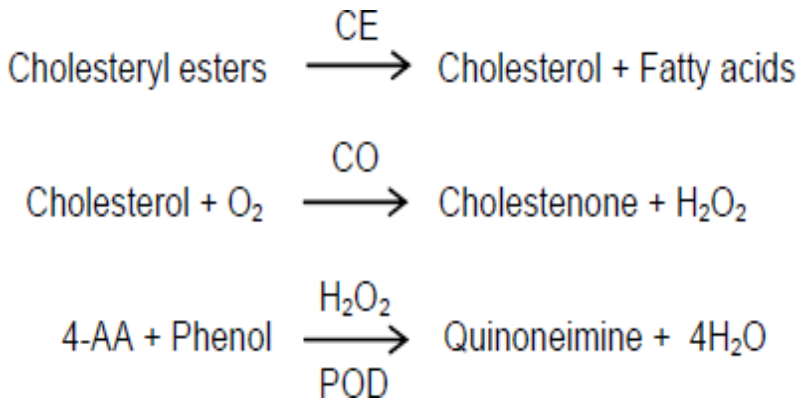
2.4. Estimation of Lipid Profile

2.4.1. Estimation of Total Cholesterol

Serum cholesterol was determined by the using of enzymatic colorimetric method, and by using a ready kit from reliable scientific company.

Principle

This method for the measurement of total cholesterol in serum involves the use of three enzymes: cholesterol esterase (CE), cholesterol oxidase (CO) and peroxidase (POD). In the presence of the former the mixture of phenol and 4-aminoantipyrine (4-AA) are condensed by hydrogen peroxide to form a quinoneimine dye proportional to the concentration of cholesterol in the sample[(157)].



Reagent composition

Monoreagent (R1): Pipes 200 mmol/l pH 7.0, sodium cholate 1 mmol/l, cholesterol esterase > 250 U/L, cholesterol oxidase > 250 U/L, peroxidase > 1 KU/l, 4-aminoantipyrine 0.33 mmol/L, phenol 4 mmol/l, non-ionic tensioactives 2 g/L (w/v).

Cholesterol standard: Cholesterol 200 mg/dl.

Procedure

- Bring reagents and samples to room temperature.

- Pipette into labeled tubes:

Tube	Blank	Sample	Standard
R1 monoreagent	1 ml	1 ml	1 ml
Sample	-	10 µl	-
Standard	-	-	10 µl

- Mix and let the tubes stand 10 minutes at room temperature or 5 minutes at 37 ºC.
- Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

Calculations

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = \text{mg/dl total cholesterol}$$

2.4.2. Estimation of Triglycerides

Serum triglyceride was determined by the using of enzymatic colorimetric method, and by using a ready kit from reliable scientific company.

Principle

The method is based on the enzymatic hydrolysis of serum or plasma triglyceride to glycerol and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosin triphosphate (ATP) in the presence of glycerolkinase (GK) to form glycerol-3-phosphate (G-3-P) and adenosine diphosphate (ADP). G-3-P is oxidized by glycerophosphate oxidase (GPO) to form dihydroxyacetone phosphate (DHAP) and hydrogen peroxide.

A red chromogen is produced by the peroxidase (POD) catalyzed coupling of 4-aminoantipyrine (4-AA) and phenol with hydrogen peroxide (H2O2), proportional to the concentration of triglyceride in the sample[158].

$$\text{Triglycerides} + 3\,H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\,\text{FFA}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-P} + \text{ADP}$$

$$\text{Glycerol-3-P} + O_2 \xrightarrow{\text{GPO}} \text{DHAP} + H_2O_2$$

$$\text{4-AA} + \text{4 Phenol} \xrightarrow[\text{POD}]{H_2O_2} \text{Quinoneimine} + H_2O$$

Reagent composition.

Monoreagent (R1): Pipes buffer 50 mmol/L pH 6.8, LPL≥ 12 KU/L, GK ≥ 1 KU/L, GPO ≥ 10 KU/L, ATP 2.0 mmol/L, Mg^{2+} 40 mmol/L, POD ≥ 2.5 KU/L, 4-AA 0.5 mmol/L, phenol 3 mmol/L, non-ionic tensioactives 2 g/L (w/v).

Triglycerides standard: glycerol trioleate 200 mg/dl.

Procedure

- Bring reagents and samples to room temperature.

- Pipette into labeled tubes:

Tube	Blank	Sample	Standard
R1 monoreagent	1 ml	1 ml	1 ml
Sample	-	10 µl	-
Standard	-	-	10 µl

- Mix and let the tubes stand 10 minutes at room temperature or 5 minutes at 37 ºC.

- Read the absorbance (A) of the samples and the standard at 500 nm against the reagent blank.

The color is stable for at least 30 minutes protected from light.

Calculations

$$\frac{A_{Sample}}{A_{Standard}} \times C_{Standard} = \text{mg/dl triglyceride}$$

2.4.3. Estimation of LDL-Cholesterol.

Serum LDL-Cholesterol was determined by the use of differential precipitation enzymatic colorimetric test, and by using a ready kit from reliable scientific company.

Principle

This technique uses a separation method based on the specific precipitation of low-density lipoproteins (LDL) by polyvinyl sulfate in whole serum, sedimentation of the precipitant by centrifugation, and subsequent test as residual cholesterol of the rest of lipoproteins (VLDL+ HDL) remaining in the clear supernatant[(159)].

Reagent composition.

Precipitating reagent: Polyvinylsulfate 1 g/L, polyethylenglicol 170 g/L. Stabilizers.

LDL-Cholesterol standard: Cholesterol 50 mg/dl.

Procedure

***Precipitation.**

1. Bring reagents and samples to room temperature.

2. Pipette into labeled centrifuge tubes:

Sample or standard	0.2 ml
Precipitating reagent	1 ml

1. Vortex and allow to stand for 10 minutes at room temperature.
2. Centrifuge for 10 minutes at 6000 r.p.m., or 2 minutes at 12000 r.p.m
3. Remove an aliquot of the supernatant for measurement of cholesterol.

***Colorimetry.**

1. Bring the components of the kit and the components of the Cholesterol MR to room temperature.

2. Prepare two series of tests to measure in parallel the total cholesterol of the sample and the remaining cholesterol in the

supernatant. Follow for total cholesterol the instructions of the insert.

3. Pipette into labeled tubes:

Tube	Blank	Sample supernatant	Standard
Monoreagent	1 ml	1 ml	1 ml
Supernatant	-	50 µl	-
Standard	-	-	50 µl

The color is stable for at least 30 minutes protected from light.

Calculations

$$\frac{A_{Supernatant}}{A_{Standard}} \times C_{Standard} = \text{mg/dl cholesterol}$$

LDL-Cholesterol = mg/dl $\text{Cholesterol}_{Total}$ – mg/dl $\text{Cholesterol}_{supernatant}$

2.4.4. Estimation of HDL-Cholesterol.

Serum LDL-Cholesterol was determined by the using of differential precipitation enzymatic colorimetric test, and by using a ready kit from reliable scientific company.

Principle

This technique uses a separation method based on the selective precipitation of apoliprotein B-containing lipoproteins (VLDL and LDL) by phosphotungstic acid/MgCl2, sedimentation of the precipitant by centrifugation, and subsequent enzymatic analysis of high density lipoproteins (HDL) as residual cholesterol remaining in the clear supernatant[(160)].

Reagent composition.

Precipitating reagent: Phosphotungstic acid 0.63 m mol/L, magnesium chloride 25 m mol/L. Stabilizers.

LDL-Cholesterol standard: Cholesterol 50 mg/dl.

Procedure

***Precipitation.**

1. Bring reagents and samples to room temperature.
2. Pipette into labeled centrifuge tubes:

Sample or standard	0.2 ml
Precipitating reagent	0.4 ml

1. Vortex and allow to stand for 10 minutes at room temperature.
2. Centrifuge for 10 minutes at 6000 r.p.m., or two minutes at 12000 r.p.m
3. Separate off the clear supernatant within 2 hours.

***Colorimetry.**

1. Bring the components of the kit and the components of the Cholesterol MR to room temperature.
2. Prepare two series of tests to measure in parallel the total cholesterol of the sample and the remaining cholesterol in the supernatant. Follow for total cholesterol the instructions of the insert.
3. Pipette into labeled tubes:

Tube	Blank	Sample supernatant	Standard
Monoreagent	1 ml	1 ml	1 ml
Supernatant	-	50 µl	-

Standard	-	-	50 µl

The color is stable for at least 30 minutes protected from light.

Calculations

$$\frac{A_{Supernatant}}{A_{Standard}} \times C_{Standard} = \text{mg/dl HDL-Cholesterol}$$

2.5. Estimation of Thyroid hormones.

2.5.1. Estimation of Serum T3.

This method is a quantitative determination of total triiodothyronine concentration in human serum or plasma by a Microplate Enzyme Immunoassay.

Principle

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained[(161)].

Reagents

A/ Human Serum References : 1ml/vial - Icons A-F Six (6) vials of serum reference for triiodothyronine at concentrations of 0 (A), 0.5 (B), 1.0 (C), 2.5 (D), 5.0(E) and 7.5(F) ng/ml. Store at 2-8°C. A preservative has been added. For SI units: ng/ml x 1.536 = nmol/L.

B/ T3 Enzyme Reagent: 1.5ml/vial – Icon. One(1) vial of T3-horseradish peroxidase (HRP) conjugate in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C/ T3/T4 Conjugate Buffer : 13ml – Icon. One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D/ T3 Antibody Coated Plate : 96 wells – Icon. One 96-well microplate coated with Sheep anti-T3 serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E/ Wash Solution Concentrate : 20ml – Icon. One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F/ Substrate A : 7 ml/vial – Icon. One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G/ Substrate B : 7 ml/vial – Icon. One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

H/ Stop Solution : 8ml/vial - Icon STOP. One (1) bottle of stop solution containing a strong acid (1N HCL). Store at 2-30°C.

I/ product instructions.

Reagent preparation

1. Working Reagent A : T3-enzyme Conjugate Solution Dilute the T3-enzyme conjugate 1:11 with T3/T4 conjugate buffer in a suitable ontainer.

For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2- 8°C.

General Formula:

Amount of Buffer required = Number of wells * 0.1

Quantity of T3-Enzyme necessary = # of wells * 0.01

i.e.= 16 x 0.1 = 1.6ml for Total T3/T4 Conjugate Buffer

16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate.

2. Wash Buffer : Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.

3. Working Substrate Solution : Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2-8°C.

Procedure

Bring all reagents, serum, controls to room temperature.

1. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
2. Add 0.100 ml (100µl) of Working Reagent A, T3 Enzyme Reagent to all wells (see Reagent Preparation Section).
3. Swirl the microplate gently for 20-30 seconds to mix and cover.

4. Incubate 60 minutes at room temperature.

5. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

6. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

7. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

8. Incubate at room temperature for fifteen (15) minutes.

9. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.

10. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader.

Calculations

1. Record the absorbance obtained from the printout of the microplate reader .
2. Plot the absorbance for each serum reference versus the corresponding T3 concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.

4. Determine the concentration of T3 for an unknown by using the standard curve.

2.5.2. Estimation of Serum T4.

This method is a quantitative determination of total thyroxine concentration in human serum or plasma by a Microplate Enzyme Immunoassay.

Principle

The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen. Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained[(162)].

Reagents

A. Human Serum References - 1ml/vial - Icons A-F

Six (6) vials of serum reference for thyroxine at concentrations of 0 (A), 2.0 (B), 5.0 (C), 10.0 (D), 15.0 (E) and 25.0 (F) µg/dl. Store at 2-8°C. A preservative has been added.

B. T4-Enzyme Reagent - 1.5ml/vial - Icon E

One(1) vial of thyroxine-horseradish peroxidase (HRP) conjugate in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3/T4 Conjugate Buffer - 13 ml - Icon B

One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T4 Antibody Coated Plate - 96 wells - Icon

One 96-well microplate coated with sheep anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate - 20ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A - 7ml/vial - Icon SA

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B - 7ml/vial - Icon SB

One (1) bottle containing hydrogen peroxide (H2O2) in buffer. Store at 2-8°C.

H. Stop Solution - 8ml/vial – Icon

One (1) bottle containing a strong acid (1.0N HCl). Store at 2-8°C.

Reagent preparation

1. Working Reagent A = T4-Enzyme Conjugate Solution

Dilute the T4-enzyme conjugate 1:11 with Total T3/T4 conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within twenty-four hours for maximum performance of the assay. Store at 2-8°C.

General Formula:

Amount of Buffer required = Number of wells 0.1

Quantity of T4 Enzyme necessary = No. of wells 0.01

i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 conjugate buffer

16 x 0.01 = 0.16ml (160µl) for T4 enzyme conjugate 2.

2. Wash Buffer.

Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.

3. Working Substrate Solution.

Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Procedure

1. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.

2. Add 0.100 ml (100µl) of Working Reagent A, T4 Enzyme Reagent to all wells (see Reagent Preparation Section).

3. Swirl the microplate gently for 20-30 seconds to mix and cover.

4. Incubate 60 minutes at room temperature.

5. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.

6. Add 350µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.

7. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).

8. Incubate at room temperature for fifteen (15) minutes.
9. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds.

10. Read the absorbance in each well at 450nm in a microplate reader.

Calculations

1. Record the absorbance obtained from the printout of the microplate reader .

2. Plot the absorbance for each serum reference versus the corresponding T4 concentration in ng/ml on linear graph paper.

3. Draw the best-fit curve through the plotted points.

4. Determine the concentration of T4 for an unknown by using the standard curve.

2.5.3. Estimation of Serum TSH.

This method is a quantitative determination of total TSH concentration in human serum or plasma by a Microplate Enzyme Immunoassay.

Principle

The essential reagents required for an immune enzymometric assay include high affinity and specificity antibodies (enzyme conjugated and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen,

reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex[163].

Reagents

A. Thyrotropin Calibrators - 1ml/vial - Icons A-G

Seven (7) vials of references for TSH Antigen at levels of 0(A), 0.5(B), 2.5(C), 5.0(D), 10(E), 20(F) and 40(G) µIU/ml. Store at 2-8°C. A preservative has been added.

B. TSH Enzyme Reagent - 13ml/vial - Icon E

One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate - 96 wells

One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate - 20 ml - Icon

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A - 7ml/vial - Icon SA

One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B - 7ml/vial - Icon SB

One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution - 8ml/vial – Icon

One (1) bottle containing a strong acid (1N HCl). Store at 2-8°C.

Reagents preparation

1. Wash Buffer

Dilute contents of wash concentrate to 1000ml with distilled or de-ionized water in a suitable storage container. Store at 2-

30°C for up to 60 days.

2. Working Substrate Solution

Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Procedure

1. Pipette 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
2. Add 0.100 ml (100µl) of the TSH Enzyme Reagent to each well.
3. Swirl the microplate gently for 20-30 seconds to mix and cover.
4. Incubate 60 minutes at room temperature.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 350µl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
7. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section).
8. Incubate at room temperature for fifteen (15) minutes.
9. Add 0.050ml (50µl) of stop solution to each well and mix gently for 15-20 seconds.
10. Read the absorbance in each well at 450nm in a microplate reader.

Calculations

1. Record the absorbance obtained from the printout of the microplate reader
2. Plot the absorbance for each serum reference versus the corresponding TSH concentration in µIU/ml on linear graph paper
3. Draw the best-fit curve through the plotted points.

concentration of TSH for an unknown by using the standard curve Determine the.

2.6. Treatment by Narraw-Band UVB Phototherapy

2.6.1. Study design

The study was planned to follow up and collect data for patients with vitiligo who visited private dermatologic clinic in Ramadi for a period of about 4months from july 2018 to october 2018 as per the limited time schedule allowed for the project.

2.6.2. Treatment given to the patients

After diagnosis of vitiligo by specialist dermatologist, folow up collection of data were done for 20 patients (12 women and 8 men) with age range (10-48) years .The patients characteristics are shown in the questionnaire form.

The treatment of 20 patients are expousure to Narrow-band ultraviolet B NB-UVB (311 nm) twice weekly.

The dermatologist explained thoroughly the details of the protocol for each option of therapy.

These patients should visit the physician once every two weeks for monitoring and to check if any adverse effects appears.

2.6.3. Instrument

The instrument used with its supplier is shown in Table (2-3) and Figure (2-2)

Table (2-3) The instrument used and its supplier

Instrument	Supplier
HOUVA III UHU-322 TL100W/01/FS72	National Biological / USA
Camera Nikon D7200	Japan

Figure (2-2) The photos show HOUVA III instrument used in the present study.

2.6.4. Treatment process by NB-UVB

Before beginning the treatment, the paients were asked to be naked (except the under wears) and stand in the phototherapy unit .

The painent was given an instruction to wear eyes goggles to protect eyes .once the machine is switched on, the patient was given a calculated dose of NB-UVB .The paient may feel a warm sensation during treatment, but without pain. The starting dose of NB-UVB may only be a few seconds and then gradually be bulit up. The skin condition decided the frequency of the visited.

2.6.5. Measurement of Repigmentation

Efficacy was assessed in way vitiligo aera scoring index (VASI). The way involved monthly estimation of the surface area of vitiligo using VASI. The tool is a simple scale that allows patients and clinicians to speak a common language when discussing how vitiligo will respond to treatment[164]. The extent of pigmentation was scored as excellent, good, or poor depending on the percentage of the repigmentation in the previously depigmented site. The color of the repigmented area was compared with the adjacent normally pigmented area. The VASI score is a composite estimate of the overall area of vitiligo patches at baseline and the degree of macular repigmentation within these patches over time, ranging from 0-100%. The time taken until initial repigmentation, as well as overall repigmentation achieved, and adverse effects. For each body region, the VASI was determined by the product of the area of vitiligo in hand units (which was set at1% per unit) and the extent of depigmentation within each hand unit–measured patch (possible values of 0, 10%, 25%, 50%, 75%,90%, or 100%). The total body VASI was then calculated using the following formula by considering the contributions of all body regions (possible range, 0-100) [165].

$$\text{VASI} = \sum_{\text{all body sites}} [\text{Hand Units}] \times [\text{Residual Depigmentation}]$$

2.7. Statistical Analysis:

The program SPSS of the 22th edition is used, the following statistical parameters were obtained: standerd deviation, mean, and t-test for independent samples .Pearson ̇s correlation has been use to test the linear relationship between parameters. P values of < 0.05 were considered significant[166].

Chapter Three

Results

&

Discussion

Chapter Three

3. Results and discussion

3.1. Epidemiology.

Questions have been asked to each vitiligo patient. these questions include: age, accommodation, occupation, chronic diseases, time of infection ,emotional and psychological disorders, smoking, the most common diet, the most common drinks, the duration of disease, the season that exacerbate the disease, family history.

The study includes 120 persons. Among them 80 had vitiligo (30 males and 50 females) and 40 were healthy controls (8 males and 32 females). Their ages are divided into different groups:-

- Group A (10-20) years
- Group B (21-40) years
- Group C (41-70) years

The study shows the distribution of patients participated among AL-Anbar cities

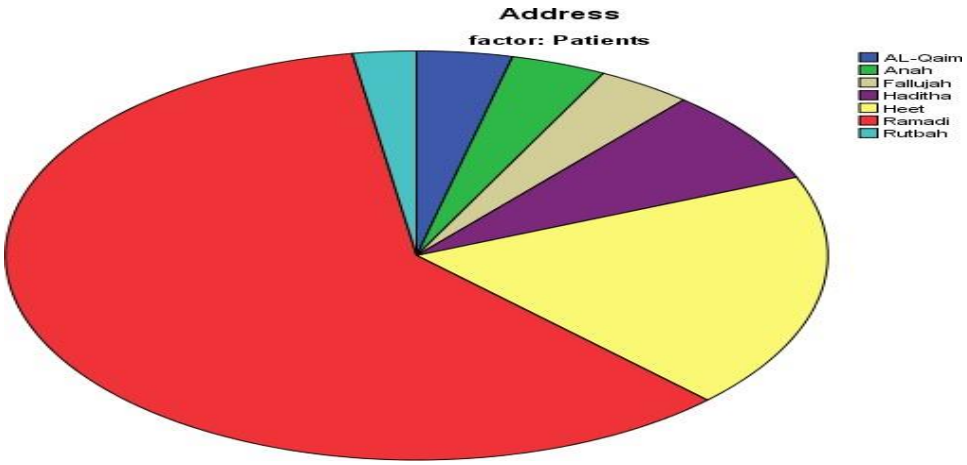


Figure (3.1): The distribution of patients participated among AL-Anbar cities

Family history of vitiligo was positive in a percentage 0f (30%) of the patients. The duration of disease ranged between 1 month to 20 years . History of seasonal variation of disease was positive in(60%) patients. Out of these (10%) noticed exacerbation of disease in winter while (50%) in summer season. Bad emotional state exacerbates of about (70%) of vitiligo patients, while the other (30%) does not affect.

This study showed that there is no relationship between the occupation, accommodation, most common diet, most common drinks and spiritual side with vitiligo.

3.2. Estimation of Hemoglobin Concentration and Packed Cells Volume (P.C.V).

The table (3-1) shows the results of Hemoglobin Concentration and Packed cells Volume for the patients and controls.

Table (3-1) the concentrations of Hemoglobin(g/dl) and Packed cells volume(%).

Parameters	Condition	Mean ± SD	t- value	P-value
H.b g/dl	Patient	12.82±1.29	-2.569	0.011
	Control	13.58±1.91		
P.C.V %	Patient	39.46±395	-5.327	0.000
	Control	44.51±6.40		

In the patient's group hemoglobin concentrations and packed cells volume were significantly lower than those of control group ($P \leq 0.05$).

Almost one quarter of vitiligo patients had anemia but did not statistically differ from their control[167]. Although[168] reported a lower prevalence (9.9%) of anemia in vitiligo patients; this prevalence was similarly not statistically different from the control. On the other hand[169],found a statistically higher prevalence of anemia among vitiligo patients (20%) as compared to control (3%). Interestingly, vitiligo patients in our study were may be more suscepitble to have microcytic anemia with a significantly lower mean (PCV) value when compared to controls. Vitiligo patients may be more susceptible to develop celiac disease , an autoimmune disorder known to be associated with iron deficiency[170].

3.3. Oxidative Stress Biomarkers

3.3.1. Estimation of Serum Lipid Peroxide Malondialdehyde (MDA).

The mean (±SD) values of serum MDA levels in patient's group and control are listed in table (3-2).

Table (3-2) Serum MDA concentration (µmol/l) in vitiligo patients compared with the control group.

Parameters	Condition	Mean ± SD	t- value	P-value
Malondialdehyde (µmol/l)	Patient	4.36±0.78	7.279	0.000
	Control	3.22±0.87		

The data in the table (3-2) showed that MDA concentration in the serum of patient's group was significantly higher than the control group ($P \leq 0.05$).

The problem of vitiligo is basically a cosmetic problem and more often the disease leads to social and emotional consequences in addition to depression and social anxiety . Vitiligo is a very complex disease and so far is not known why[171][172]. Several studies have shown oxidative stress have very important roles in appearance of vitiligo. In the destruction of melanocytes it

is believed that oxidative stress has a significant role in the process[173][174]. In normal conditions, antioxidants have major roles within cells in keeping cellular levels reactive oxygen species by remaining low levels because low levels of free radicals are harmless to cells. On the contrary, high levels of reactive oxygen in humans, there are harmful effects of cell components such as protein, fat and DNA [175][176]. Any disturbance or imbalance between cells producing reactive oxygen and levels of antioxidants lead to a condition called oxidative stress, which have a large role in inductions of many cancers and various diseases, including vitiligo [177][178].

MDA is an end-product of lipid peroxidation induced by reactive oxygen species (ROS). It is well correlated with the degree of lipid peroxidation and is an indicator of oxidative stress[179]. Our results are consistent with Abbas et al.(2019)[180] and Yildirim et al.(2004)[179] and Kamel et al.(2010)[181] as it found a significant increase of MDA in vitiligo patients compared with control group they explained this that it is a condition of oxidative stress. Moreover, Dammak et al.(2009)[182] added that lipid peroxidation in the cellular membrane of melanocytes may play an important role in rate of depigmentation observed in the skin of patients with active vitiligo.

3.3.2. Estimation of total serum bilirubin

The mean (±SD) values of serum total bilirubin levels in patient's group and control are listed in table (3-3).

Table (3-3) Serum total bilirubin concentration (mg/dl) in vitiligo patients compared with the control group.

Parameters	Condition	Mean ± SD	t- value	P-value
Total bilirubin (mg/dl)	Patient	0.48±0.21	-0.228	0.820
	Control	0.49±0.29		

The data in table (3-3) showed that total bilirubin concentration in the serum of patient's group and control was not significant statistically ($P \geq 0.05$).

Serum bilirubin was one of the blood parameter that was analyzed among all vitiligo patients and compared with the healthy persons. The results agree with Amin et al.,(2012)[183], there were no significant changes observed in this study. This finding could be suggested that vitiligo is not associated with jaundice.

3.3.3. Estimation of Serum Uric acid

The mean (±SD) values of serum uric acid levels in patient's group and control are listed in table (3-4).

Table (3-4) Serum Uric acid concentration (mg/dl) in vitiligo patients compared with the control group.

Parameters	Condition	Mean ± SD	t- value	P-value
Uric acid (mg/dl)	Patient	6.25±1.42	2.722	0.007
	Control	5.58±0.93		

The data in table (3-4) showed that uric acid concentration in the serum of patient's group significantly higher than those in control group ($P \leq 0.05$).

Our findings agree with Amin et al.(2012)[183] that serum uric acid levels were significantly changed in age group 11-20 and 31-40 compared with the healthy control. Uric acid has proven to be a selective non-enzymatic and chain breaking antioxidant . It contributes as much as two third of all free radical scavenging capacity in plasma. It serves as protective physiological role by preventing lipid peroxidation[184]. In a variety of organs and vascular beds, local uric acid concentration increases during acute oxidative stress and ischemia, and the increased concentrations might be a compensatory mechanism that confers protection against increased free radical activity[185]. Koca et al.(2004)[186] showed significantly higher xanthine oxidase levels in

vitiligo patients than in controls. We consider that the increased xanthine oxidase levels could contribute to the oxidative stress in the aetiopathogenesis of vitiligo. Our result disagrees with Jain et al.(2008)[187] who was found significantly decrease in level of uric acid in vitiligo patients compared to age matched healthy controls.

3.3.4. Estimation of Glutathion Concentration in Blood Serum

The mean (±SD) values of serum glutathion levels in patient's group and control are listed in table (3-5).

Table (3-5) Serum Glutathion concentration (µmol/l) in vitiligo patients compared with the control group.

parameters	Condition	Mean ± SD	t- value	P-value
Glutathion (µmol/l)	Patient	620.84±85.91	7.339	0.000
	Control	491.58±100.39		

The data in table (3-5) showed that glutathion concentration in the serum of patient's group significantly higher than those in control group ($P \leq 0.05$).

Our results disagree with Shamsa (2012)[188] and Shin et al.(2010)[189] ,who found a significant lower serum and tissue GSH levels in vitiligo patients in comparison to controls. The decreased level of GSH in vitiligo may be explained by more production of free radicals which leads to consumption of antioxidant defense system including GSH. On the other hand, our result disagree with the results obtained by Picardo et al.(1994)[190] who found no significant difference in GSH levels in vitiligo patients and controls . Human cells possess an efficient antioxidants system for protection against and control of toxic effects of the free radicals . Assessment of serum antioxidants may give an idea about the oxidative status of the body[191]. In the present study plasma GSH levels are significantly higher in vitiligo

patients compared to controls because some vitiligo patients are using drugs containing GSH .

3.3.5. Estimation of Vitamin E (α-Tocopherol) in blood serum

The mean (±SD) values of serum vitamin E levels in patient's group and control are listed in table (3-6).

Table (3-6) Serum Vitamin E concentration (µmol/l) in vitiligo patients compared with the control group.

Parameters	Condition	Mean ± SD	t- value	P-value
Vitamin E (µmol/l)	Patient	10.44±1.26	0.539	0.591
	Control	10.31±1.26		

The data in table (3-6) showed that vitamin E concentration in the serum of patient's group and control was not significant statistically ($P \geq 0.05$).

Our results agree with Agrawal et al.,(2014)[192] and (Boisseau) et al..(2002)[193] and Agrawal et al.,(2004)[194] they do not find significant difference between the two groups. Alpha-tocopherol is an efficient scavenger of lipid peroxyl radicals and, hence, it is able to break peroxyl chain propagation reactions. The unpaired electron of the tocopheroxyl radical thus formed tends to be delocalized rendering the radical more stable. Vitamin E forms complexes with the lysophospholipids and free fatty acids liberated by the action of membrane lipid hydrolysis. Both these products form 1:1 stoichiometric complexes with vitamin E and as a consequence the overall blance of hydrophobic: hydrophilic affinity within the membrane is restord. In this way, vitamin E is thought to negate the detergent-like properties of the hydrolytic products that would otherwise disrupt membrane stability[195]. However, Passi et al.,(1998)[196] and Khan et al.,(2009)[197] founded significantly lower levels of vitamin E among active vitiligo

patients as compared to the controls. Vitamin E and carotenoids can be useful in vitiligo treatment[198].

3.3.6. Estimation of Serum ascorbic acid (Vitamin-C)

The mean (±SD) values of serum vitamin C levels in patient's group and control are listed in table (3-7).

Table (3-7) Serum Vitamin C concentration (µmol/l) in vitiligo patients compared with the control group.

Parameters	Condition	Mean ± SD	t- value	P-value
Vitamin C (µmol/l)	Patient	82.26±3.22	-0.377	0.707
	Control	82.49±2.90		

The data in table (3-7) showed that vitamin C concentration in the serum of patient's group and control was not significant statistically ($P \geq 0.05$).

Our results agree with Agrawal et al.,(2014)[192] and Kumar et al.,(2019)[199] they do not find significant difference between the two groups. Vitamin C is a water-soluble, chain breaking antioxidants. As an antioxidants, it scavenges free radicals and reactive oxygen molecules by donating its electrons to prevent other compound from being oxidized[200]. However, controversies exist regarding serum ascorbic acid measurement in vitiligo, a few studies have found lower serum ascorbic acid in vitiligo patients than the control[201][202][197]. According to the general Ayurvedic system of medicine in India, citrus fruits may worsen the condition. Nutritional strategies suggest the potential benefits of a diet rich in vitamin C as a preventive tool for patients with skin diseases[203][204].

3.3.7. Determination of copper and zinc.

The concentrations of copper and zinc are shown in the table(3-8).

Table (3-8) Serum concentrations of copper and zinc (µmol/l) in vitiligo patients compared with the control group.

Parameters	Condition	Mean ± SD	t- value	P-value
Zn(µmol/l)	Patient	10.38±2.39	3.473	0.001
	Control	8.75±2.50		
Cu(µmol/l)	Patient	10.88±0.99	6.328	0.000
	Control	9.45±1.46		

The statistical analysis results showed a significant increase in the concentrations of zinc and copper for vitiligo patients compared with healthy individuals ($P \leq 0.05$).

Copper and zinc are essential trace elements involved in many biochemical processes, such as cellular respiration, DNA and RNA reproduction, maintenance of cell membrane integrity and sequestration of free radicals[205].

The abnormalities of Cu and Zn participate in the pathogenesis of many skin diseases. Numbers of studies have suggested that alterations of Cu and Zn could be found in patients of acrodermatitis enteropathica, bullous pemphigoid, decubitus ulcer, alopecia areata, psoriasis, skin cancer and vitiligo[206][207][208].

Our results agree with Helmy et al.,(2004)[209] showed that serum Zn and Cu levels were significantly higher in active vitiligo patients compared to controls. The author thought that the increased serum Zn and Cu levels came from the release of Zn and Cu, led by increased apoptosis of peripheral blood mononuclear cells in active vitiligo patients. Furthermore, melanins are colloidal pigments and have a high affinity for metal ions; therefore, Cu,

Zn and other metal ions are found in high levels in pigmented tissues involved in melanin synthesis. As melanocytes degenerate in vitiligo patients, less Cu and Zn are utilized for the melanin synthesis, which consequently raise levels of Cu and Zn in serum[206][209]. In other studies, Zeng et al.,(2014)[205], Arora et al.,(2002)[210] and Haider et al.,(2010)[211] and Wang et al.,(2012)[212] did not find significant alteration in serum Zn level in vitiligo. This possibly supports the autoimmune theory of vitiligo.

However, studies of Shameer et al.,(2005)[213], Brüske and Salfed(1987)[214] and Tsiskarishvili(2005)[215] indicated that serum level of Cu and Zn decreased, which causes vitiligo. The roles of Cu and Zn are mainly twofold: to promote melanogenesis and to provide a physiological defense against oxidative stress[216].

3.4. Correlation between Oxidants and Antioxidants.

The correlation coefficient (r) between MDA and antioxidants was carried out in vitiligo patients according to table (3-9).

Table (3-9) Correlations between Oxidants and Antioxidants

Sex				Vit.C (µmol/l)	Vit.E (µmol/l)	MDA (µmol/l)	GSH (µmol/l).	Zn (µmol/l)	Cu (µmol/l)
Male		MDA	Pearson Correlation	-0.005	-0.245	1	-0.253	0.229	0.107
			Sig. (2-tailed)	0.977	0.193		0.177	0.223	0.575
			N	30	30	30	30	30	30
Female		MDA	Pearson Correlation	0.231	-0.266	1	-0.447-**	.011	0.409**
			Sig. (2-tailed)	0.106	0.061		0.001	0.937	0.003
			N	50	50	50	50	50	50

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

MDA and antioxidants (vitamin C, vitamin E, Zn) have no statistical state correlation between them. This may be due to ratio of deterioration in antioxidants as a result of their activity toward the oxidants has not direct proportion with oxidants byproducts (i.e MDA). This observation may be due to the antioxidant activity which was the direct products of its reaction with oxidant while the oxidation byproduct generation was a secondary product of reaction of oxidants with the biomolecules.

There was a negative correlation between MDA and Glutathione (r=-0.447) at significant (p≤0.01), and this can be found by regression plots in figure(3-2):

Our results agree with Shin et al.,(2010)[189] who found malondialdehyde increase with glutathione decrease . These data could further prove that low glutathione level are associated with increased lipid peroxidation which may cause melanocyte destruction. These results were in accordance with many other studies[194][217][218].

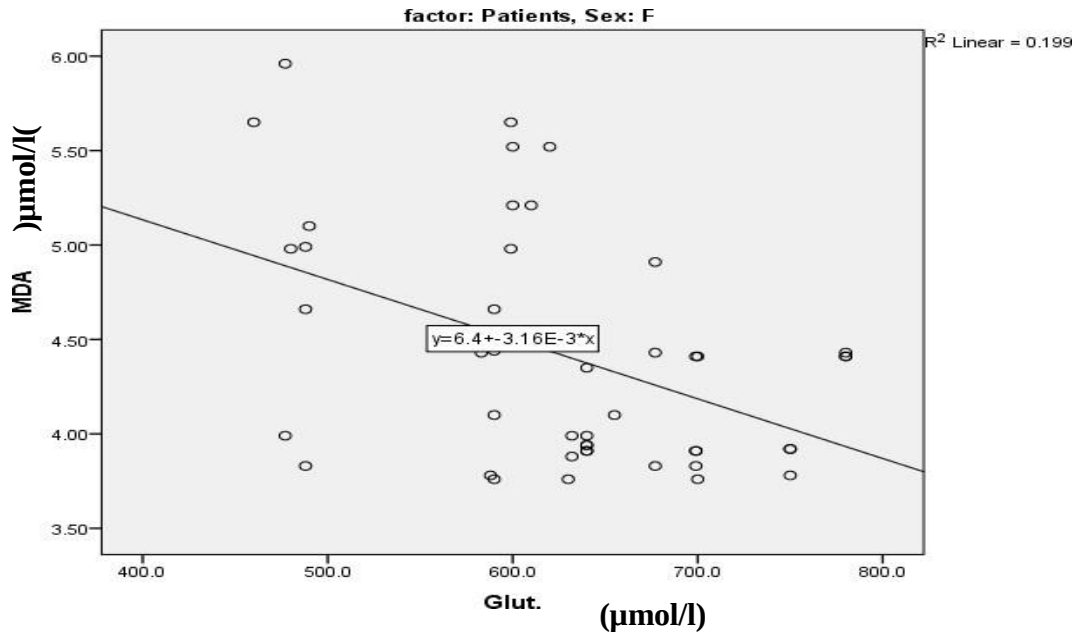


Figure (3-2) Correlations between MDA and Glutathione.

There was a positive correlation between MDA and Cu (r=0.409) at significant (p≤0.01), and this can be found by regression plots in figure(3-3)

Our results disagree with Jain et al.,(2008)[187] who found MDA increase with Ceruloplasmin decrease. This demonstrates the presence of an imbalance in the oxidant-antioxidant system in the blood of vitiligo patients.

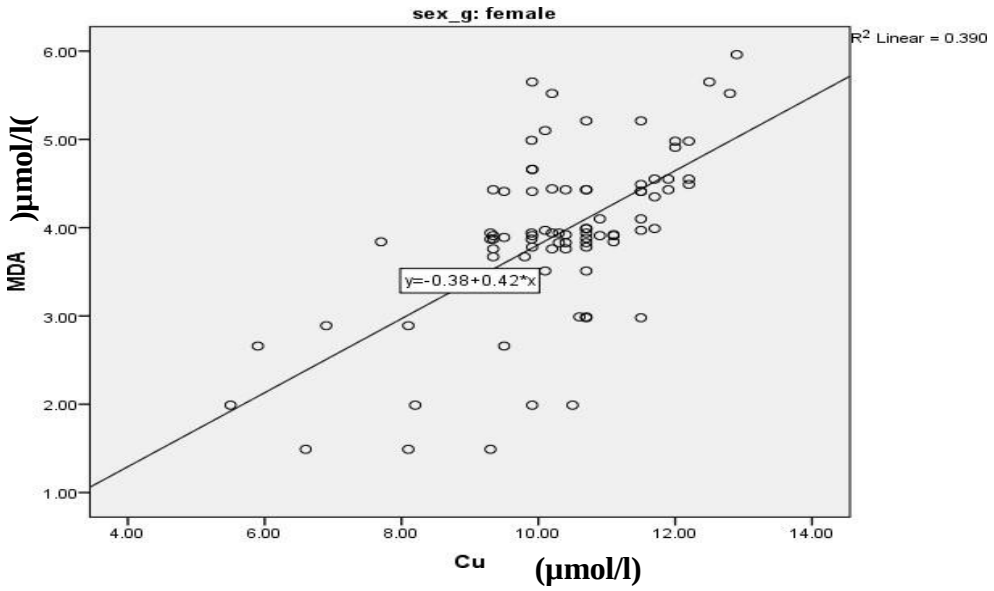


Figure (3-3) Correlations between MDA and Cu.

3.5. Determination of Lipid Profile.

The table (3-10) shows the results of lipid profile for the patients and controls.

Table (3-10) Serum concentrations of lipid profile mg/dl .

Parameters	Condition	Mean ± SD	t- value	P-value
Total cholesterol	Patient	193.47±27.23))	1.264	0.209
	Control	186.07±35.57))		
Triglycerides	Patient	100.29±15.71))	0.770	0.443
	Control	97.92±16.21))		
HDL	Patient	49.81±7.36))	-0.794	0.429
	Control	50.99±8.37))		
LDL	Patient	123.14±22.16))	1.593	0.114
	Control	115.95±25.46))		
VLDL	Patient	20.14±3.06))	0.915	0.362
	Control	(19.58±3.23)		

In the patient's group serum cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-cholesterol), low density lipoprotein cholesterol (LDL-cholesterol) and very low density lipoprotein cholesterol (VLDL-cholesterol) were not significant statistically than those in control group ($P \geq 0.05$).

The stratum corneum consists of corneocytes and intracellular lipids, mainly ceramides, sterols, and free fatty acids which form the barrier for diffusion of substances into the skin[219][220][221][222]. The lipids are organized into multilamellar intercellular membranes derived from glycerophospholipids, glucocerebrosides, sphingomyelin, of the stratum granulosum-stratum corneum interface[223]. Then the precursors are converted to ceramides and free fatty acids by the hydrolytic enzymes[224][225].

Many researches about vitiligo and lipids profile have been done with different results. One of these Metta et al.,(2016)[226] showed that the analyzing of lipid profile in vitiligo patients, a significantly lower LDL-cholesterol and VLDL-cholesterol levels were observed, whereas other parameters did not show much change, in comparison with controls. The significantly higher LDL and VLDL levels in vitiligo patients suggest lipid disturbances, which according to Karadage et al.,(2011)[227] may be owing to the complex interaction of inflammatory, cytotoxic, and immunological factors in vitiligo patients inducing the systemic disturbances. However, Pietrzak et al.,(2000)[228] in their study on girls with vitiligo have reported similar findings of disturbances in the lipid profile. However, they had observed significantly lower level of HDL-cholesterol in vitiligo patients along with significantly higher levels of LDL and VLDL. The differences in the studies may be owing to other associated confounding factors that are likely to interfere with lipid disturbances.

3.6. Estimation of Thyroid hormones.

The results of thyroid hormones (T3, T4, TSH) are shown in the table (3-11).

Table (3-11) The activity of thyroid hormones .

Parameters	Condition	Mean ± SD	t- value	P-value
T3 ng/ml	Patient	1.03±0.30	-0.429	0.669
	Control	1.05±0.17		
T4 µg/dl	Patient	7.69±1.86	0.000	1.000
	Control	7.69±1.55		
TSH µIU/ml	Patient	3.33±2.27	2.920	0.004
	Control	2.23±0.96		

In the patient's group T3 and T4 were not significant statistically than those in control group ($P \geq 0.05$). While TSH was significantly higher than those in control group($P \leq 0.05$).

Thyroid functional disorders and autoimmune thyroid diseases have been reported in association with vitiligo, and it seems that the incidence of clinical and subclinical thyroid involvement the incidence of clinical and subclinical thyroid involvement is more common in vitiligo patients than controls[229][230][231].

Many researches about vitiligo and thyroid disease have been done with different results, one of these Mubki et al.,(2017)[167] showed that thyroid functional abnormalities were generally found more in vitiligo patients were approximately 1.6 times more likely to have abnormal TSH than control. The mean TSH level was overall higher in the vitiligo group. Both high TSH and low TSH levels were seen more frequently in vitiligo patients. The vitiligo group had significantly higher prevalence (5%) of primary hypothyroidism (high TSH and low T4) as compared to the control group. Alissa et al.,(2011)[232] and Akay et al.,(2010)[233] showed the predominance of females among vitiligo patients can be attributed to the fact that females

are more conscious about their cosmetic appearance and thus more likely to seek medical attention.

Vitiligo seems to be commonly associated with autoimmune diseases . Two studies have reported associated autoimmune disease in (19%) and (23%) of vitiligo patients[234][235].

One of the most commonly reported associations is thyroid disease, especially Hashimoto ’s thyroiditis[236]. The reported prevalence of thyroid disease in the literatur ranged from (4%) to (21%) to even higher in other studies[235][237][238].

3.7. Degree of response to treatment by exposure to NB-UVB.

The degree of response to treatment by NB-UVB varies from excellent to moderate depending on the percent of vitiligo repigmentation .As shown in Table (3-12), and Figure(3-4); a response of 80-90%was given grade excellent, a response of 70-80% was given grade good and a response of 60-70% was given grade moderate, a response of 50-60% was given grade mild, a response of 40-50% was given grade poor. As shown in Table (3-12); about 35% of the patients had excellent response, about 25% had good response, about 20% had moderate response, about 15% had mild response and about 10% had poor response.

Table (3-12): Degree of response to treatment by exposure to NB-UVB.

Response	Percent of vitiligo repigmentation	No. of patients
Excellent	80%-90%	7
Good	70%-80%	5
Moderate	60%-70%	3
Mild	50%-60%	3
Poor	40%-50%	2

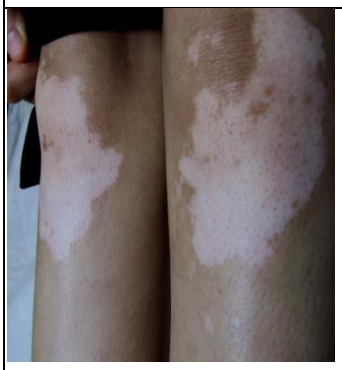
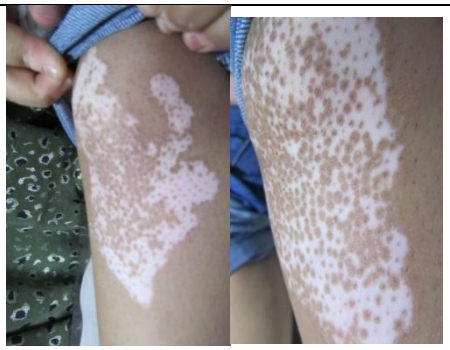
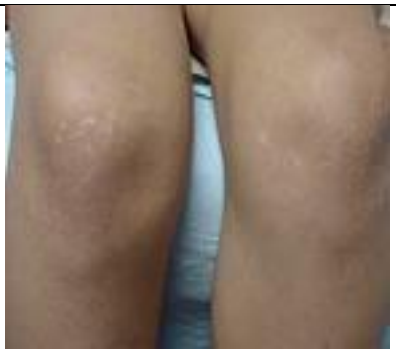
First visit, before treatment	After 8 weeks of treatment	After 16 weeks Of treatment
	 Repigmention in some of the area	 Repigmention in the area and almost complete healing of about 90%.

Figure (3-4) Photos show the effect (repigmentation) before and after treatment by exposure to NB UVB.

Narrow-band UVB phototherapy has been found to be effective and safe for vitiligo[(239)].

NB UVB is now considered as the gold standard of treatment for vitiligo covering more than 20% of the body. Vitiligo is the most common dermatological diseases treated with phototherapy. Phototherapy is the exposure to nonionizing radiation for therapeutic benefits. It involves exposure to ultraviolet A (UVA) or ultraviolet B (UVB)[(240)(241)].

NB UVB uses the portion of the UVB spectrum form 311-312 nm. This region has been determined to help in stimulation of pigment cell to produce melanin in less time than psoralen and exposure to sun ligh[(242)(243)].

Our results degree with Al-Saedy et al., (2012)[(244)] and Ameen(2011)[(245)] both studies reach the same conclusion that NB-UVB is safer and more effective for treatment of vitiligo than psoralen plus exposure to sun light. NB-UVB has been advocated as more applicable than psoralen plus sun light as it dose not need psoralen, and is as effective as psoralen plus sun light.

Conclusions and Recommendations

Conclusions:-

The results found in this study enabled to conclude the following points:

1. The pathogenesis of vitiligo in AL-Anbar governorate differs from that of others governorate, and than that of other country because of the difference in the results obtained compared to other studies in different governorates and countries.
2. There is no relationships between the disease and the age because it catches all the ages.
3. The disease may be transmitted via the genetic informations but in a small ratio. Our results showed that only (30%) of patients have a relationship with heredity.
4. The disease is exacerbated for most patients in summer, from this we conclude that the seasonal variations have an effect on the severity of the disease.
5. Bad emotional and psychological state exacerbates the disease of about (70%) of the patients, therefore we expect that anti-depression drugs may improve the disease.
6. There is no relationship between the pathogenesis of vitiligo and accommodation, occupation, most common diet, and most common drinks, therefore we conclude that the disease is not caused by a contamination, certain diet, and certain drink.
7. Our study showed that the vitiligo patients were may be more susceptible to have anemia with a significantly lower mean (PCV) and more susceptible to develop celiac disease , an autoimmune disorder known to be associated with iron deficiency.
8. The oxidants such as MDA are significantly increased along disease activity, that leads to play an important role in rate of depigmentation observed in the skin of patients with active vitiligo.

9. Our study showed no relationship between the concentrations of vitamin E and C and total bilirubin and lipid profile and the pathogenesis of vitiligo.
10. A strong relationship has been concluded from our results between the pathogenesis of vitiligo, trace elements and uric acid and glutathione.
11. From our results, we conclude that a strong relationship is found between the thyroid hormones and the pathogenesis of vitiligo.
12. NB-UVB is an effective and well-tolerated treatment option for patients with vitiligo.

Recommendations:-

1. We recommend to complete the study with different parameters and on different type of vitiligo.
2. We have not to rely on studies performed in other countries in the diagnosis and treatment of vitiligo because of the variation between our results and them.
3. Studying the effect of phytochemicals and supplemental antioxidants on treatment of vitiligo patients.
4. Studying of the immunological and genetic side and their effects on pathogenesis of vitiligo.
5. Establishing specialized dermatological centers equipped with NB-UVB phototherapy unites.

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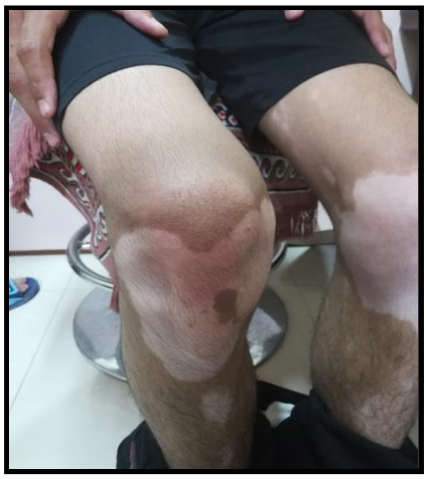
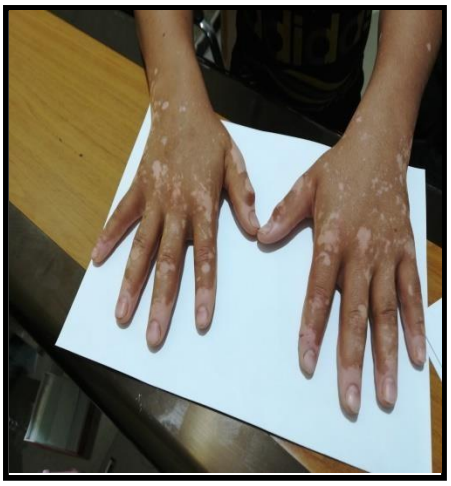
Appendixes

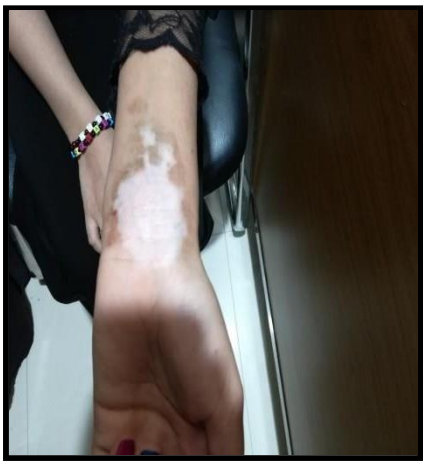
Appendixes:

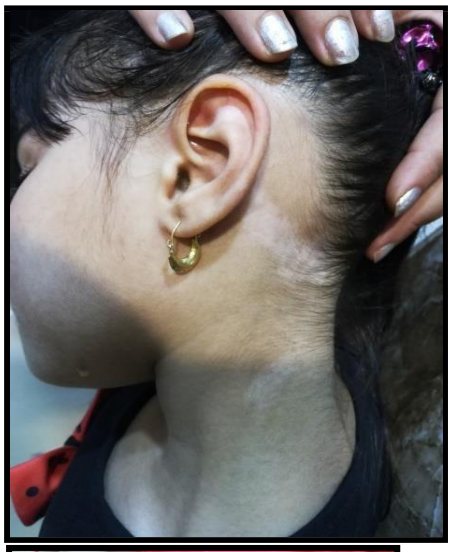
Appendix 1. Questionnaire form

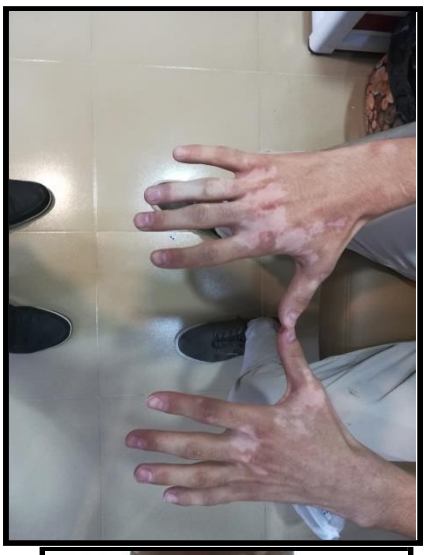
Name		Age years	
Sex		Address	
Contact			
Alcoholic		Smoking	
MEDICAL HISTORY			
Vitiligo diseases			
Duration			
Sign and Symptoms			
Follow up			
The effect of sunlight investigation			
Anemia			
Diabetic			
Thyroid disorders			
Liver Function disease			
Hyperlipidemia			
Mental state			
Other disease			
Treatments & Drugs that he used			
Family history of the disease			
Others			

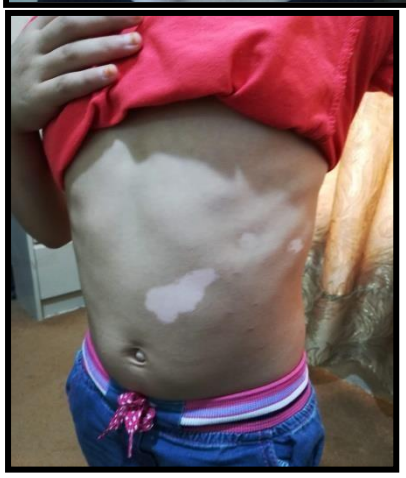


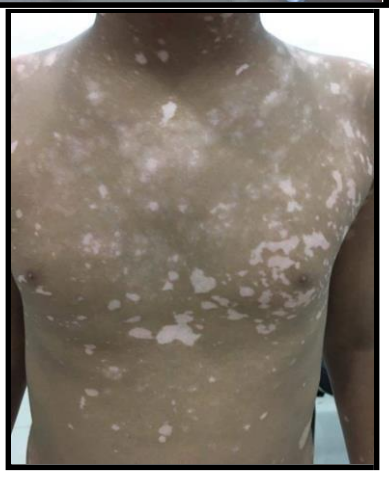


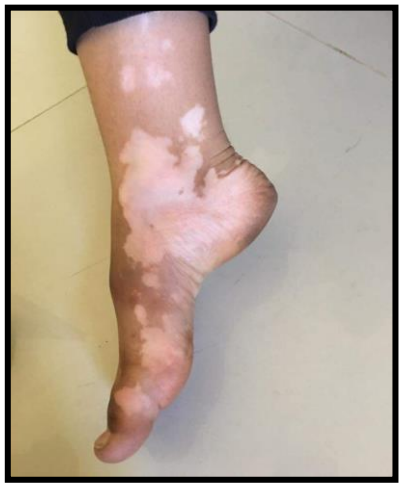
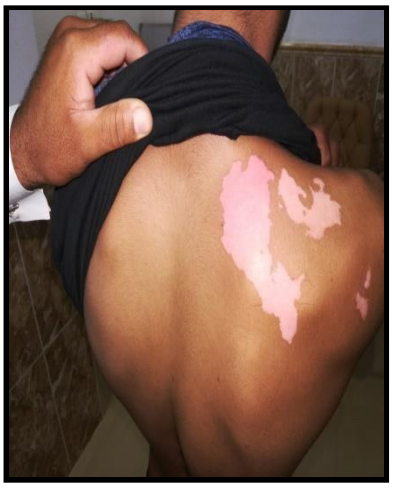
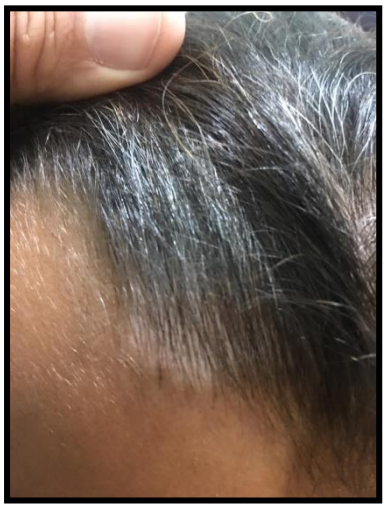
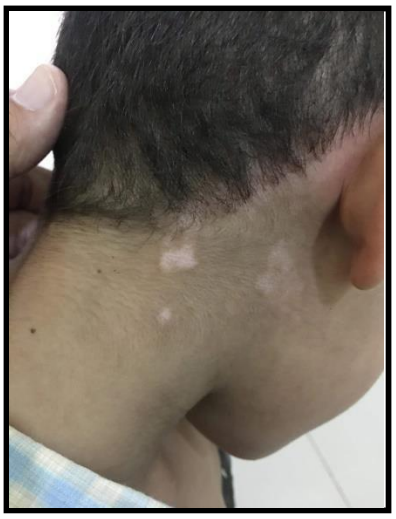




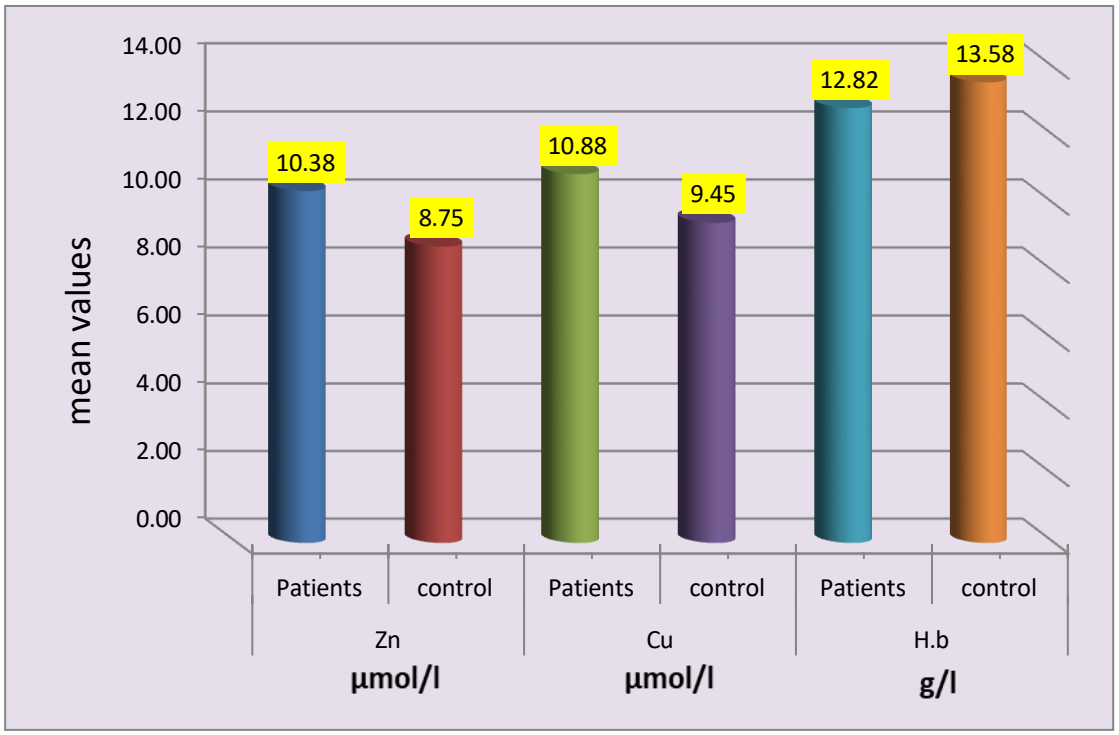




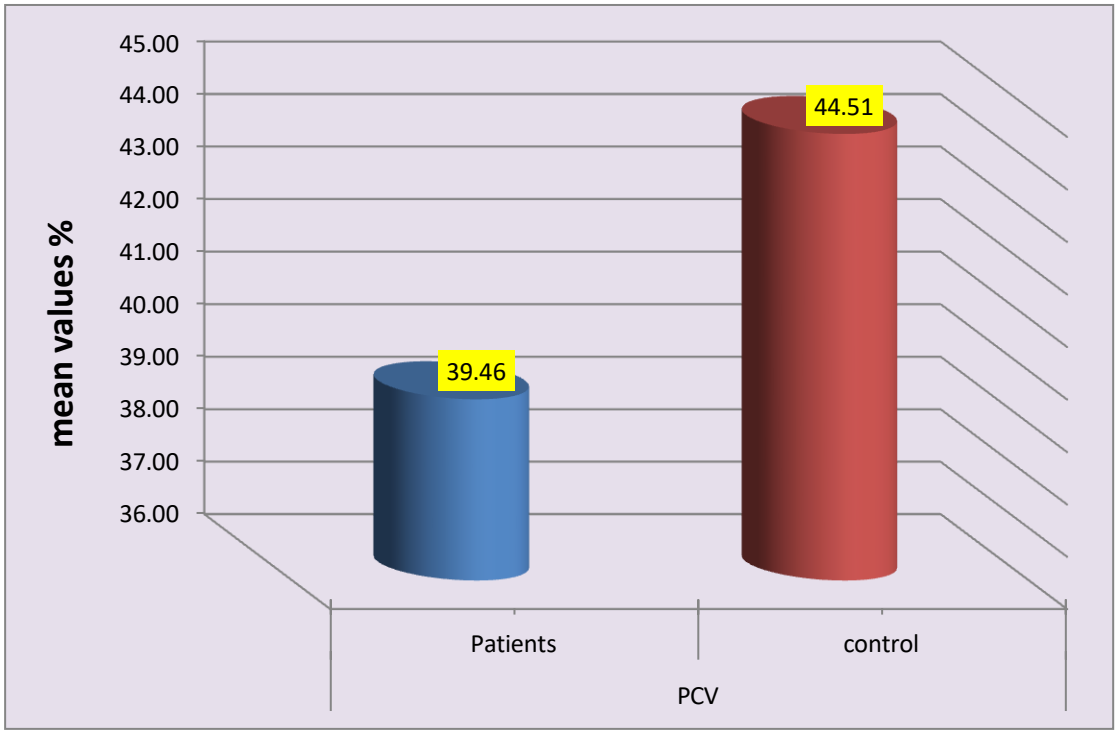


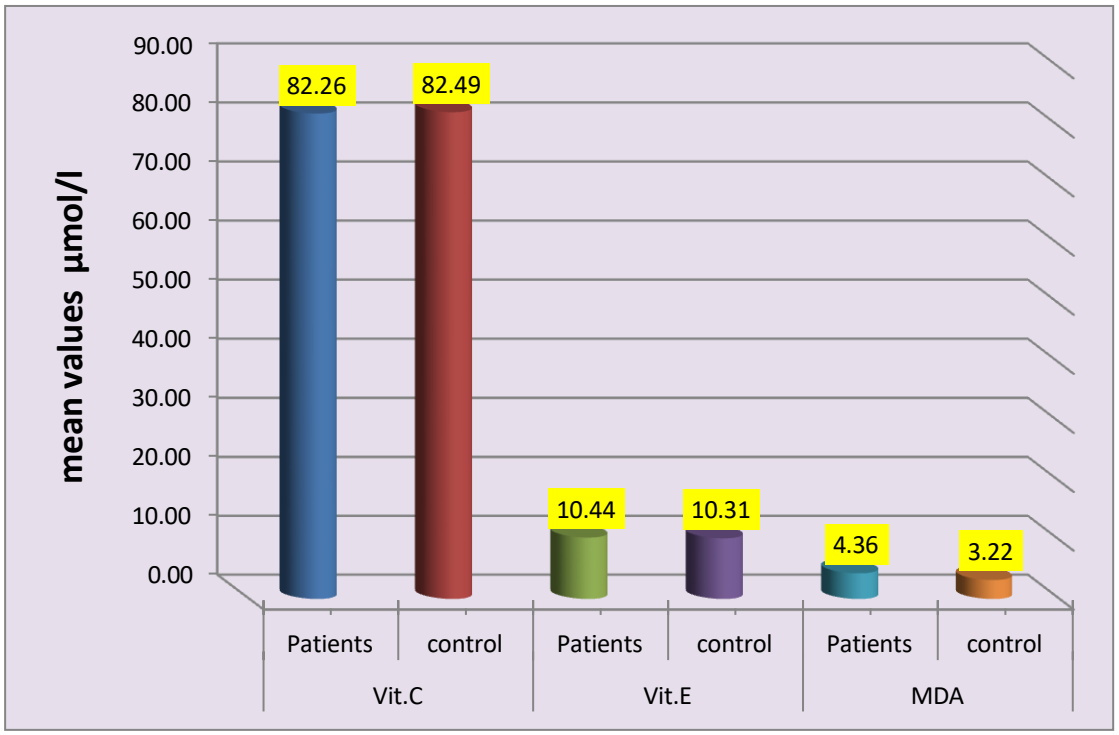
Appendix (2) vitiligo patients images.



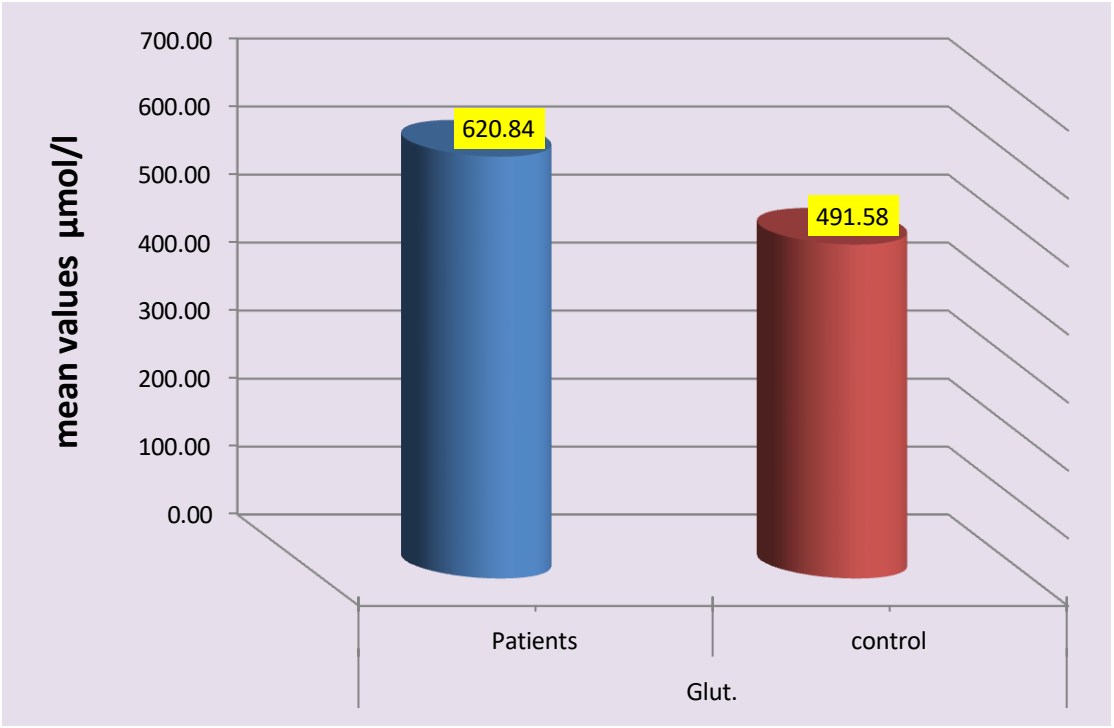
Appendix (3): the mean values of patients and control trace elements and hemoglobin concentration.



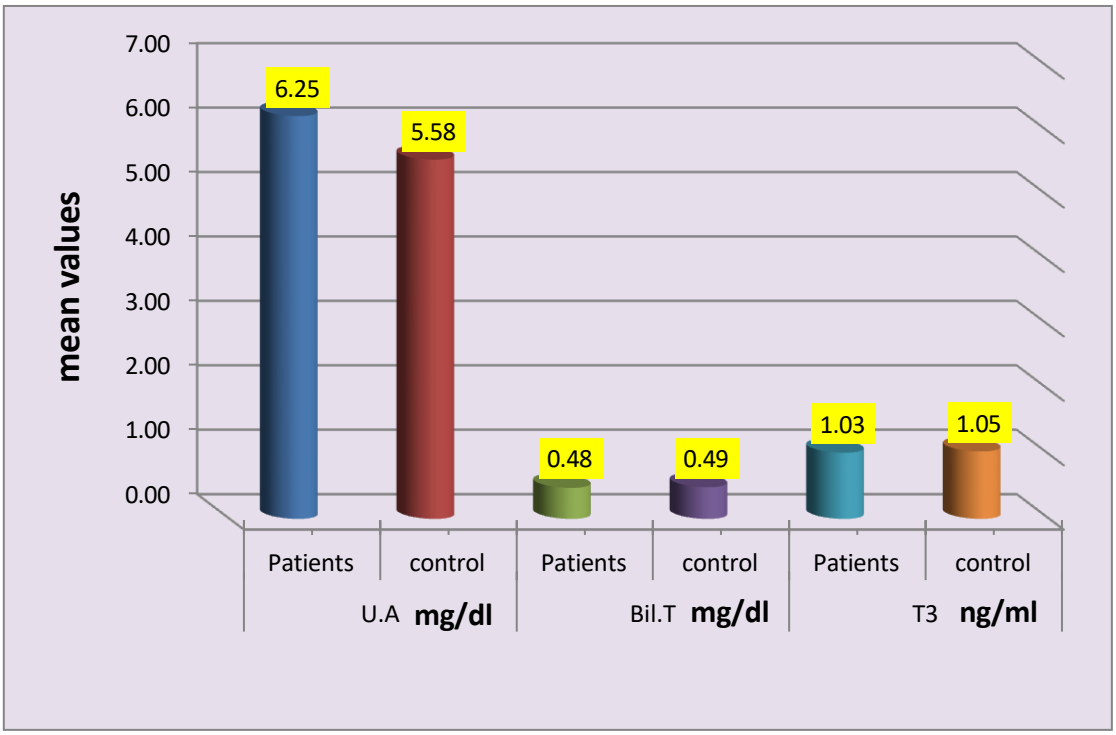
Appendix (4): the mean values of packed cells volume.



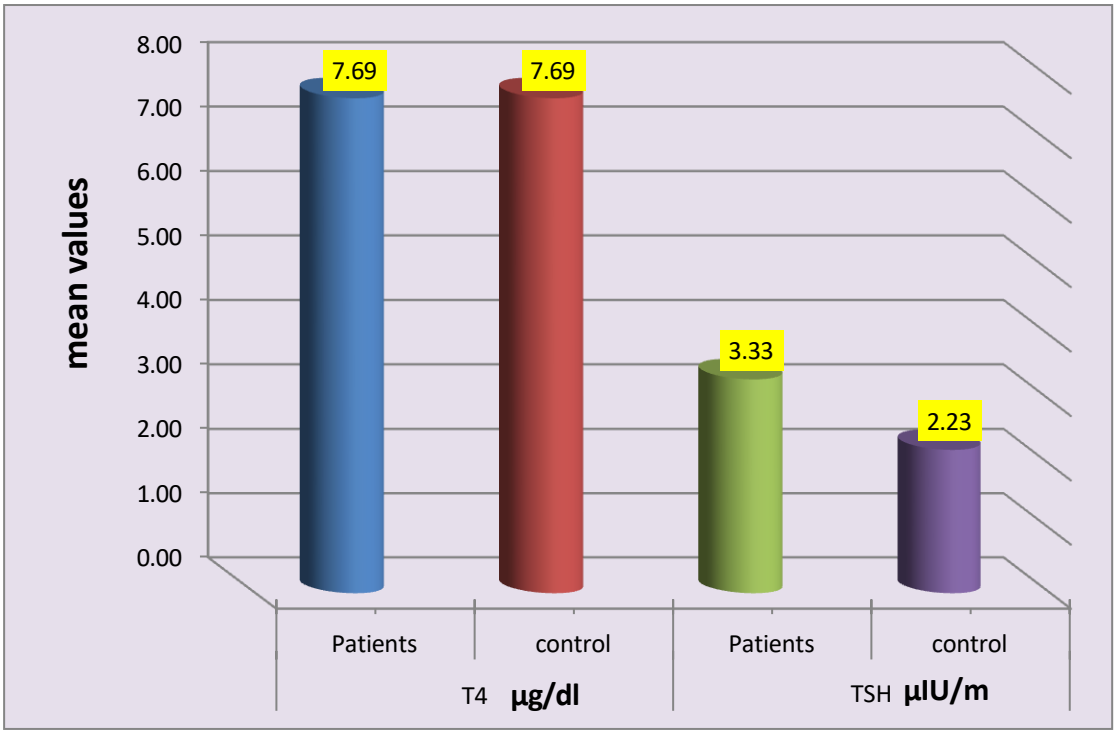
Appendix (5): the mean values of vitamins C and E and MDA.



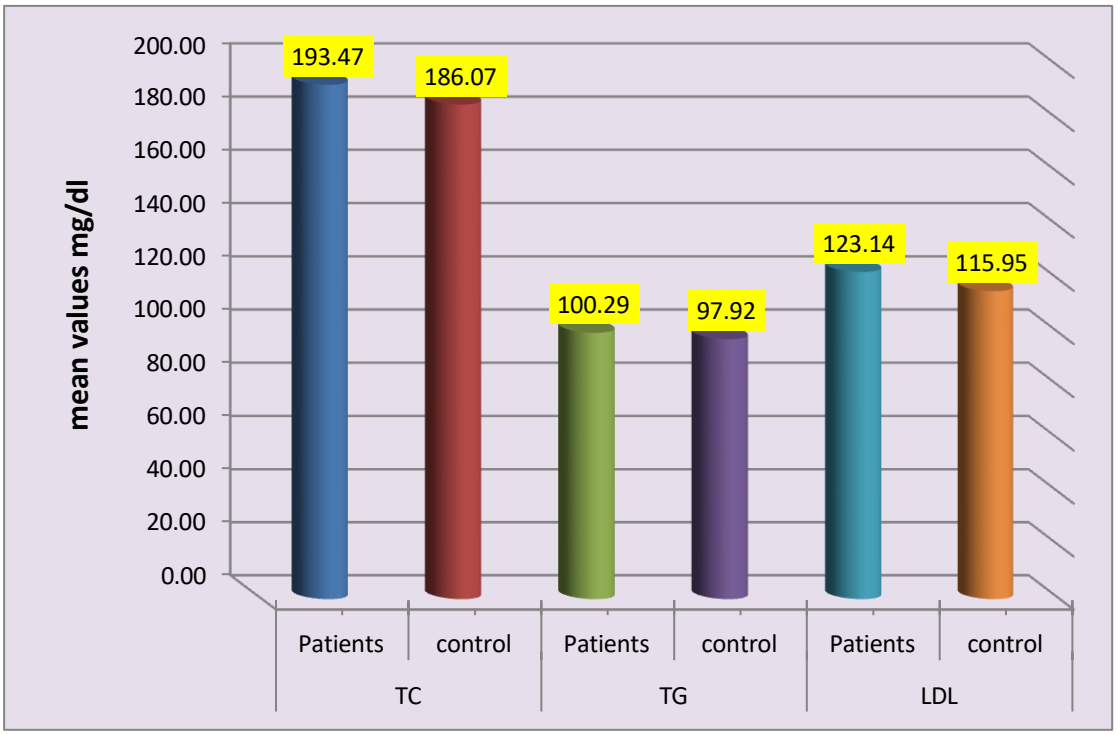
Appendix (6): the mean values of glutathione.



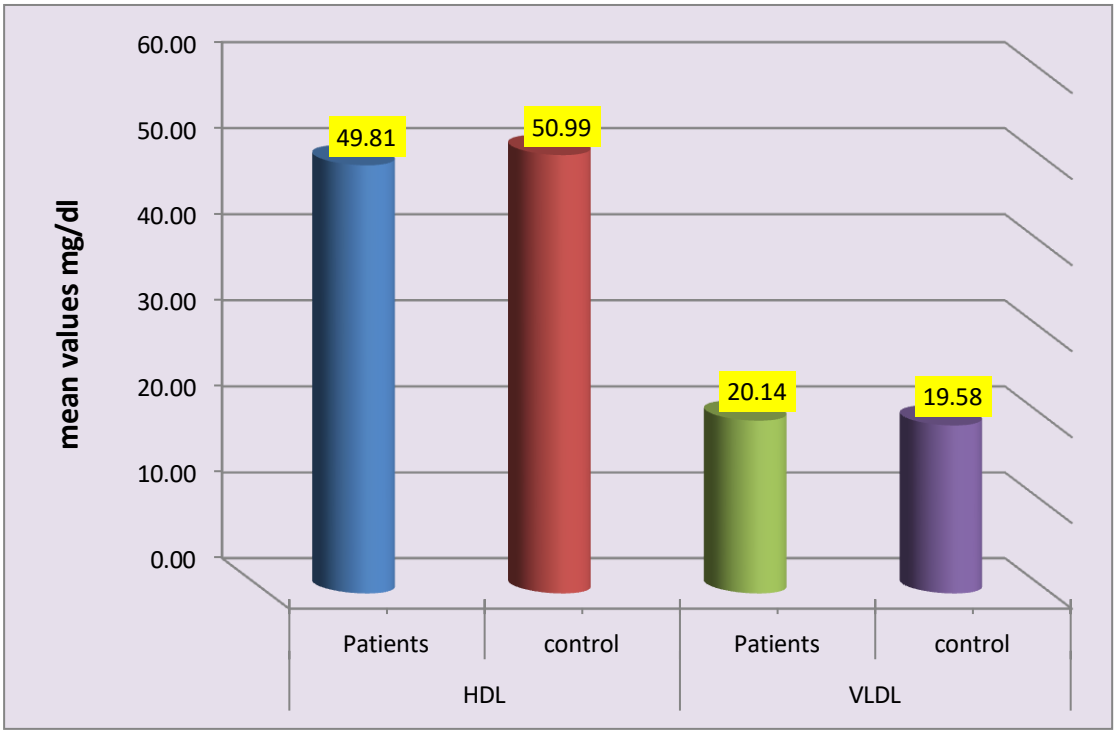
Appendix (7): the mean values of uric acid and bilirubin and T3.



Appendix (8): the mean values of T4 and TSH.



Appendix (9): the mean values of lipid profile.



Appendix (10): the mean values of lipid profile.

الخالصة:-

اإلجهاد التأكسدي هو حالة عدم التوازن بين المؤكسدات ومضادات االكسدة، يلعب دورا هاما في بدء وتطور البهاق، البهاق هو اضطراب مزمن ومكتسب لإل ازلة التصبغ في الجلد. وينتج المرض عن الفقدان االنتقائي للخاليا الميالنية. والهدف من هذه الد ارسة هو تقييم تأثير بعض مؤش ارت االجهاد التأكسدي والحرارة والضوء في معالجة المرضى المصابون في البهاق في محافظة االنبار.

تضمنت الد ارسة جمع مصل الدم من 80 مريض مصاب بالبهاق)30 ذكر و 50 أنثى(وكذلك جمع مصل الدم من 40)8 ذكر و 32 أنثى(شخص سليم غير مصاب، عدة اختبا ارت كيموحيوية أنجزت لكل من المرضى واالصحاء لمعرفة سبب المرض. باإلضافة الى ذلك عدة أسئلة وجهت لكل مريض بغية الحصول على معلومات تفيد في معرفة سبب المرض. أستمرت مدة جمع العينات 6 أشهر ابتداء من تموز 2018 ولحد كانون الثاني 2019.

قسمت أعمارهم الى مجاميع مختلفة: مجموعة)أ(تت اروح أعمارهم مابين)10-20(سنة ، مجموعة)ب(تت اروح أعمارهم مابين)21-40(سنة ، مجموعة)ج(تت اروح أعمارهم مابين)41-70(سنة. تاريخ العائلة كان إيجابي بنسبة)30%(من المرضى. مدة اإلصابة بالبهاق للمرضى تحت الد ارسة كان يت اروح بين شهر واحد الى 20 سنة . نسبة التأثر بالموسم كانت 60% ، من بينها 10% تزداد سوء الحالة لديهم في فصل الشتاء و50% في فصل الصيف، اما النسبة المتبقية فال يتأثرون بعامل الموسم. نسبة التأثر بالحالة العاطفية السيئة كانت 70% من مرضى البهاق، بينما 30% ال يتأثرون.

اظهرت الد ارسة عدم وجود ت اربط بين المرض من جهة والمهنة, والسكن, االكل, الشرب, والجانب الديني من جهة اخرى.

تم تقدير تركيز الهيموغلوبين وحجم الخاليا المت ارصة لدى المرضى اقل مما هو عليه في ألشخاص االصحاء وبمستوى احتمالية ($P \leq 0.05$).

أظهرت نتائج الد ارسة ان هناك زيادة معنوية في تركيز المالون داي الديهايد)MDA(مع مستوى احتمالية($P \leq 0.05$) في مرضى البهاق مقارنة مع مجموعة السيطرة وأظهرت عدم وجود فروق معنوية في تركيز البليروبين)Bilirubin(الكلي مع مستوى احتمالية ($P \geq 0.05$) في مرضى البهاق مقارنة مع مجموعة السيطرة واظهرت زيادة معنوية في تركيز اليورك اسد) Uric acid(مع مستوى احتمالية($P \leq 0.05$) في مرضى البهاق مقارنة مع مجموعة السيطرة واظهرت زيادة معنوية في تركيز الكلوتاثايون)Glutathion(مع مستوى احتمالية ($P \leq 0.05$) في مرضى البهاق مقارنة مع مجموعة السيطرة.

اظهرت نتائج التحليل االحصائي عند قياس بعض الفيتامينات عدم وجود فروق معنوية في ت اركيز فيتامين E وC مع مستوى احتمالية ($P \geq 0.05$) في مرضى البهاق مقارنة مع مجموعة السيطرة.

أظهرت نتائج التحليل االحصائي عند قياس بعض العناصر النزرة زيادة معنوية في ت اركيز الزنك والنحاس مع مستوى احتمالية ($P \leq 0.05$) في مرضى البهاق مقارنة مع مجموعة السيطرة.

كما أظهرت الد ارسة عدم وجود عالقة ارتباط بين المؤكسدات MDA ومضادات االكسدة)فيتامين E، فيتامين C، Zn (. لكن أظهرت عالقة عكسية بين MDA والـكلـوتاثايون (r=- 0.447) عند مستوى احتمالية ($p\leq0.01$) وعالقة طردية بين MDA وCu (r=0.409) عند مستوى احتمالية ($p\leq0.01$).

تم تقدير ملف الدهون وبينت النتائج إلحصائية ان تركيز الكوليسترول)TC(، الت اري كليسي اريد)TG(، الدهون البروتينية العالية الكثافة)HDL(، الدهون البروتينية الواطئة الكثافة)LDL(،

والدهون البروتينية واطئة الكثافة جدا)VLDL(ال توجد فروق معنوية من تلك الموجودة في مجموعة السيطرة مع مستوى احتـماليـــــة ($P \geq 0.05$).

تم تقدير هرمونات الغدة الدرقية فكان تركيز هرموني ايودو ثايرونين)T3(والثايروكسين)T4) لدى المرضى ال توجد فروق معنوية من تلك الموجودة في مجموعة السيطرة مع مستوى احتماليـــــــــــــــــة ($P \geq 0.05$) بينما تركيز الهرمون المحفز للدرقية)TSH(كان اعلى مما هو عليه في مجموعة االصحاء مع مستوى احتمالية ($P \leq 0.05$).

تعنى هذه الدراسة بدراسة تأثير االشعة الفوق البنفسجية الضيقة النطاق نوع B على 20 مريض مصابون بالبهاق)12 نساء و 8 رجال(. كانت االستجابة من 80-90% أعطت درجة ممتازة واالستجابة من 70-80% أعطت درجة استجابة جيدة و االستجابة من 60-70% أعطت درجة متوسطة بينما االستجابة من 50-60% أعطت درجة معتدلة واالستجابة من 40-50% أعطت درجة ضعيفة.

مجهورية العراق
وزارة التعليم العايل والبحث العلمي
جامعة االنبار - كلية العلوم
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دراسة تأثير االجهاد التأكسدي في المرضى المصابين بالبهاق في محافظة االنبار

اطروحة

مقدمة إلى مجلس كلية العلوم في جامعة ألنبار وهي جزء من
متطلبات الحصول على درجة الدكتوراه فلسفة
في علوم الكيمياء

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