A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF ÇANKIRI KARATEKİN UNIVERSITY

EVALUATION OF THE EFFECT OF HYPOTHYROIDISM ON INFLAMMATORY CYTOKINES STIMULATION ASSOCIATED WITH OXIDATIVE STRESS IN IRAQI PATIENTS

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
CHEMISTRY

BY

MURTADHA HADI JAWAD ALBAYATI

ÇANKIRI

2024

EVALUATION OF THE EFFECT OF HYPOTHYROIDISM ON INFLAMMATORY CYTOKINES STIMULATION ASSOCIATED WITH OXIDATIVE STRESS IN IRAQI PATIENTS

By Murtadha Hadi Jawad ALBAYATI

January 2024

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

Advisor : Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor : Asst. Prof. Dr. Nawal Khinteel JABBAR

Examining Committee Members:

Chairman : Prof. Dr. Volkan EYÜPOĞLU
Chemistry
Çankırı Karatekin University

Member : Prof. Dr. Şevki ADEM
Chemistry
Çankırı Karatekin University

Member : Asst. Prof. Dr. Ümit YIRTICI
Medical Laboratory
Kırıkkale University

Approved for the Graduate School of Natural and Applied Sciences

Prof. Dr. Hamit ALYAR
Director of Graduate School

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Murtadha Hadi Jawad ALBAYATI

ABSTRACT

EVALUATION OF THE EFFECT OF HYPOTHYROIDISM ON INFLAMMATORY CYTOKINES STIMULATION ASSOCIATED WITH OXIDATIVE STRESS IN IRAQI PATIENTS

Murtadha Hadi Jawad ALBAYATI
Master of Science in Chemistry
Advisor: Prof. Dr. Volkan EYÜPOĞLU
Co-Advisor: Asst. Prof. Dr. Nawal Khinteel JABBAR
January 2024

The current study aims to evaluate the relationship of hypothyroidism to the stimulation of inflammatory cytokines and oxidative stress and to study the role of levothyroxine as a treatment for hypothyroidism and its effect in alleviating these side effects by raising the levels of thyroid hormones to the normal level. The study groups were distributed into three groups, two groups for hypothyroidism patients and one for healthy people. The first group (G1) was for healthy people, the second group (G2) was for hypothyroidism patients with treatment of levothyroxine according to the doctor's instructions, and the third group (G3) was for hypothyroidism patients who were without treatment and those who are newly diagnosed with hypothyroidism. Samples were collected, serum was obtained and tests were conducted for Thyroid Stimulating Hormone (TSH), and thyroid hormones Triiodothyronine (T3), and Thyroxine (T4) to diagnose the disease or not. Analysis of triglycerides(TG), cholesterol(TC), low-lipoprotein(LDL), high-density lipoprotein(HDL), and very low-density lipoprotein(VLDL) were performed, to evaluate the lipid profile of patients related to hypothyroidism. Antioxidant status has been evaluated by determination of Superoxide dismutase (SOD) and Catalase (CAT) as well as assessment of oxidative stress biomarkers such as Malondialdehyde (MDA), and Advanced oxidation protein products (AOPP). Levels of inflammatory cytokines C-reactive protein (CRP), and Tumor necrosis factor alpha (TNF-α) to evaluate its relationship to the progression of disease, and estimate the role of treatment in changing the levels of these biochemical factors in the group of patients receiving levothyroxine as therapeutic for hypothyroidism state. The results of the study showed increase significantly level of TSH in the patient groups compared to the control group (G1), but decreased values of T3 and T4 ($P<0.001$). a significant increase in body mass patient groups compared to control, as well as in the values of TC, TG, LDL, and VLDL concentrations but decrease in HDL level in patient groups compared to control ($P<0.001$). Increased level of oxidative markers Malondialdehyde (MDA), and Advanced oxidation protein products(AOPP) in patient groups (G2 and G3) compared to G1 ($P<0.001$), regarding the activity of antioxidant enzymes, it was found that there was a non-significant difference in the activity of superoxide dismutase between all groups but there is significantly increase the activity of catalase in patients groups compared to control ($P<0.001$). Increased levels of inflammatory markers, Tumor Necrosis Factor Alpha (TNF-α), and C-reactive protein(CRP) significantly in patient groups compared to the control group ($P<0.001$). When comparing between the two groups of patients, there are also significant differences in the results. TSH level significantly decreased in G2 compared to G3. T4, and T3 increased in G2 compared to G3 ($P<0.001$). Oxidative stress (MDA, AOPP) and inflammatory markers (TNF-α, CRP) significantly decreased in G2 compared to G3 ($P<0.001$). Regarding antioxidant enzyme activities, CAT activity decreased significantly ($P<0.001$) in G2 compared to G3 but there are no significant differences in SOD activity. The results show the extent of the positive effect of treatment, not only in enhancing thyroid hormone levels, but also in helping reduce the effect of oxidative stress and inflammation that may occur as side effects of the disease by reducing the levels of inflammatory cytokines, despite not reaching the ideal condition, as was seen in the results of the healthy group.

2024, 71 pages

Keywords: Hypothyroidism, Triiodothyronine, Tetraiodothyronine, Thyroid-stimulating hormone, Antioxidant enzyme, Oxidative stress, Inflammatory cytokine

ÖZET

IRAKLI HASTALARDA HİPOTİROİDİZMİN OKSİDATİF STRES İLE İLİŞKİLİ İNFLAMATUAR SİTOKİN Stimülasyonu ÜZERİNDEKİ ETKİSİNİN DEĞERLENDİRİLMESİ

Murtadha Hadi Jawad ALBAYATI

Kimya, Yüksek Lisans

Tez Danışmanı: Prof. Dr. Volkan EYÜPOĞLU

Eş Danışman: Dr. Öğr. Üyesi Nawal Khinteel JABBAR

Ocak 2024

Bu çalışma, hipotiroidizmin inflamatuar sitokinlerin uyarılması ve oksidatif stres ile ilişkisini değerlendirmeyi ve levotiroksinin hipotiroidizm tedavisindeki rolünü ve tiroid hormon düzeylerini normal seviyeye yükselterek bu yan etkileri hafifletmedeki etkisini araştırmayı amaçlamaktadır. . Çalışma grupları iki grup hipotiroidi hastaları ve bir grup sağlıklı kişiler olmak üzere üç gruba ayrıldı. Birinci grup (G1) sağlıklı kişiler, ikinci grup (G2) doktorun talimatıyla levotiroksin tedavisi gören hipotiroidi hastaları, üçüncü grup (G3) ise tedavisi olmayan hipotiroidi hastaları ve hipotiroidi hastalarıydı. Hipotiroidizm tanısı yeni konuldu. Hastalığa tanı koymak veya teşhis koymak için numuneler alındı, serum alındı ve Tiroid Uyarıcı Hormon (TSH), tiroid hormonları Triiyodotironin (T3) ve Tiroksin (T4) testleri yapıldı. Hipotiroidizmle ilişkili hastaların lipit profilini değerlendirmek amacıyla trigliserit(TG), kolesterol(TC), düşük lipoprotein(LDL), yüksek yoğunluklu lipoprotein(HDL) ve çok düşük yoğunluklu lipoprotein(VLDL) analizleri yapıldı. Antioksidan durumu, Süperoksit dismutaz (SOD) ve Katalazın (CAT) belirlenmesinin yanı sıra Malondialdehit (MDA) ve Gelişmiş oksidasyon protein ürünleri (AOPP) gibi oksidatif stres biyobelirteçlerinin değerlendirilmesiyle değerlendirilmiştir. İnflamatuar sitokinler C-reaktif protein (CRP) ve Tümör nekroz faktörü alfa (TNF-α) düzeylerinin, hastalığın ilerlemesi ile ilişkisini değerlendirmek ve bu gruptaki bu biyokimyasal faktörlerin düzeylerini değiştirmede tedavinin rolünü tahmin etmek. Hipotiroidizm durumu için terapötik olarak levotiroksin alan hastalar. Çalışmanın sonuçları hasta gruplarında TSH düzeyinin kontrol grubuna

(G1) göre anlamlı düzeyde arttığını, ancak T3 ve T4 değerlerinde azalma olduğunu gösterdi (P<0.001). hasta gruplarında kontrole göre vücut kitlesinde, TC, TG, LDL ve VLDL konsantrasyonlarında anlamlı artış görülürken, hasta gruplarında HDL düzeyinde kontrole göre azalma görüldü (P<0.001). Hasta gruplarında (G2 ve G3) oksidatif belirteç Malondialdehit (MDA) ve İleri Oksidasyon Protein Ürünleri (AOPP) düzeylerinin G1'e göre arttığı (P<0.001), antioksidan enzimlerin aktivitesi açısından - süperoksit dismutaz aktivitesinde tüm gruplar arasında anlamlı fark vardı ancak hasta gruplarında katalaz aktivitesinde kontrol grubuyla karşılaştırıldığında anlamlı bir artış vardı (P<0.001). Kontrol grubuyla karşılaştırıldığında hasta gruplarında inflamatuar belirteçler, Tümör Nekroz Faktörü Alfa (TNF-α) ve C-reaktif protein (CRP) düzeyleri anlamlı derecede arttı (P<0.001). İki hasta grubu karşılaştırıldığında sonuçlarda da önemli farklılıklar vardır. TSH düzeyi G2'de G3'e kıyasla önemli ölçüde azaldı. T4 ve T3, G2'de G3'e kıyasla arttı (P<0.001). Oksidatif stres (MDA, AOPP) ve inflamatuar belirteçler (TNF-α, CRP), G2'de G3'e kıyasla önemli ölçüde azaldı (P<0.001). Antioksidan enzim aktiviteleri açısından, CAT aktivitesi G2'de G3'e kıyasla önemli ölçüde azaldı (P<0.001), ancak SOD aktivitesinde anlamlı bir fark yok. Sonuçlar, tedavinin yalnızca tiroid hormon düzeylerini artırmada değil, aynı zamanda inflamatuar sitokin düzeylerini azaltarak hastalığın yan etkisi olarak ortaya çıkabilecek oksidatif stres ve inflamasyonun etkisinin azaltılmasına yardımcı olma konusundaki olumlu etkisinin boyutunu göstermektedir. sağlıklı grubun sonuçlarında görüldüğü gibi ideal duruma ulaşılamıyor.

2024, 71 sayfa

Anahtar Kelimeler: Hipotiroidizm, Triiyodotironin, Tetraiyodotironin, Tiroid uyarıcı hormon, Antioksidan enzim, Oksidatif stres, İnflamatuar sitokin

PREFACE AND ACKNOWLEDGEMENTS

I would like to thank my thesis advisor, Prof. Dr. Volkan EYÜPOĞLU, for his patience, guidance and understanding.

I would also like to thank my thesis co-advisor, Asst Prof. Dr. Nawal K. JABBAR, for her help and support

I thank my family for supporting me during my studies.

Murtadha Hadi Jawad ALBAYATI

Çankırı-2024

CONTENTS

LIST OF SYMBOLS

g	Gram
μL	Microliter
mL	Milliliter
nm	Nanometer

LIST OF ABBREVIATIONS

TMB	(3,3', 5,5;-tetramethylbenzidine) chromogen solution
CH3COOH	Acetic acid
AOPP	Advanced oxidation protein products
$RO^{\cdot}$	Alkoxy radical
NHCl	Ammonium chloride
BMI	Body mass index
CAT	Catalase
TC	Cholesterol
Cu/Zn-SOD	Copper, Zinc- superoxide dismutase
CRP	C-reactive protein
DNA	Deoxyribonucleic acid
Na_2EDTA	Disodium ethylenediaminetetraacetate dihydrate
ELISA	Enzyme-linked Immune Sorbent Assay
GSH	Glutathione
GSSG	Glutathione disulfide
GPx	Glutathione peroxidase
GR	Glutathione Reductase
GM-CSF	Granulocyte-macrophage colony stimulating factor
HDL	High- density lipoprotein
HCl	Hydrochloric acid
H_2O_2	Hydrogen peroxide
$OH^{\cdot}$	Hydroxyl radical
HOCl	Hypochlorous acid
INF	Interferon gamma
IL-1	Interleukin-1
IL-12	Interleukin-12
IL-18	Interleukin-18
IL-6	Interleukin-6
D	Iodothyronine deiodinase enzymes
LPO	Lipid peroxidation
LDL	Low-density lipoprotein
MDA	Malondialdehyde
Mn-SOD	Manganese- superoxide dismutase
NADPH	Nicotinamide adenine dinucleotide phosphate
NH_2	Nitrenium ion
NO	Nitric oxide
OD	Optical density
OS	Oxidative stress
$ROO^{\cdot}$	Peroxyl radical

$ONOO^-$	Peroxynitrite
PBS	Phosphate buffer solution
KI	Potassium iodide
PKC	Protein kinase C
ROS	Reactive oxygen species
$^1O_2$	Singlet oxygen
sTNF-α	Soluble tumor necrosis factor-alpha
$O_2^{-\cdot}$	Superoxide
SOD	Superoxide dismutase
Th	T helper cells
HRP	The enzyme horseradish peroxidase
TBA	Thiobarbituric acid
TBARS	Thiobarbituric Acid Reactive Species
TSH	Thyroid-stimulating hormone
TRH	Thyrotropin-releasing hormone
T4	Thyroxine
tmTNF-α	Transmembrane tumor necrosis factor-alpha
TCA	Trichloroacetic acid
TG	Triglyceride
T3	Triiodothyronine
TNF-α	Tumor necrosis factor-alpha
TACE	Tumor necrosis factor-alpha converting enzyme
TNFR1	Tumor necrosis factor-alpha receptors 1
TNFR2	Tumor necrosis factor-alpha receptors 2
VLDL	Very-low-density lipoprotein

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Nearly 10% of the global population suffers from hypothyroidism; the majority of those affected are female. This condition is frequent among endocrine gland problems. Low levels of the thyroid hormones T3 and T4 cause hypothyroidism, a condition that, if left untreated, may cause serious health problems or even death.

Hypothyroidism is divided into two types: primary hypothyroidism when the thyroid gland cannot produce sufficient amounts of thyroid hormone, and secondary hypothyroidism when the thyroid gland itself is normal, but the defect is linked to the pituitary gland or hypothalamus, this kind is less common (Chiovato *et al.* 2019). The most common reason for hypothyroidism globally is a lack of dietary iodine. A simple blood test for hormones may identify hypothyroidism, and the exogenous thyroid hormone is a viable treatment option. Thyroid-stimulating hormone. Symptoms of the disease vary from one person to another, the patient's presentation ranges from an asymptomatic disease to the occurrence of myxedema, which may lead to coma (Suarez and Anastasopoulou 2023).

High levels of cytokines, such as C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6), are a hallmark of primary hypothyroidism, an inflammatory condition (Tayde *et al.* 2017).

These inflammatory cytokines play a part in the development of hypothyroidism-related problems by attracting or activating inflammatory cells, which in turn triggers endothelial dysfunction.

Levo-thyroxine is an artificial hormone with a chemical structure analogous to natural endogenous T4, that is a substitutive treatment for any of the situations linked with hypothyroidism, thyroxine therapy lowers the raised TSH levels into an acceptable range with regulation of triiodothyronine (T3) and T4 (T4) levels (Mateo and Hennessey 2019).

The role of extra TSH levels is suggested to directly enhance oxidative stress, hypothyroidism is related to improved response to oxidative stress. Therapy with L-thyroxine is active in carrying a drop in the stress factor levels such as MDA (Chakrabarti *et al.* 2016).

Levothyroxine therapy has a significant effect on variations in the inflammatory state, by reducing pro-inflammatory and stimulating anti-inflammatory cytokines. Besides the observed reduction of oxidative stress biomarkers (Marchiori *et al.* 2015).

1.1 Aim of the Study

- To assess the role of thyroid hormone levels on metabolic regulation such as body weight and lipid profile.
- To evaluate the activity of the antioxidant system by measurement of superoxide dismutase and catalase activities in women patients with hypothyroidism.
- To evaluate the change in oxidative stress biomarkers like MDA, and AOPP in women patients with hypothyroidism.
- To assess the inflammatory markers (CRP, and TNF-α) levels as a biomarker of the inflammatory state in hypothyroidism diseases.
- Studying the correlation of low levels of thyroid hormones with oxidative stress and inflammatory markers.
- To evaluate the consequence of levothyroxine treatment on oxidative stress and inflammatory markers, in addition to improving the levels of thyroid hormones in hypothyroidism patients.

2. LITERATURE REVIEW

2.1 Thyroid Gland

The thyroid gland is an organ with the shape of a butterfly that consists of two lobes, the left and right, joined by a thin tissue band known as a "isthmus". In adults, it weighs 25 grams. The gland, which is often larger in women than in men consider an important endocrine hormone gland, it has a significant impact on how the human body grows, develops, and regulates metabolism. Thyroid hormones are continuously released into the bloodstream at a consistent rate, aiding in the regulation of numerous bodily processes. The thyroid gland generates more hormones when the body requires more energy, such as when it is growing or cold or when a woman is pregnant (Maenhaut *et al.* 2015).

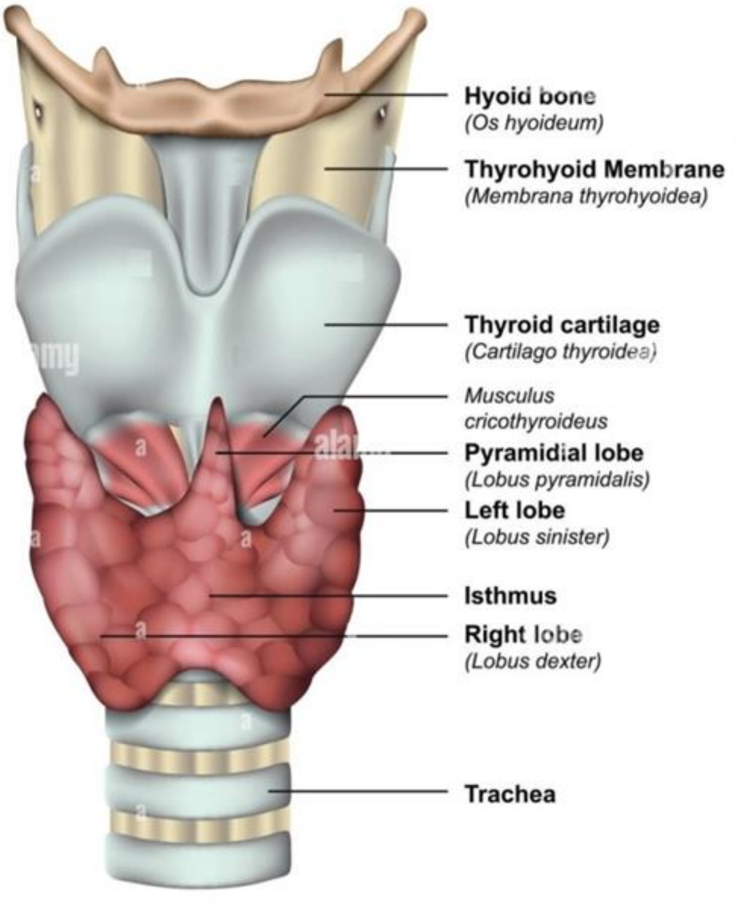


Figure 2.1 An illustration of the details of the human thyroid gland (Snead 2022)

The thyroid gland secretes three hormones: triiodothyronine (T3), thyroxine (T4), and calcitonin, a peptide hormone. The production of both thyroid hormones is regulated by the anterior pituitary gland's release of thyroid-stimulating hormone (TSH). TSH levels are regulated by the hypothalamus-produced thyrotropin-releasing hormone (TRH) (Samuels *et al.* 2018).

2.1.1 Thyroid hormones

The thyroid gland produces triiodothyronine and thyroxine, which are iodinated dipeptides. A complex sequence of mechanisms, including bidirectional transport from and to the lumen of thyroid follicles, manufacture, store, and release these hormones. So, thyrocytes can't secrete thyroid hormone unless they form and can keep a tight membrane around themselves (Borsò *et al.* 2022).

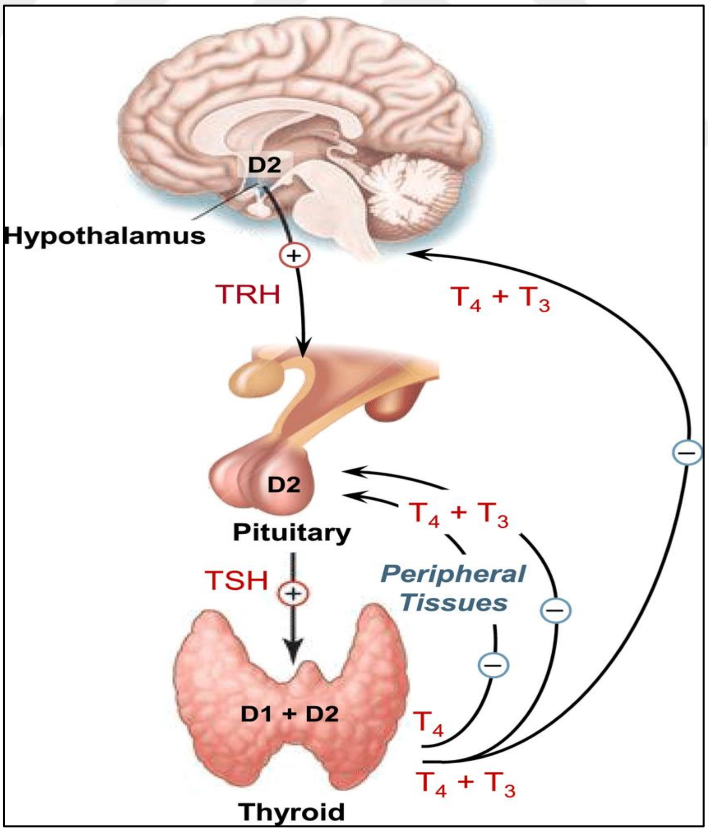


Figure 2.2 Diagram of the system that shows the roles of thyroid hormones (Borsò *et al.* 2022)

2.1.2 The importance of the thyroid gland

In addition to regulating vital physiological processes like growth and energy consumption, the thyroid gland and the hormones it produces play an important role in the development of several organs in vertebrates. The thyroid gland's follicular cells manufacture the hormones tetraiodothyronine (T4) and triiodothyronine (T3), which modify the expression of several gene products and act on almost every bodily tissue via their interactions with nuclear receptors (Alzahrani *et al.* 2020). Thyroxine (T4) and 3,5,3-ltriiodothyronine (T3), two pro-hormones produced by the thyroid gland, are not fully functional. The iodothyronine deiodinase enzymes D1 and D2 convert T4 to T3 via 5 monodeiodination, however most circulating T3 is produced by pre-receptor ligand metabolism. The D3 enzyme, in contrast, monodeiodinates five times, destroying T3 and T4. T3 levels inside cells are therefore dictated by the relative activities of these three deiodinases (Al-Suhaimi and Al-Khater 2019).

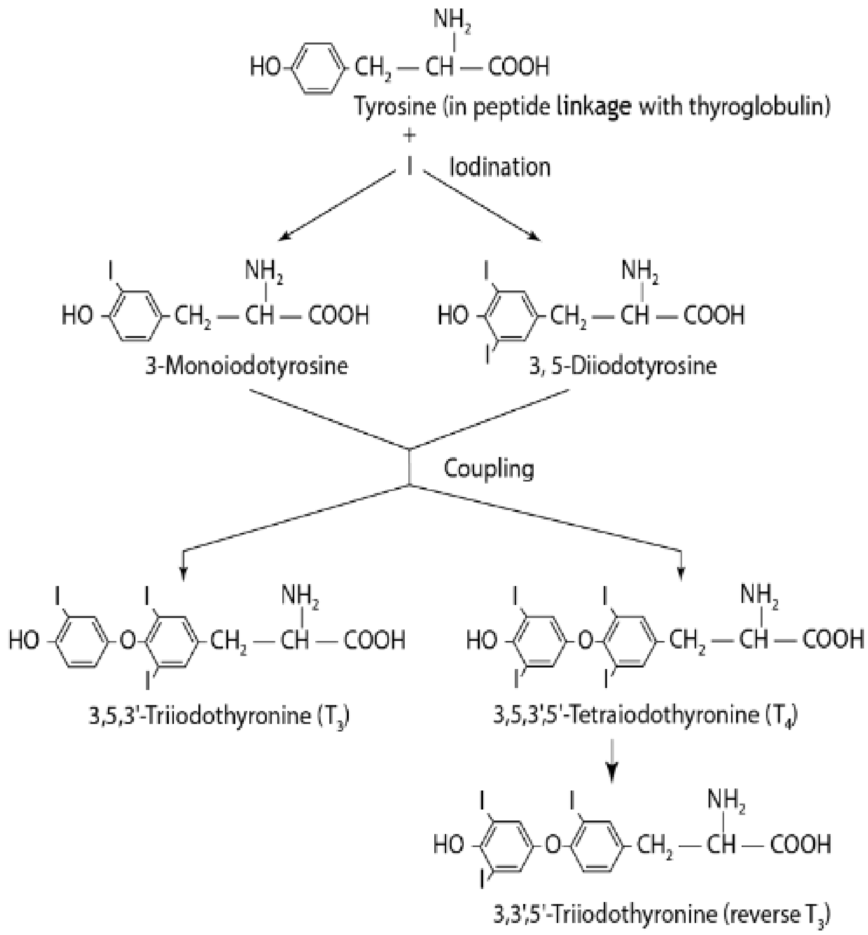


Figure 2.3 Synthesis and release of thyroid hormones (Babić Leko *et al.* 2021)

2.2 Disorders Related to Thyroid Gland

There are broad varieties of thyroid diseases, and each has its own causes and symptoms. A deficiency in the thyroid gland's function produces abnormalities in the body's functioning. A person may experience one or several species simultaneously, including those involving genetic influences, low thyroid function brought on by insufficient free thyroid hormones is known as hypothyroidism, In return, hyperthyroidism caused by there are too many thyroid hormones, also structural abnormalities, most frequently a goiter and tumors that may or may not be malignant (Al-Suhaimi and Al-Khater 2019).

2.2.1 Hypothyroidism

Reports indicate 200 million people are affected by thyroid problems globally, and up to 60% of those suffering from thyroid dysfunction are ignorant of their illness. Iodine insufficiency is the most frequent cause of thyroid dysfunction, and two billion people worldwide are thought to consume insufficient amounts of iodine (Tuttle *et al.* 2019). The most frequent causes of thyroid problems, however, are autoimmune thyroid disorders in nations where iodine supplementation is popular. Hypothyroidism occurs when the thyroid gland does not produce enough thyroid hormones, which reduces the metabolic activity of the target tissue (La Vignera and Vita, 2018). When hypothyroidism is left untreated, it can lead to additional long-health issues, such as hypertension, cognitive impairment, dyslipidemia, neuromuscular dysfunction and infertility (La Vignera *et al.* 2017).

Although it can be treated, hypothyroidism is an endocrine disorder that requires constant medicine supplementation and careful monitoring of the thyroid profile to prevent treatment adverse effects (Duntas and Yen 2019). Often, it takes a long time to regularize the thyroid profile with medication treatment. Despite receiving therapy for hypothyroidism, patients continue to have an increased risk of morbidity from multiple cardiovascular and cerebrovascular illnesses (Idrees *et al.* 2020).

2.2.1.1 Clinical features of hypothyroidism

Many important physiologic processes are regulated by thyroid hormone receptors. Consequently, a wide range of symptoms and clinical signs may be brought on by hypothyroidism. Generally speaking, the intensity of these symptoms typically reflects the level of thyroid malfunction and the rate at which hypothyroidism developed (Tuttle *et al.* 2019). Often the signs and symptoms of hypothyroidism are undefined. These include menstruation irregularities, weight gain, exhaustion, poor attention, sadness, and widespread muscular ache. high specificity (Kim *et al.* 2018).

2.2.1.2 Causes of hypothyroidism

Hypothyroidism results if the thyroid gland is unable to produce enough hormones, there are numerous potential causes of hypothyroidism (La Vignera *et al.* 2017). Hashimoto's thyroiditis, an autoimmune condition, is the most typical cause of hypothyroidism. Thyroid gland may be harmed by radiation used to treat head and neck malignancies, which could result in hypothyroidism. Some newborns may also be born without a thyroid gland or with a malfunctioning thyroid gland. A pituitary gland issue, which is very uncommon, can lead to hypothyroidism, which is when the pituitary gland is unable to produce enough TSH. Some pregnant women experience hypothyroidism after or during delivery. A lack of iodine, it is one of the factors that contribute to hypothyroidism since it is necessary for the creation of thyroid hormones (Thayakaran *et al.* 2019).

2.2.1.3 Screening and diagnosis

Screening of asymptomatic people may be considered, with those who have hypothyroidism risk factors, like autoimmune disease history, (La Vignera and Vita 2018). A serum TSH test is the best laboratory evaluation of thyroid function and the preferred test for identifying primary hypothyroidism. If the TSH is high, a test should be performed T4. The presence of both a low serum T4 level and an increased serum TSH level suggests overt primary hypothyroidism. Secondary hypothyroidism is consistent with low T4 levels and low TSH levels. These conditions are typically accompanied by additional signs of hypothalamic-pituitary insufficiency (Alzahrani *et al.* 2020).

2.3 Free Radical, Antioxidants System and Oxidative Stress Related Disease

2.3.1 Free radical

Free radicals are chemicals (ions, atoms, or molecules) whose outer orbitals include one or more unpaired electrons, making them highly reactive chemicals that the body produces in reaction to stimuli from the environment and other factors. Considered reactive nitrogen species RNS as well reactive oxygen species ROS from free radicals (Di Meo and Venditti 2020). Millions of free radical reactions can happen in a matter of seconds due to smoking, prolonged sun exposure, emotional or psychological stress, and poor eating habits, unless free radicals are neutralized. Free radical production is related to aerobic cells' proper metabolism. Due to the oxygen consumption necessary for cell growth, a number of oxygen-free radicals get produced (Vadgama 2021). That an increase in ROS causes damage to cells, lipids, proteins, or DNA, which inhibits normal function. As a result, oxidative stress is becoming linked to a growing number of diseases. The relation between disease and ROS can serve to explain by the notion of oxidative stress (Seven *et al.* 2012).

2.3.2 Reactive oxygen species (ROS)

Reactive oxygen species are a class of oxidants,The formation of free radicals was the same or molecular species that can produce free radicals. Nitric oxide (NO) radicals and super oxide radicals make up the majority of ROS. Transformation reactions change both $O_2^{\cdot -}$ and $NO^{\cdot}$ radicals into potent oxidizing radicals including alkoxy radical ($RO^{\cdot}$), the hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2$), peroxyl radical ($ROO^{\cdot}$), and alkoxy radical ($RO^{\cdot}$), where some of the types can be changed into molecular oxidants such as hypochlorous acid (HOCl), peroxynitrite ($ONOO^-$), hydrogen peroxide (H_2O_2), and other substances (Winterbourn 2008).

2.3.3 Antioxidants system

Antioxidants are substances that aid in preventing or minimizing cell damage brought on by free radicals. Low molecular weight anti-oxidants can safely interact with free radicals, prevent the chain-reaction from damaging crucial components. It is possible for antioxidants to give hydrogen atoms into free radicals produced by cellular metabolism or by external sources, preventing starting the chain reaction , resulting in DNA, lipid, and amino acid damage, and finally cell death (Hasanuzzaman *et al.* 2020).

Antioxidants are characterize as substances that prevent the oxidation effect to diverse compounds. Antioxidants contain enzymes that consist catalase (CAT), superoxide dismutase(SOD), and other peroxidases, as well non-enzyme compound such as minerals, vitamins, thiols like polyphenols, tocopherol, retinol derivatives, glutathione, and other compounds (Hasanuzzaman *et al.* 2020).

2.3.3.1 Antioxidant enzymes

Superoxide dismutase (EC 1.15.1.1) is a essential enzyme that eliminates the reactive superoxide radical in the human body by converting it to hydrogen peroxide, there are two types of SOD present: Mn-SOD is found in the mitochondria and Cu/Zn-SOD is present in extracellular space and the cytosol. The superoxide radical can produce a variety of reactive species, including alkoxyl and lipid peroxyl radicals, and peroxynitrite, that which can cause extensive damage to a lot of human tissue. Superoxide radical is neutralized by the SOD enzyme, which also protects cellular proteins, lipids, and DNA against ROS-induced deterioration (Jha *et al.* 2018).

Catalase has a quick response rate, each second, millions from molecules of hydrogen peroxide (H_2O_2) are broken down. It counteracts H_2O_2's maybe fatal effects. Iron and manganese are both cofactors in CAT Equation (2.1) (Czyzewska and Trusek 2018).

$$2H_2O_2 \xrightarrow{\textbf{Catalase}} 2H_2O + O_2 \quad (2.1)$$

Catalase (EC 1.11.1.6) is Exists in the peroxisomes, erythrocytes, and extracellular spaces. Human erythrocyte and liver have the highest levels of catalase, whereas the brain and heart have the lowest levels (Kar and Sinha 2014).

The Selenium contain Glutathione peroxidase (GPx) the glutathione (GSH)useing as an electron granter to decrease ROS as well change GSH into glutathione disulfide (GSSG), which primarily occurs in the mitochondria but also occurs in the cytoplasm, converts lipid peroxides into their conformable alcohols and H_2O_2 to H_2O (Ungati *et al.* 2019).

GSH and Glutathione peroxidase are connecting through the oxidation 6-phosphogluconate, and glucose-6-phosphat, from that it results NADPH, which glutathione Reductase (GR) uses to convert GSSG into GSH Equation (2.2) and Equation (2.3) (Gonul Baltaci *et al.* 2019).

$$\mathrm{GSH} + \mathrm{H_2O_2} \xrightarrow{\mathbf{GPx}} \mathrm{GSSG} + \mathrm{H_2O} \tag{2.2}$$

$$\mathrm{GSSG} + \mathrm{NADPH} \xrightarrow{\mathbf{GR}} \mathrm{GSH} + \mathrm{NADP^+} \tag{2.3}$$

Glutathione-S-transferase is a vital enzyme that aids in the antioxidant metabolism to thiol compounds, protecting cells from xenobiotics, oxidative stress and free radical-induced cellular damage (Ungati *et al.* 2019).

2.3.3.2 Superoxide dismutase (SOD)

Superoxide dismutase is a crucial enzyme in the living family that spontaneously dismutates the $O^{-\bullet 2}$ anion to produce O_2 and H_2O in order to maintain normal physiological conditions and to cope with stress. Superoxide $O^{-\bullet 2}$ is mostly quenched by Superoxide Dismutase Equation (2.4) (Mi *et al.* 2018).

$$2H^{+} + 2O_2-.+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (2.4)$$

There are different types of superoxide dismutase; one kind is found in the matrix of mitochondria and contains manganese; the other type is found in the cytoplasm and contains copper and zinc. In response to excessive oxidative stress, cells have the capacity to increase SOD production. The fluid extracellular containing SOD of a high molecular weight type, the enzyme attaches to the surface of external endothelial cells and may have a role in causing free radical damage (Dworzański *et al.* 2020).

To each type, it is believed that the mechanism of catalysis is the same, involve a protein pocket surrounded by residues of positively charged amino acids that operate as an electrostatic basin to draw superoxide anion radicals to the active site. When the transition metal is at the active site, it performs an alternate oxidation-reduction reaction and transfers one electron between two superoxide radicals (Reier *et al.* 2017).

2.3.3.3 Catalase (CAT)

Catalase is the type of enzymes, that catalyze breaks down hydrogen peroxide into oxygen and water. Hydrogen peroxide's potentially fatal effects are mitigated by catalase Equation (2.5) (Tehrani and Moosavi-Movahedi 2018).

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \qquad (2.5)$$

All aerobics eukaryotes contain on the enzyme and is important in is crucial for removing hydrogen peroxide produced in peroxisomes by oxidase interested in B-oxidation long fatty acid. There are three isoenzymes that are located individually in mitochondria, cytosol, and peroxisomes (Shahid *et al.* 2022).

2.3.3.4 Non–enzymatic antioxidants

Through non-enzymatic processes, free radical scavengers transform free radicals into a nonradical, harmless state. Antioxidant substances work as free radical scavengers, giving the radical an atom of hydrogen to bring it back to equilibrium. Antioxidants decrease free radicals and become oxidized as a result. A conjugated double bond allows the aromatic ring structure of the vitamins E, C, carotenoids, and flavonoids, which are all dietary and endogenous free radical scavengers. (Jain 2021).

2.3.4 Oxidative stress

Oxidative stress (OS) is a popular term that expresses oxidation's impact when the level of ROS is abnormal, like the free radical (hydroxyl, superoxide, nitric acid) and the non-radicals (lipid peroxide, hydrogen peroxide) and we can determine the severity of oxidative stress by balancing the rate at which oxidative damage occurs and the rate which it is repaired (Hasanuzzaman *et al.* 2020). A specific steady-state ROS level is provided by the balance. The ROS rises during disease, infections, exposure to pollutants, exercise, smoking, ionizing radiation, UV light ...etc. Because the increase in ROS can harm cells, DNA or lipids proteins and impair normal function, Due to the association of oxidative stress with many diseases. ROS can activate a variety of transcriptional factors that promote inflammation and produce cytokines and chemokines (Bigagli and Lodovici 2019).

2.4 Oxidative Stress with Hypothyroidism

Oxidative stress results from an imbalance between the production of free radicals and the antioxidant state. It has been connected to a number of pathologies includes thyroid disease (Reddy *et al.* 2013).

Oxidative stress has a long time linked with hyperthyroidism in many studies due to increased metabolic rate and oxygen consumption this leads to the formation of free radicals (Nanda 2016).

Oxidative stress was description in hypothyroidism. Demonstrated a connection between oxidative stress and hyperlipidemia and increased lipid risk factors for cardiovascular disease in hypothyroid individuals, wherefore, oxidative stress could be one of possible mechanisms to future complications from atherosclerosis in hypothyrodisim patients (Reddy *et al.* 2013).

Low-density lipoprotein (LDL) that has undergone oxidation is a known contributor to atherosclerosis, that LDL from hypothyroidism patients is more affected for oxidation, a sign of oxidative stress (Nanda 2016).

2.4.1 Advanced oxidation protein products (AOPP)

Because oxidative stress damages proteins more easily than it should, AOPPs were created. AOPPs are considered a novel marker of protein degradation caused by oxidants, AOPP are proteolysis-resistant and connected with dityrosine level, which is mostly generated under oxidative stress by interaction of plasma albumin with chlorinated oxidants. Found advanced-oxidation protein products at first in the patient's plasma that metabolic disorder. It was found that, AOPPs can increase stimulate atherosclerosis and vascular endothelial dysfunction by increasing oxidative stress as well inflammatory factors expression (Cheignon *et al.* 2018).

Advanced-oxidation protein products have been demonstrated to increase the agitation of mesangial cells by PKC activation dependent-NADPH oxidase, and the accumulation of AOPPs causes the reduction of NADPH oxidase depending podocytes during a p53-Bax regular cells death pathway. Additionally, AOPPs have the ability to inhibit pre-adipocyte development and cause cellular inflammation (Zhou *et al.* 2010).

2.4.2 Malondialdehyde (MDA)

Malondialdehyde is a very unstable and reactive compound, hence no commercial standard is available. MDA is a chemically active compound that can interact with DNA and the amino acids found in protein moieties. Due to the two aldehyde groups' reactivity with nucleophiles, MDA can generate adducts that cause biomolecular damage. The TBARS assay, also known as thiobarbituric acid reactive Species compounds, is a widely used technique for detecting lipid peroxidation (Mas-Bargues *et al.* 2021).

2.5 Inflammatory Response to Hypothyroidism

Inflammation is an unspecific immunological response for any form of physiological damage. The inflammatory response know is a defense mechanics that evolved in the higher organisms to keep them from injury and infection. Its goal is to locate the harmful agent, get rid of it, and get rid of the parts of the injured tissue so that the body may start to heal. The response consist from changes in blood flow, an rise in permeability of blood vessels, and the transmigration of fluid, white blood cells and proteins from the circulation to the site of harm to tissue. Acute inflammation is an inflammatory response usually lasts only a few days, while chronic inflammation is an inflammatory response which lasts longer (Marchiori *et al.* 2015). In some conditions, the normally self-limiting inflammatory process, becomes chronic, leading to the development of chronic inflammatory diseases (Li *et al.* 2018).

With the progression of hypothyroidism, inflammation increases, which may contribute to improved oxidative stress. Oxidative stress leads to an increase in the risk of atherosclerosis and cardiovascular illnesses in hypothyroid individuals, which may be brought on by inflammation (Marchiori *et al.* 2015).

An inflammatory illness known as hypothyroidism is marked by elevated levels of cytokines such as C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL 6) (Sencar *et al.* 2019). These inflammatory indicators may play a part in the etiology of certain hypothyroidism-related problems via triggering inflammatory cell recruitment and activation, smooth muscle cell migration and proliferation, and endothelial dysfunction. Additionally, they mediate apoptosis and encourage the synthesis of interferon-gamma (Marchiori *et al.* 2015).

2.5.1 Inflammatory cytokines

Some cell types, including immune cells, emit cytokines that promote inflammation; one such class is the inflammatory cytokine. When it comes to inducing inflammatory reactions, the primary makers of inflammatory cytokines are macrophages and helper cells (Th) (Scarpioni *et al.* 2016). Among them are tumour necrosis factor alpha (TNF-α), interferon gamma (IFN), and granulocyte-macrophage colony stimulating factor (GM-CSF). Additionally, IL-1, IL-6, IL-12, and IL-18 are all part of this group (Ojima *et al.* 2013).

Cytokines are essentially, low-molecular-weight proteins secreted by stromal cells and activated immune cells, and it has important functions, that are crucial for immune system control and have been embroiled to causing a number of disturbances. After attaching to certain receptors, they trigger intracellular messengers that regulate gene transcription. In this way, cytokines regulate the production and function of inflammatory cytokines, which may have a positive or negative effect on inflammation. Their effects on immune cell function, differentiation, proliferation, and survival are notable as well. They have been associated to a range of infections and diseases that impact the immune system through both pro- and anti-inflammatory pathways; they are important mediators that control the immune response; and their expression in immune cells is influenced by various factors like pain, sickness, hormonal changes, and related gene polymorphisms (Scarpioni *et al.* 2016).

2.5.1.1 C-Reactive protein

One member of the pentraxin family of plasma proteins is C-reactive protein, or CRP. Hepatocytes produce CRP in reaction to pro-inflammatory cytokines, especially IL-6, and it is often employed as a general inflammatory marker because of this. The acute phase response is triggered by a wide range of inflammatory illnesses, both chronic and acute. These include infections caused by bacteria, viruses, or fungi; inflammatory disorders such as rheumatoid arthritis; cancer; trauma; or tissue necrosis. Under these conditions, cytokines like interleukin-6 and tumour necrosis factor-alpha are released, which stimulate the liver to produce CRP and fibrinogen (Pan *et al.* 2017). A new research found that compared to healthy controls, hypothyroid individuals had significantly higher blood CRP levels (Newling *et al.* 2018).

2.5.1.2 Tumor necrosis factor-alpha (TNF-α)

Pro-inflammatory cytokines are increased in inflammatory injuries and help to promote and maintain chronic inflammation in a variety of inflammatory illnesses (Shi *et al.* 2019).

TNF is a key component of a broad family of cytokines that is crucial for controlling of the adaptive and innate immune systems. TNF is a crucial component in the beginning and management of immunity and inflammation. TNF is generally recognized as a potent proinflammatory cytokine that stimulates the majority of immune system cells. TNF- (26 kDa) is first formed in adipocytes as a transmembrane protein (tmTNF-α). The help of matrix metalloprotease stimulate breakdown of extracellular domain of tmTNF-α with the assistance of matrix metalloprotease, a TNF-α-converting enzyme (TACE) that leaves soluble TNF-α (17 kDa). Both tmTNF and sTNF are biologically active and function as signal transducer by interacting with two receptors, TNFR1 and TNF-R2. while oligomerization of TNF-α occur via specific protein-protein interactions that connection both receptors with adapter molecules, it provides two cellular responses; first one is induction of cell death of by apoptosis and the second one is the gene transcription activation for cell survival (Coperchini *et al.* 2021).

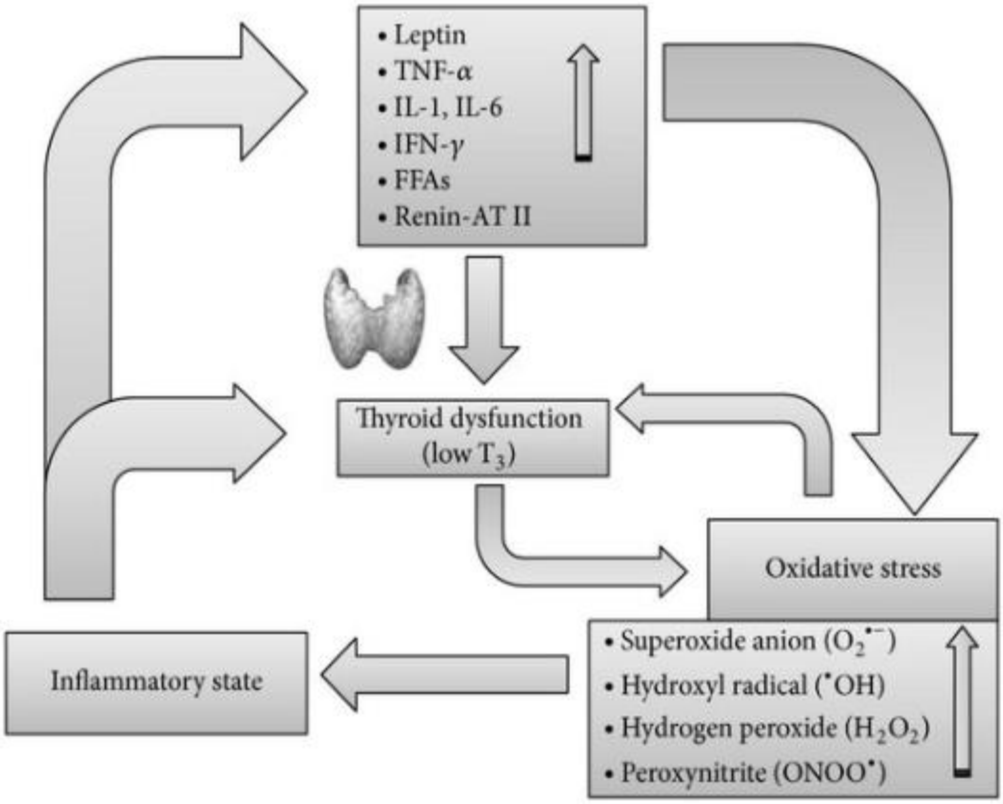


Figure 2.4 Proposed model of the interrelationships between inflammation, oxidative stress, and thyroid derangement (Mancini *et al.* 2016)

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Tools and instruments

All instruments and equipment that used in the study are listed in the Table 3.1.

Table 3.1 Tnstruments and tools that are used in the study

No.	Instrument / Equipment	Company	Origin
1	Micropipette 0.5-10, 5-50,100-1000µl	CBPP	U.K
2	96-Well plate	Elabscience	China
3	Absorbance Microplate Reader	Biotech	USA
4	Centrifuge	Kokusan	Germany
6	Eppendorf tubes	Eppendorf	Germany
7	Gel Tubes	Afco	Jordan
8	Incubator	Memmert	Germany
9	Microtube Rack	ThermoFisher	USA
10	pH meter	Hanna	Italy
11	Sensitive Balance	Sartorius	Germany
12	Spectrophotometer	Spectrolab	Germany
13	Disposable Syringe (10ml)	MEDECO	UAE
14	T80 UV - VIS Spectrometer	PG	UK
15	Timer	Wondfo	China
16	Water bath	Techne Junior TE-8J	England
17	Freezer -20° C	Arcelik	Turkey
19	Reusable Ice Packs	J.L. CHILDRESS	USA

3.1.2 Kits

The kits that used in the study are listed in the Table 3.2.

Table 3.2 The kits used in the study

No.	Kit	Company	Country
1	T3 ELISA Kit	Cortez	USA
2	T4 ELISA Kit	Cortez	USA
3	TSH ELISA Kit	Elabscience	USA
4	Human TNF-α (Tumor Necrosis Factor α) ELISA Kit	Elabscience	USA
5	NycoCard CRP	Abbott	Norway
6	Cholesterol	Beckman coulter	Ireland
7	Triglyceride	Beckman coulter	Ireland
8	HDL- Cholesterol	Beckman coulter	Ireland
9	LDL- Cholesterol	Beckman coulter	Ireland

3.1.3 Chemicals

The chemical and biological agents used in the current work are shown in Table 3.3.

Table 3.3 The table lists the chemicals that were employed in this study

No.	Chemicals Materials	Company	Origin
2	Chloramine-T	BDH	England
4	Disodium ethylenediaminetetraacetic acid	Fluka. Company	Switzerland
5	Disodium hydrogen phosphate	Fluka. Company	Switzerland
6	Epinephrine	Sigma. Aldrich.	UK
8	Glacial acetic acid	Scharlau	Spain
9	Hydrochloric acid	BDH	England
10	Hydrogen peroxide	Scharlau	Spain
12	potassium iodide	Sigma. Aldrich.	UK
13	Potassium dihydrogen phosphate	BDH	England
14	Sodium bicarbonate	BDH	England
15	Sodium hydroxide	BDH.	England
16	Thiobarbutric acid	AVONCHEM	UK
17	Tri Chloroacetic acid	Merek	Germany

3.2 Design of Study

In this (case and control) study. This study is carried out in Biochemistry Laboratory in the Department of Chemistry/College of Science/University of Al- Qadisiyah and AL-

Diwaniyah Teaching Hospital in Qadisiyah, through the period between (May 2022) to (September 2022). The study included (120) subjects, (40) healthy with mean age (mean age ±SD), (40) hypothyroidism Patients previously diagnosed and stick with treatment by levothyroxine ((mean age ±SD), (40) hypothyroidism Patients newly diagnosis without treatment (mean age ±SD).

General data: gender, age, body mass index (BMI) was calculated as body weight (kg) divided by squared height (in meters), and period of hypothyroidism for the patients, The information was recorded.

3.2.1 Inclusion criteria

In this study, three groups comprise a healthy group with normal blood biochemical values, and two patient groups with hypothyroidism . Patients were choose after screening by Endocrinology specialists for thyroiditis and determine the degree of severity, as well as the diagnosis was confirmed by clinical features and biochemical tests of TSH, T3, and T4.

3.2.2 Exclusion criteria

Patients sufferance from autoimmune disease, diabetic, pregnancy, renal disease, tumor, liver disease and acute illness or infection were excluded from the study.

3.3 Whole Blood Collection

Whole venous blood samples were aseptically collected by venous puncture using a sterile 6ml disposable syringe. Blood were put in gel tubes and allowed to clot for 15 minutes and then separated by centrifuge for 10 minutes at (4000 rpm). Sera were separated and transferred to Eppendorf tubes, which were labeled and stored in a deep freezer at -20 ℃ until they were used for measurement parameters in this study, except oxidative stress parameters were examined at the same time.

Thyroid hormones, lipid profile, Blood pressure, Antioxidant enzymes like SOD and CAT, oxidative stress markers such as MDA, and AOPP, and inflammatory markers like C-Reactive protein, and TNF-α levels were measured.

3.3.1 Ethical approval

Prior to collecting specimens, verbal agreement was obtained from every patient. The publishing ethics committee of Çankırı Karatekin University's Fen Bilimleri Enstitüsü and the ethical committee at Diwaniyah Teaching Hospital both reviewed and approved this work.

The study plan is shown in the Figure 3.1

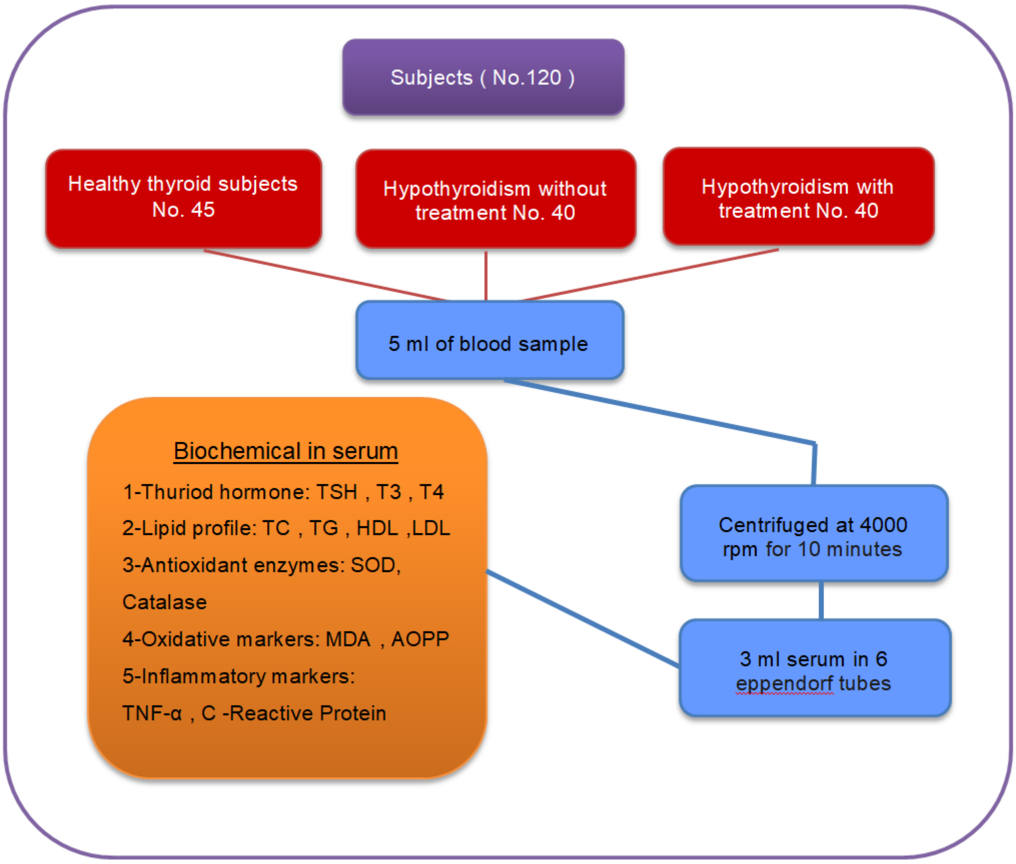


Figure 3.1 Experimental design

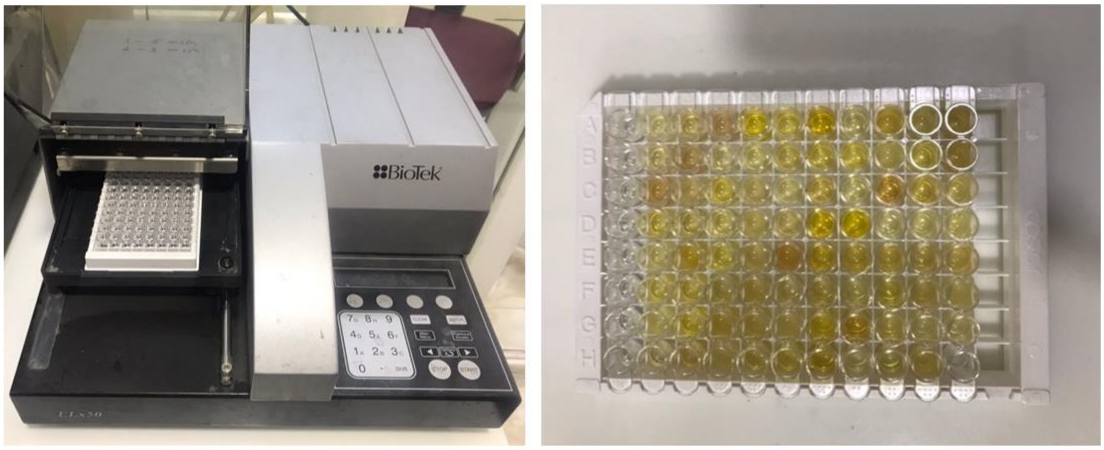
3.4 Biochemical Analysis

3.4.1 Use the ELISA to enzyme-linked ımmune sorbent assay

Principle: In the hormones ELISA, a specific quantity of anti-hormone antibody is placed on microtiter wells. A determined amount of patient serum and a consistent quantity of hormone conjugated and horseradish peroxidase were, applied to the microtiter wells.

During incubation, it happens the anti-hormone antibody binds to the second antibody in the wells, and also the conjugated hormone competes for the anti-hormone antibody's restricted binding sites. Following a 60-minute incubation to wells at room temperature, and the wells are washed five times with water to eliminate unbound hormone conjugate. After that, a TMB solution is added and it is incubated for 20 minutes, leading to the appearance of blue color. The addition of 2NHCl to stops the color development, absorbance is measured spectrophotometrically using a wavelength of 450 nm. The strength of the color generated is proportional to the quantity of enzyme present and is inversely linked to the amount of unrecognized hormone in the sample. Using a set of hormone criteria that were all tested in the same way, The hormone concentration in an unknown sample is determined.

The hormones that were tested in this method are Triiodothyronine (T3) and Thyroxine (T4).



(A) (B)

Figure 3.2 (A) ELISA device used to measure thyroid hormones and TNF-alpha (B) ELISA plate

3.4.2 Thyroid stimulating hormone (TSH) by enzyme-linked immune sorbent assay

Principle: Is a quantitative in vitro enzymelinked immunosorbent test for human TSH in blood, and plasma. This test makes use of a 96-well plate covered with an antibody specifically for human TSH. Using the immobilised antibody, samples and standards are introduced into the wells, and the TSH present in a sample is bound to the wells. Applying a biotinylated anti-human TSH antibody follows the washing of the wells. The wells are prepared by pipetting HRP-conjugated streptavidin into them after washing away any unattached biotinylated antibody. After rinsing the wells again, a substrate TMB solution is added, and colour develops according to the amount of bound TSH. Colour intensity is measured at 450 nm after the Stop Solution changes the colour from blue to yellow.

3.4.3 Lipid profile by spectrophotometry and potentiometry by beckman coulter AU480 auto analysis

Principle: Spectrophotometry and potentiometry are the principles of the “Backman Coulter biochemistory analyser”. Submerging a particle or cell in a conductive liquid (diluent) and then measuring the changes in electrical resistance as it passes through a small hole is the basis of the Coulter Principle. Discrete insulators are particles or cells that are suspended in a conducting liquid. An electrical pulse is produced when a diluted particle solution is drawn through a small cylinder-shaped aperture, and the motion of each cell alters the resistance of the electrical circuit connecting the two submerged electrodes on each side of the aperture.

The analyzes conducted with this technique are the analysis of cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and very-low-density lipoprotein (VLDL).

3.4.4 Determination of serum advanced oxidation of protein product (AOPP) concentration

Principle: “The Advanced oxidation of protein product” test directly quantifies the amount of AOPP in biological specimens. To begin the color enhancement method, unidentified samples with AOPP or Chloramine standards are coupled with an assay reaction substance. The AOPP levels in unidentified samples may be estimated by comparing them to a predefined Chloramine reference curve. The I_3^- yield from the reaction of samples a solution of potassium iodide at wavelength 340nm is utilized to compute AOPP concentrations at μmol/L of Chloramine-T equivalent Equation (3.1) (Liu *et al.* 2020).

$$RNHCl + 3I^- \text{ (colorless)} + H^+ \leftrightarrow I_3^- \text{ (yellow-brown)} + RNH_2 + Cl^- \quad (3.1)$$

Reagents

- PBS (0.1M, pH7.4)
- 19.256 gm from “KI (MW= 166.003 gm/mol)” was dissolved in 100 ml from distilled water to make KI (1.16 M).
- CH_3COOH.
- Solution of Chloramine-T 150 µM standard was prepared by dissolved 4.215mg from Chloramine-T (MW= 281.69gm/mol) in hundred ml of PBS
- Two hundred µl from specimen serum was diluted in PBS in a ratio of 1:5.
- The solution of chloramine-T was diluted with PBS to the desired concentrations (0, 10, 20, 40, 60, 80, 100,120, and 140 µM) before drawing the standard curve.

Procedure: The following three tube sets were prepared Table 3.4.

Table 3.4 Preparation for the AOPP mixture test

Reagent	**Blank**	**Standard**	**Sample**
Serum	—	—	1000 µl
Chloramine-T	—	1000 µl	—
Phosphate buffer	1000 µl	—	—
Potassium iodide	10 µl	10 µl	10 µl
	Kept for 2 min at 25 °C		
Acetic acid	20 µl	20 µl	20 µl

A standard curve was used to determine the concentration of AOPP. As previously stated, 20µl acetic acid and 10µl of KI two minutes later had been added to every standard concentration (1000 µl). The absorbance of standards at 340 nm was measured using a T80 UV - VIS Spectrometer (PG, UK). Were measured AOPP concentrations in µmol/L for Chloramine-T equivalents Table 3.4.

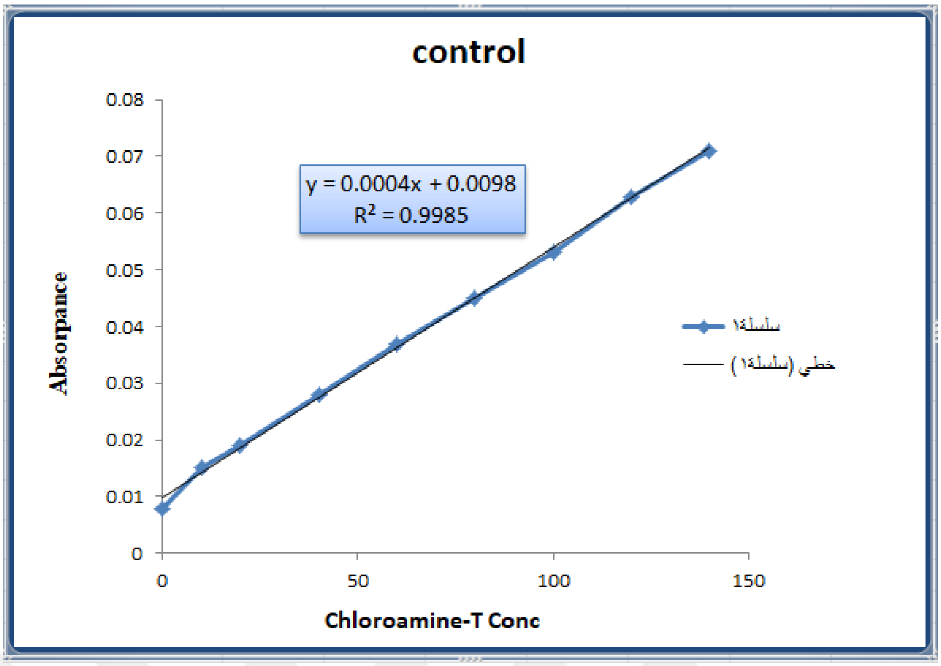


Figure 3.3 Standard curve calculation of the concentration of chloramine-T

3.4.5 Determination of serum malondialdehyde (MDA)

Principle: Lipid peroxidation (LPO) was measured using the thiobarbituric acid technique. The product of LPO that reacts with thiobarbituric acid (TBA) in coexisting trichloroacetic acid (TCA) Under high temperature (90-100 °C) and acidic conditions, to create a pink chromophore absorbing at 532 nm was identified as malondialdehyde (MDA) generated from breakdown the polyunsaturated fatty acids. MDA concentrations were measured using "the molar extinction coefficient of MDA of 1.56x105 M(-1) cm(-1)" (Islam *et al.* 2018). Malondialdehyde is a helpful biomarker of peroxidation processes.

Reagents

- 0.6 % Thiobarbituric acid (TBA)
- 70 % Trichloroacetic acid (TCA)
- 17.5% Trichloroacetic acid (TCA)

Procedure: The tubes listed below were constructed in two sets Table 3.5.

Table 3.5 MDA mixture test preparation

Reagent	Sample	Blank
Serum	150 µl	—
TCA (17.5%)	1000 µl	1000 µl
TBA (0.6%)	1000 µl	1000 µl
All tubes were vortexed well before being incubated in a water bath that was boiling for (15) minutes and Then leave to cool		
TCA (70%)	1000 µl	1000 µl

The combination was allowed to stand at room temperature for twenty minutes. before being passed through a centrifuge at 450X g for fifteen seconds, and the supernatant was collected to evaluate the absorbance of the specimen at 532 nm using a spectrophotometer.

Calculation, Equation (3.2).

$$\text{The conc. of MDA} = \frac{\textbf{A}\text{ of sample at 532nm}}{\text{L X } \varepsilon} \times \text{ D} \qquad (3.2)$$

Where:

- L = light path (1cm)
- ε = Extinction coefficient ($1.56x10^5 M^{-1} cm^{-1}$)
- D = Dilution factor

3.4.6 Determination of serum superoxide dismutase (SOD)

Principle: The ability to inhibit the spontaneous oxidation from adrenaline to adrenochrome in alkaline pH is used to measure serum superoxide dismutase (SOD) activity. A reaction occurs when SOD comes into contact with $O_2^{\cdot -}$. Which is produced as a byproduct of epinephrine oxidation, lowering both the pace and amount of adrenochrome produced. One unit of SOD activity is defined as the concentration of enzyme in blood that resulted in a 50% reduction in the auto-oxidation of epinephrine (Dworzański *et al.* 2020).

Reagents

- Working carbonate buffer (0.05M) pH 10.2
- Epinephrine 0.01M.
- HCl (0.020 M)
- Na_2EDTA (1×10^{-4} M)

Procedure: The tubes listed below were constructed in Three sets

Table 3.6 SOD mixture test preparation

Reagent	Blank		Control		Sample
Serum	----		----		100 µl
Carbonate buffer	1600 µl		1700 µl		1600 µl
Na2EDTA	1000 µl		1000 µl		1000 µl
Epinephrine	----		300 µl		300 µl

A spectrophotometer was used to detect absorbance at 480nm immediately after adding epinephrine and after five minutes had passed.

Calculation: The inhibition % was computed as follows:

$$\text{Inhibition\%} = \left(\frac{\Delta\, A\ control - \Delta\, A\ sample}{\Delta\, A\ contro\ l}\, x\ 100\right)$$

One unit of (SOD) is defined as the amount of enzyme that inhibits the oxidation of 50% of adrenaline Equation (3.3).

$$\text{SOD activity (Unit/ml)} = \text{SOD (Unit)/Total Volume (ml)} \times \text{df} \qquad (3.3)$$

ΔA: The difference in absorbance after five minutes against initial absorbance.

Df: Dilution factor.

3.4.7 Determination of serum catalase (CAT)

Principle: Catalase catalyzes the dismutation of hydrogen peroxide to produce water and oxygen. The drop in absorbance caused by the loss of H2O2 was utilized to calculate its activity Equation (3.4) (Hadwan and kadhum Ali 2018).

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2 \qquad (3.4)$$

Reagents

- Phosphate-buffered solution (50 mM, pH 7.0)
- A solution of H2O2 (30 mM) was prepared by diluting 340 l of 30% H2O2 in the PBS to 100 ml and measuring the solution immediately.

Procedure

- The serum (300 μl) was dilute with 1.7 ml from PBS (50 mM, pH 7.0) before to measurement.
- The tubes were arranged as follows:

Table 3.7 Preparation for the CAT mixture test

Reagent	Sample	Blank
Serum	2000 μl	____
Phosphate-buffered solution	____	2000 μl
Hydrogen peroxide	1000 μl	1000 μl

The reaction started When adding of hydrogen peroxide, then all tubes were instantaneously mixed, and the starting absorbance (t1) after 15 seconds in 240 nm and then the final absorbance (t2) in 75 seconds were measured with a T80 UV - VIS Spectrometer (PG, UK).

Calculation: The following law was used to compute catalase activity Equation (3.5).

$$\text{Catalase activity (U/ml)} = \frac{(\Delta As - \Delta Ao) \times dF}{\in \times Tv \times \Delta t \times L} \tag{3.5}$$

- ΔA_S: The difference between the sample's absorbance after 75 seconds and its initial absorption after 15 seconds.
- ΔA_o: The difference in absorbance between 75 seconds and 15 seconds to the blank.
- Df: Dilution factor
- $\in$: Extinction coefficient (0.0436 $M^{-1}cm^{-1}$)
- Tv: Total volume
- L: light path
- Δt: is (time 2 – time 1) and it is equal to one minute.

3.4.8 C-Reactive protein by NycoCard (CRP)

Principle: Is an immunometric assay using a solid phase and a sandwich configuration. Immmobilized CRP-specific monoclonal antibodies cover a membrane in the device's test well. In order to test the device, a diluted sample is administered. Antibodies bind to C-reactive proteins as they pass through a membrane. In a sandwich-type reaction, the gold-antibody conjugate will bind to the CRP that has been trapped on the membrane. The washing solution is used to remove the unbound conjugate from the membrane. Subsequent to the membrane, a paper layer serves to soak up any surplus liquid. When there is an abnormal amount of CRP in the sample, the membrane takes on a reddish-brown hue, the intensity of which is directly related to the sample's CRP content. Color intensity was quantified using a NycoCard Reader II shown in Figure 3.4.

Material required

- Test device (TD)
- Dilution liquid (R1)
- Conjugate (R2)
- Washing solution (R3)

Procedure

- Fill a 5 μL from the patient's serum by pipet put it in the tube with R1/dilution liquid. Close the tube and mix well for 10 seconds.
- Apply 50 μL diluted sample to the TD/test device. Allow the sample to soak into the membrane (approx. 30 seconds).
- Apply one drop R2/conjugate to the TD/test device. Allow the reagent to soak into the membrane (approx. 30 seconds).
- Apply one drop R3/washing solution to the TD/test device. Allow the reagent to soak into the membrane (approx. 20 seconds).
- Read the result within 5 minutes using the NycoCard Reader II.

Figure 3.4 NycoCard Reader II device , the sandwich-format method is used to measure the analyses

3.4.9 Quantification of tumor necrosis factor-alpha (TNF-α) by enzyme-linked immune sorbent assay

Principle: The samples and standards were put in a wells micro ELISA plate that had been pre-coated with the human TNF alpha antibody. TNF alpha is injected into the wells from the sample and binds to antibodies. The sample is then incubated with the biotinylated human TNF-a Antibody, which binds to TNF alpha. Streptavidin-HRP is then used to bind for biotinylated TNF alpha antibody. All unattached Streptavidin-HRP is eliminated during the washing step following incubation. The substrate solution is then added, The color will change in direct proportion with the quantity of TNF alpha present in the individual. The process is halted with the addition of the acidic stop solution, and the absorbance at 450 nm is measured.

Reagents preparation

- Dilute a 25x Concentrated of Wash Buffer in 1x working solution.
- Dilute a reference Standard at various concentrations.
- 15 minutes before the procedure is completed, "dilute a 100x Biotinylated Detection Ab/Ag into 1x working solution".
- Dilute the 100x HRP Concentrate Conjugate for 1x working solution for 15 minutes before step 2 is completed.

Procedure

- Add 100 µL from standard or sample into each well. After that incubate in 90 minutes at 37°C.
- Remove the liquid. And add 100 µL from Biotinylated Detection Ab/Ag. After that Incubate in 1 hour at 37°C.
- wash and Aspirate for 3 times.
- Add 100 µL from HRP Conjugate. And incubate for 30 minutes in 37°C.
- wash and Aspirate for 5 times.

- Add 90 µL from Substrate Reagent. After that incubate for 15 minutes in 37°C.
- Add 50 µL from Stop Solution. And determine the OD amount at 450 nm immediately.
- Calculation of results.

Calculation: The best fit curve was formed across the points in the graph by plotting the average OD of each standard of the vertical axis (Y) against the concentration of the horizontal axis (X), after that using a slope equation, a concentration of the specimens TNF- was determined using the standard curve.

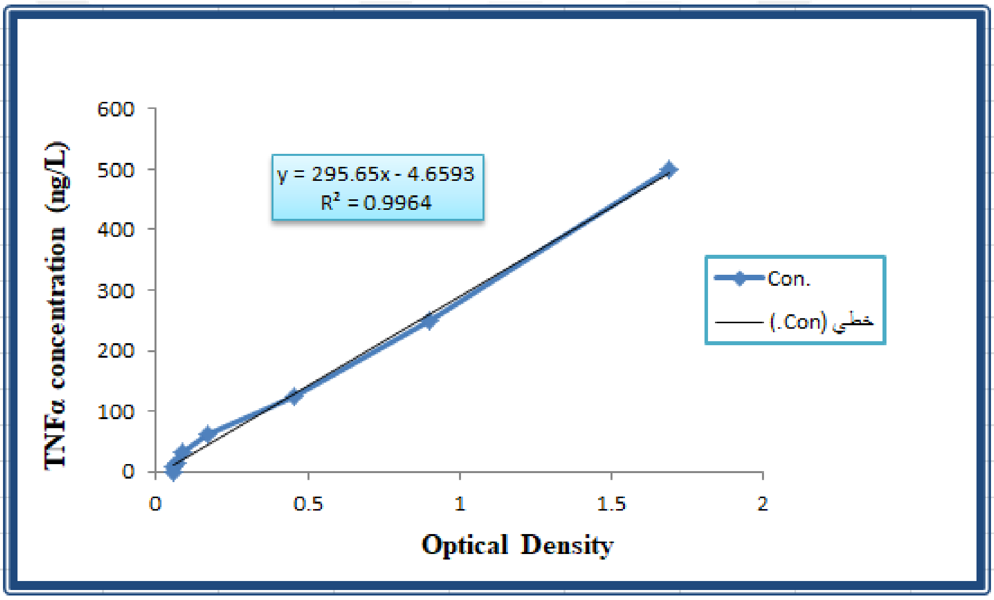


Figure 3.5 Standard curve calculation to the concentration of TNF-α

4. RESULTS AND DISCUSSION

First, the biochemical and clinical characteristics were compared. for each of control (G1), hypothyroidism patients with treatment (G2), and hypothyroidism patients without treatment (G3) as shown in Table 4.1.

Table 4.1 Biochemical, Clinical, and hemodynamic data of study groups were compared

Characteristic	G1	G2	G3	P-Value
	***n*=40**	***n*=40**	***n*=40**	
Age				
Range	19 - 50	19 - 51	21 - 50	0.616
Mean ± SD	34.22 ± 8.54 A	36.75 ± 8.91 A	36.07 ± 11.4 A	
BMI				
Range (mg/dl)	19.15 - 27.85	24.44 - 35.15	23.87 - 38.455	<0.001
Mean ± SD	24.11 ± 2.26 A	28.68 ± 2.75 B	30.74 ± 3.359 C	
TC				
Range(mg/dl)	112- 198	101- 299	115- 345	<0.001
Mean ± SD	161.675 ± 23.9 A	193.225 ± 53.85 B	216.2 ± 53.703 C	
TG				
Range(mg/dl)	68 - 150	78 - 317	76 - 241	<0.001
Mean ± SD	115.3 ± 24.674 A	155.85 ± 56.788 B	129.4 ± 32.256 B	
HDL				
Range(mg/dl)	36 - 80	25 - 58	22 - 58	<0.001
Mean ± SD	54.35 ± 11.58 A	41.775 ± 7.118 B	41.525 ± 8.783 B	
LDL				
Range(mg/dl)	20- 127	43.8- 206.8	54- 251.8	<0.001
Mean ± SD	84.265 ± 26.23 A	121.175 ± 41.35 B	148.87 ± 48.681 C	
VLDL				
Range(mg/dl)	13.6 - 30	15.6 - 63.4	15.2 - 48.2	<0.001
Mean ± SD	23.06 ± 4.934 A	31.17 ± 11.35 B	26.88± 6.45 B	

G1: Control, G2: Hypothyroidism with treatment, G3: Hypothyroidism without treatment, BMI: Body mass index, TC: Total cholesterol, TG: Triglyceride, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol, VLDL: Very low-density lipoprotein-cholesterol. The different letters indicate statistically significant differences between patient groups relative to the control group and between patient groups ($P \leq 0.05$).

All groups, ranging in age from 19 into 51 years old, were confirmed, and the mean BMI in G2 and G3 group was higher than in the control group. BMI rose considerably compared to G3, and BMI increased significantly in patient groups compared to control (p 0.001). A prior research found statistically significant variations in body mass growth in hypothyroid individuals (Al-Sultan and Al-Issa 2023). G3 has a higher level of BMI, which might be related to a metabolic condition or patients without treatment the thyroxine medication, which helps to control the level for thyroid hormones and thus has a good influence on metabolism.

Cholesterol and low density lipoprotein-cholesterol were statistically higher significantly in G2(193.225 ± 53.85, 41.775 ± 7.118) respectively and G3(216.2 ± 53.703, 148.87 ± 48.681) respectively compared to control groups(161.675 ± 23.9, 84.265 ± 26.23) respectively that agree in another study proved that the levels of total cholesterol, and LDL-C were higher in the hypothyroid patients which might be due to inhibition of hepatic low-density lipoprotein (LDL) receptors and cholesterol alpha-monooxygenase activity, resulting in decreased clearance of LDL and total cholesterol (Alsamghan *et al.* 2020). In G2 the level of cholesterol and LDL decreased significantly compared to G3 which agrees with Abbas et al, 2008., that revealed a reduction in serum TC and LDL-C levels after treatment of levothyroxine, variations in serum lipid in hypothyroid patients are associated with changes in T4 hormone.

Triglyceride and very low-density lipoprotein-cholesterol were statistical higher significantly in G2(155.85 ± 56.788, 31.17 ± 11.35) respectively and G3(129.4 ± 32.256, 25.88± 6.45) respectively compared to control groups (115.3 ±24.674, 23.06 ± 4.934), but there is non-significant difference between patients groups . An increase in the esterification of fatty acids at the hepatic level leads to an increase in triglycerides, as was shown in an earlier research. Additionally, serum TG levels rise due to low THs levels, which inhibit lipoprotein lipase (LPL), an enzyme critical for TG removal; thyroid hormone is also unable to normalise the increased intestinal absorption of cholesterol and strongly stimulates hepatic secretion of cholesterol into the blood (Sinha *et al.* 2018). In the study Patients with levothyroxine treatment have no improvement in the levels of TG and VLDL that may due to not adhering to a healthy diet, but there was a significant decrease in the TG level in previous study (Shilpi Goyal1 *et al.* 2022).

But High-density lipoprotein-cholesterol (HDL) was statistical lower significantly G2 and G3(41.775 ± 7.118, 41.525 ± 8.783) respectively compared to G1 control group (54.35 ± 11.58), Thyroid hormone performed an important role in modifiable hepatic lipase, which changes HDL-C (Pearce 2012), that is it agrees with a previous study showed had decreased HDL cholesterol in comparison with the control group (Saif *et al.* 2018), but it disagree with another study, where HDL level was found to be increased in hypothyroid cases when compared to controls (Jiskra *et al.* 2007).

4.1 Thyroid Hormones Markers

4.1.1 Serum thyroid stimulating hormone level (TSH)

The results of this study show increased levels of TSH (12.28 ± 5.831 µIU/mL) in patients with Hypothyroidism without treatment (G3) compared to Hypothyroidism with treatment (G2) (8.722 ± 4.645), and G1 (1.845 ± 0.838) µIU/mL. Our study showed a significant difference (p-value < 0.001) in the concentrations of TSH compared to all patients and control. That agree with (Hassan and Abbas 2022) showed significant increase in TSH levels in hypothyroid patients compared to the control group.

The measurement of TSH significant difference was present in mean values between G1 and G2 (p-value < 0.001), a significant difference in mean values between G1 and G3 (p-value < 0.001) a significant difference in mean values between G2 and G3 (p-value = 0.001). As shown in Figure 4.1.

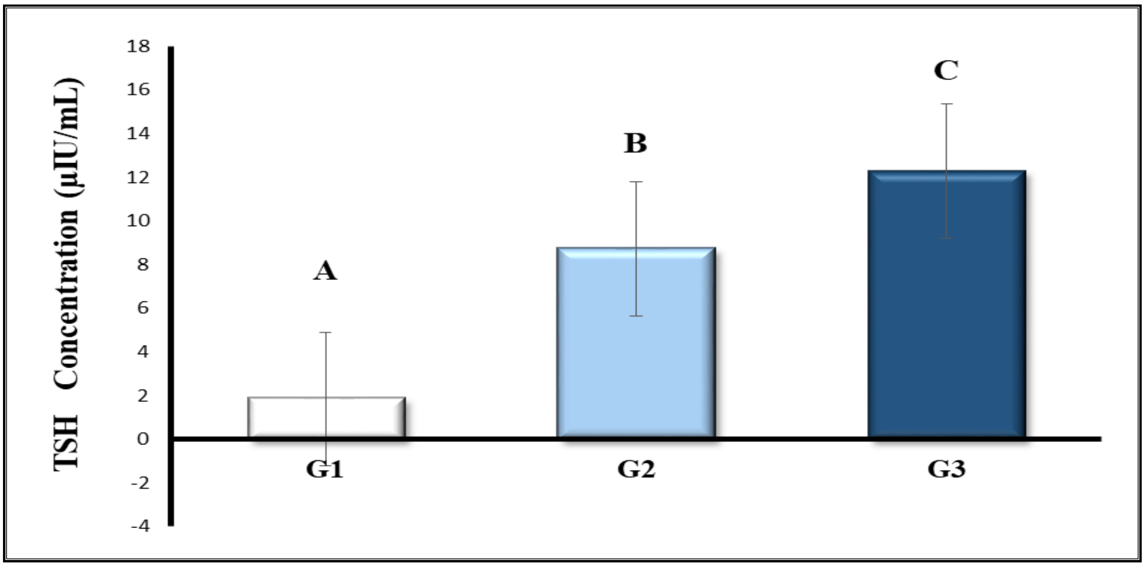


Figure 4.1 Comparison of TSH levels in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups (P < 0.05)

Figure 3.2 displays the findings of the receiver operator characteristic (ROC) curve analysis that was conducted to assess the diagnostic performance of TSH µIU/mL in individuals with hypothyroidism. Regarding TSH, the cutoff value was >4.05 µIU/mL with 97.5% sensitivity, 100% specificity, and 0.996Area

Table 4.2 Receiver operator characteristic curve for thyroid stimulating hormone

The area under the ROC curve (AUC)	0.996
Standard Error [a]	0.003
95% Confidence interval	0.990 to 1.000
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	>4.05
Sensitivity %	97.5 %
Specificity %	100 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

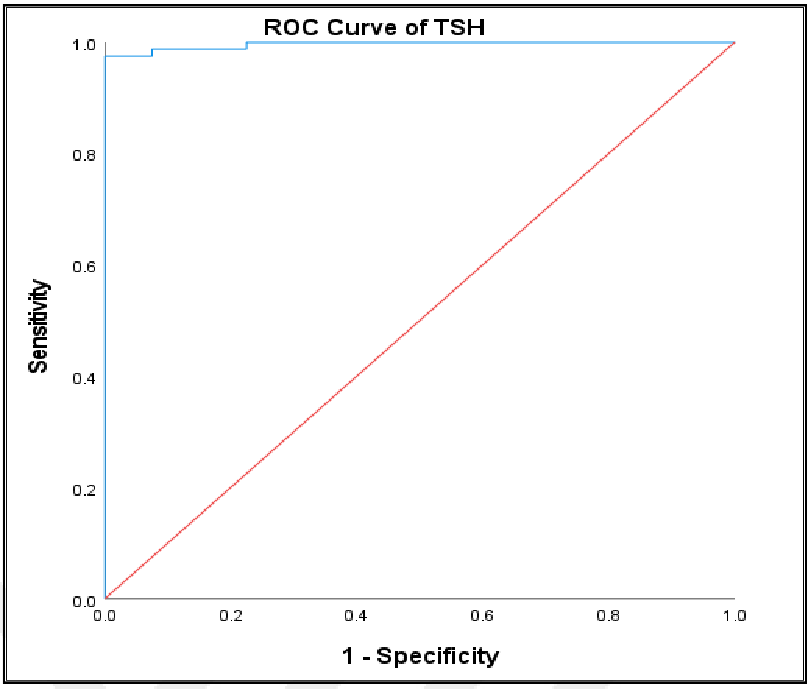


Figure 4.2 Receiver operator characteristic (ROC) curve analysis to find the best serum thyroid stimulating hormone (TSH) cutoff value that can predict a diagnosis of hypothyroidism

4.1.2 Serum triiodothyronine level (T3)

The results of this study show increased levels of T3 (1.22 ± 0.2877) ng/mL in the control (G1) compared to patients with Hypothyroidism with treatment (G2) (1.135 ± 0.9031), and Hypothyroidism without treatment (G3) (0.551 ± 0.195) ng/mL. Our study showed a significant difference (p-value < 0.001) in the concentrations of T3 compared to all patients and control. That agree with (Hassan and Abbas 2022) Findings Showed a significant decrease in serum T3 levels

The measurement of T3 ng/mL significant difference was present in mean values between G1 and G2 (p-value = 0.017), a significant difference in mean values between G1 and G3 (p-value < 0.001) a significant difference in mean values between G2 and G3 (p-value < 0.001). As shown in Figure 4.3.

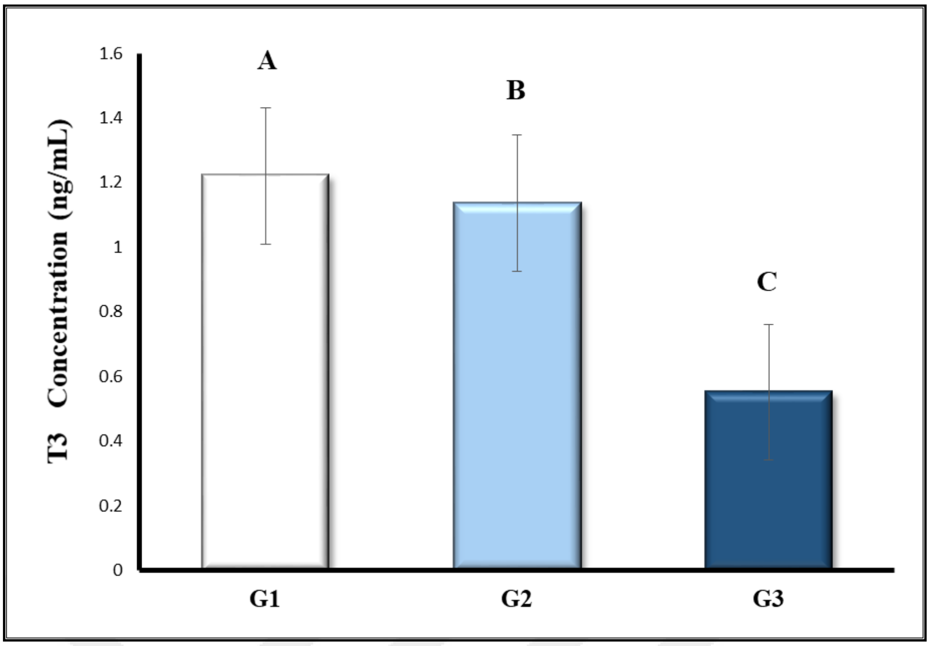


Figure 4.3 Comparison of T3 level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups ($P < 0.05$)

Figure 4.4 displays the results of a receiver operator characteristic (ROC) curve analysis that was conducted to assess the diagnostic performance of T3 ng/mL in individuals with hypothyroidism. Regarding T3, the cutoff value was < 0.79 ng/mL with 67.5 % sensitivity, 100% specificity, and 0.822 Area.

Table 4.3 Receiver operator characteristic curve for triiodothyronine

The area under the ROC curve (AUC)	0.822
Standard Error [a]	0.038
95% Confidence interval	0.747 to 0.897
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	<0.79
Sensitivity %	67.5 %
Specificity %	100 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

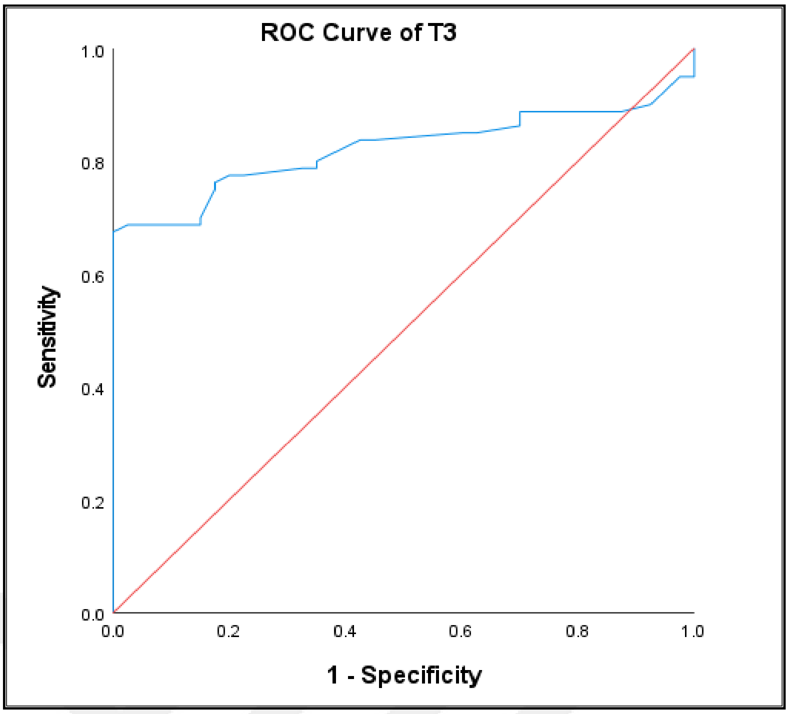


Figure 4.4 Receiver operator characteristic (ROC) curve analysis to find the best serum triiodothyronine (T3) cutoff value that can predict a diagnosis of hypothyroidism

4.1.3 Serum thyroxine level (T4)

The results of this study show increased levels of T4 (84.25 ± 17.392) ng/mL in the control (G1) compared to patients with Hypothyroidism with treatment (G2) (71.17 ± 31.852), and Hypothyroidism without treatment (G3) (41.9 ± 10.28) ng/mL. Our study showed a significant difference (p-value < 0.001) in the concentrations of T4 compared to all patients and control. That agree with (Hassan and Abbas 2022) Findings Showed a significant decrease in serum T4 levels.

The measurement of T4 ng/mL significant difference was present in mean values between G1 and G2 (p-value = 0.005), a significant difference in mean values between G1 and G3 (p-value < 0.001) a significant difference in mean values between G2 and G3 (p-value < 0.001). As shown in Figure 4.5.

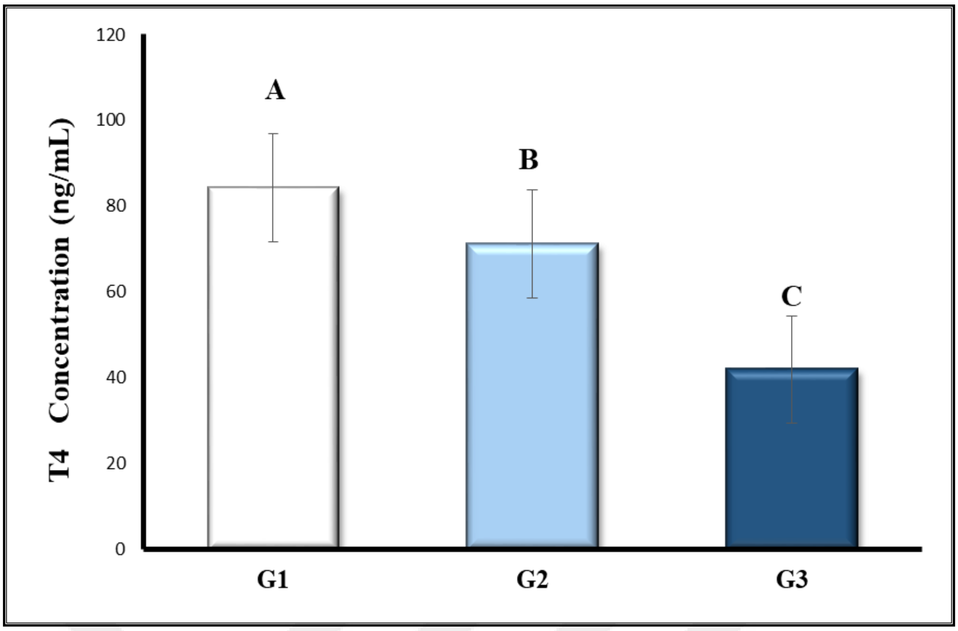


Figure 4.5 Comparison of T4 level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups ($P < 0.05$)

To evaluate the diagnostic performance of T4 ng/mL in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 4.6. Regarding T4, the cutoff value was < 53 ng/mL with 66.3 % sensitivity, 100% specificity, and 0.838 Area.

Table 4.4 Receiver operator characteristic curve for thyroxin

The area under the ROC curve (AUC)	0.838
Standard Error [a]	0.037
95% Confidence interval	0.767 to 0.910
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	< 53
Sensitivity %	66.3 %
Specificity %	100 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

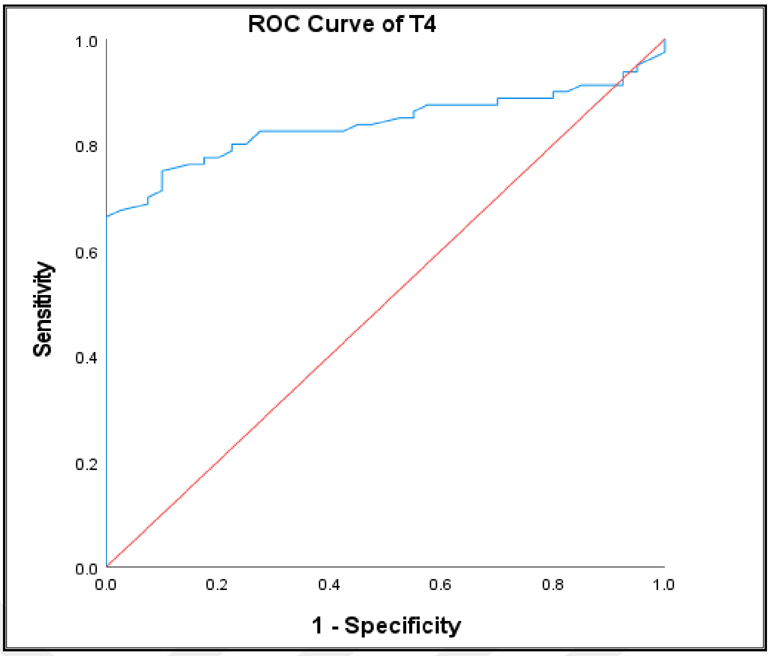


Figure 4.6 Receiver operator characteristic (ROC) curve analysis to find the best serum thyroxine (T4) cutoff value that can predict a diagnosis of hypothyroidism

It is very clear that the treated group G2 showed a significant decrease in TSH and increased thyroid hormones T3 and T4 levels as a result of the treatment effect post levothyroxine replacement treatment.

4.2 Oxidative Stress Markers

4.2.1 Serum malondialdehyde concentration (MDA)

The results of this study show decreased levels of malondialdehyde (MDA) (0.137 ± 0.020) µmole/L in the control (G1) compared to patients with Hypothyroidism without treatment (G3) (0.325 ± 0.036), and Hypothyroidism with treatment (G2) (0.272 ± 0.021) µmole/L. Our study showed a significant difference (p-value < 0.001) in the concentrations of MDA compared to all patients and control. Increased oxidative stress is being linked to hypothyroidism, as a result from increased free radical formation in hypothyroid individuals. The findings of this investigation revealed a high MDA level in hypothyroidism patients, which is consistent with previous findings (SAH *et al.* 2022) Whereas it was shown that hypothyroidism patients had much higher levels of

MDA than control individuals, indicating greater oxidative stress, thyroid hormones play a crucial role in lowering the toxicity of oxidative stress in humans.

The treatment reduced the oxidative stress in the treated group by thyroxine, which proves the positive effect of the treatment

The measurement of MDA µmole/L significant difference was present in mean values between G1 and G2 (p-value < 0.001), the significant difference in mean values between G1 and G3 (p-value < 0.001) the significant difference in mean values between G2 and G3 (p-value < 0.001). As shown in Figure 4.7.

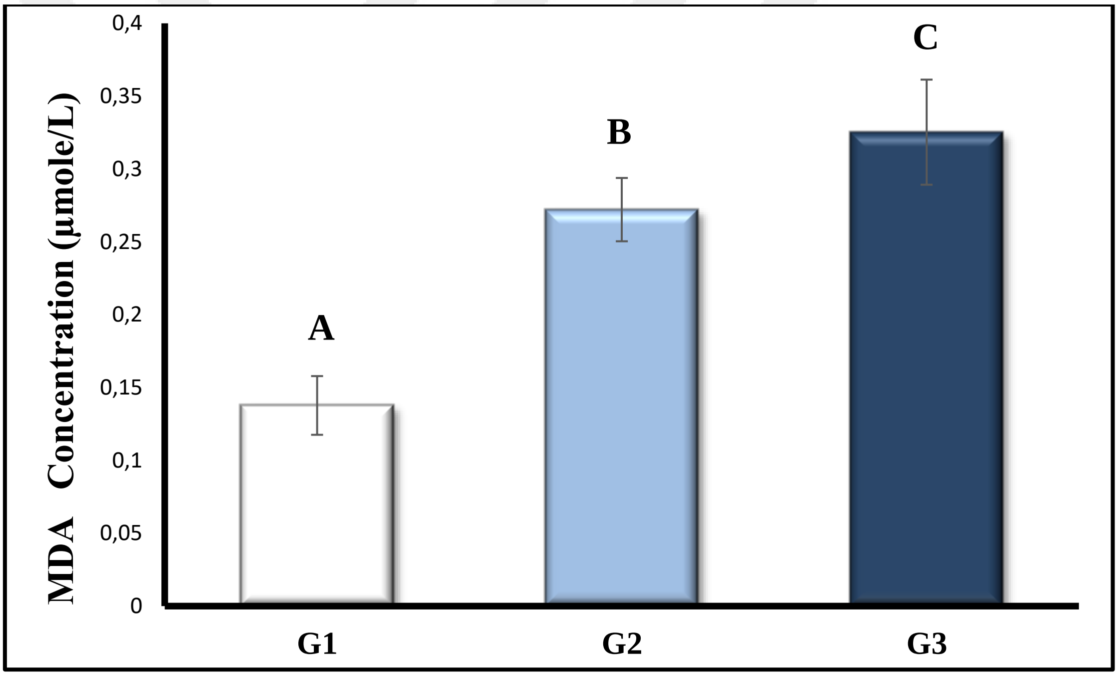


Figure 4.7 Comparison of MDA level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups (P < 0.05)

To evaluate the diagnostic performance of MDA µmole/L in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 3.8. Regarding MDA, the cutoff value was > 0.22 µmole/L with 100 % sensitivity, 100% specificity, and 1 Area.

Table 4.5 Receiver operator characteristic curve for malondialdehyde

The area under the ROC curve (AUC)	1
Standard Error [a]	0.000
95% Confidence interval	1 to 1
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	> 0.22
Sensitivity %	100 %
Specificity %	100 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

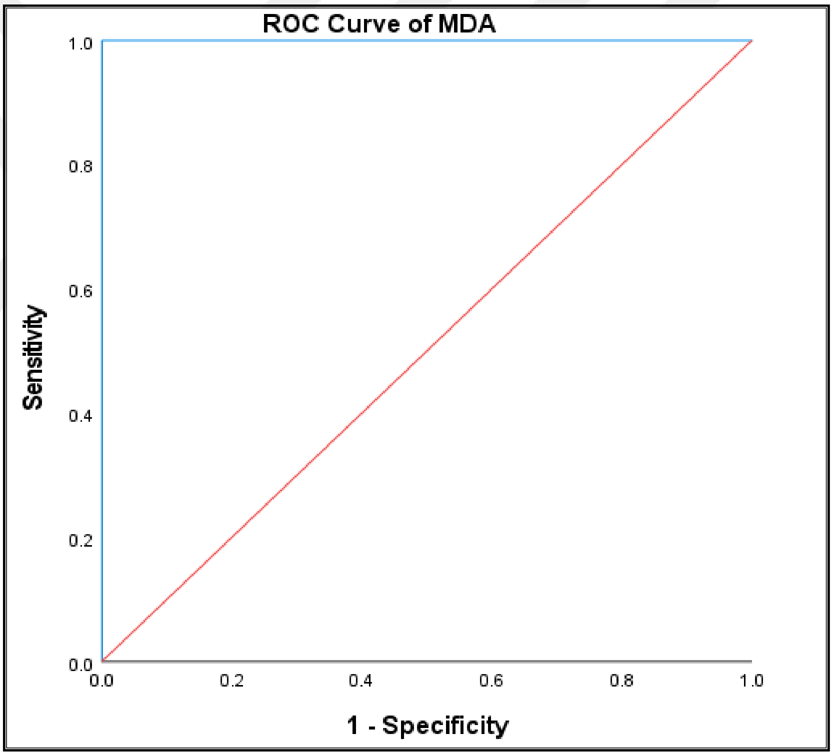


Figure 4.8 Receiver operator characteristic (ROC) curve analysis to find the best serum malondialdehyde (MDA) cutoff value that can predict a diagnosis of hypothyroidism

4.2.2 Advanced oxidation of protein product (AOPP) level

The results of this study show increased levels of AOPP (119.3 ± 24.54) µmole/L in patients with Hypothyroidism without treatment (G3) compared to Hypothyroidism with treatment (G2) (94.594 ± 25.782), and control (G1) (76.7 ± 12.99) µmole/L. Our study showed a significant difference (p-value < 0.001) in the concentrations of AOPP compared to all patients and control. That agree with (Unluhizarci *et al.* 2016) Whereas higher levels of AOPP were observed in hypothyroidism patients compared to controls, hypothyroidism also enhanced oxidative stress indicators, which were already considerably higher than in healthy people.

The measurement of AOPP µmole/L significant difference was present in mean values between G1 and G2 (p-value < 0.001), the significant difference in mean values between G1 and G3 (p-value < 0.001) the significant difference in mean values between G2 and G3 (p-value < 0.001). As shown in Figure 4.9.

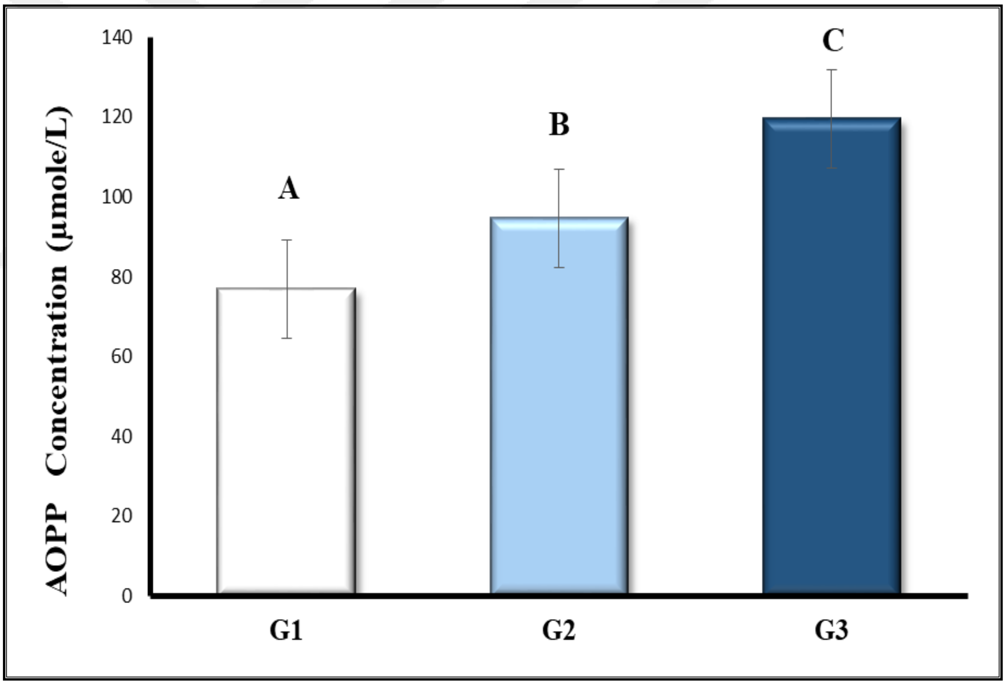


Figure 4.9 Comparison of AOPP level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups (P < 0.05)

To evaluate the diagnostic performance of AOPP µmole/L in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 3.10. Regarding AOPP, the cutoff value was > 96.75µmole/L with 66.3 % sensitivity, 97.5% specificity, and 0.827 Area.

Table 4.6 Receiver operator characteristic curve for advanced oxidation of protein product

The area under the ROC curve (AUC)	0.827
Standard Error [a]	0.036
95% Confidence interval	0.755 to 0.898
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	> 96.75
Sensitivity %	66.3 %
Specificity %	97.5 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

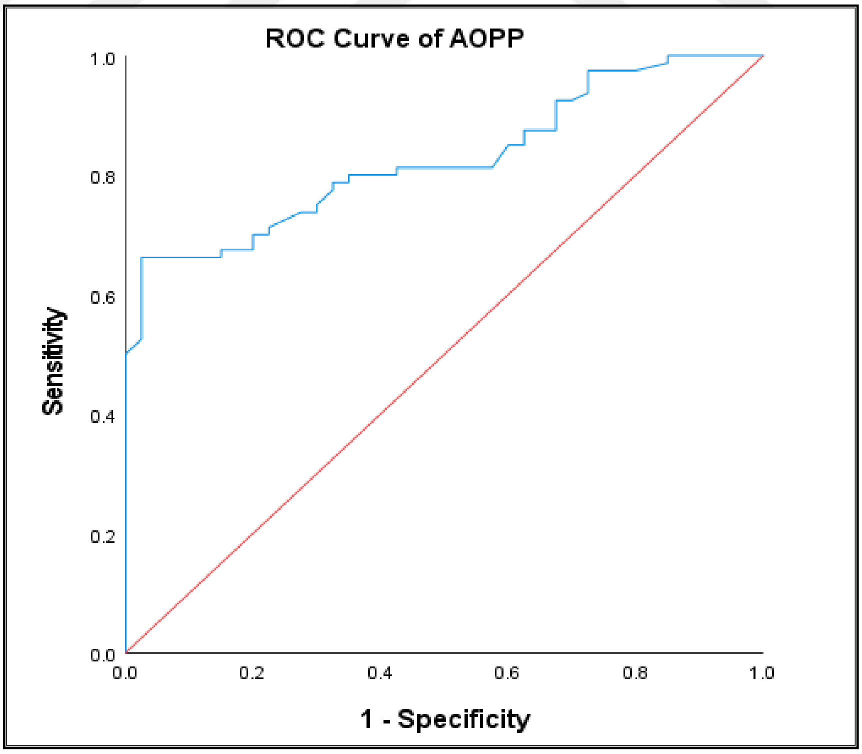


Figure 4.10 Receiver operator characteristic (ROC) curve analysis to find the best serum advanced oxidation of protein product (AOPP) cutoff value that can predict a diagnosis of hypothyroidism

4.3 Antioxidant Enzymes

4.3.1 Serum superoxide dismutase activity (SOD)

The results showed a non-significant difference (p-value = 0.24) in the activity of SOD compared to all patients and control. Levels of SOD (14.2 ± 1.742) U/mL in the control (G1) patients with Hypothyroidism with treatment (G2) (13. 75 ± 2.18), and Hypothyroidism without treatment (G3) (13.4 ± 2.362) U/mL. Our study agree with Laís Farias Masullo,*et al.*2018 (Masullo *et al.* 2018), they showed that there were no significant differences in the activity of the enzyme among hypothyroid patients, whether they receive or do not receive treatment, suggestive of no important interfering from SOD' activity in antioxidant defense.

The measurement of SOD U/mL non-significant difference was present in mean values between G1 and G2 (p-value = 0.514), a non-significant significant difference in mean values between G1 and G3 (p-value = 0.262) the non-significant difference in mean values between G2 and G3 (p-value = 0.337). As shown in Figure 4.11.

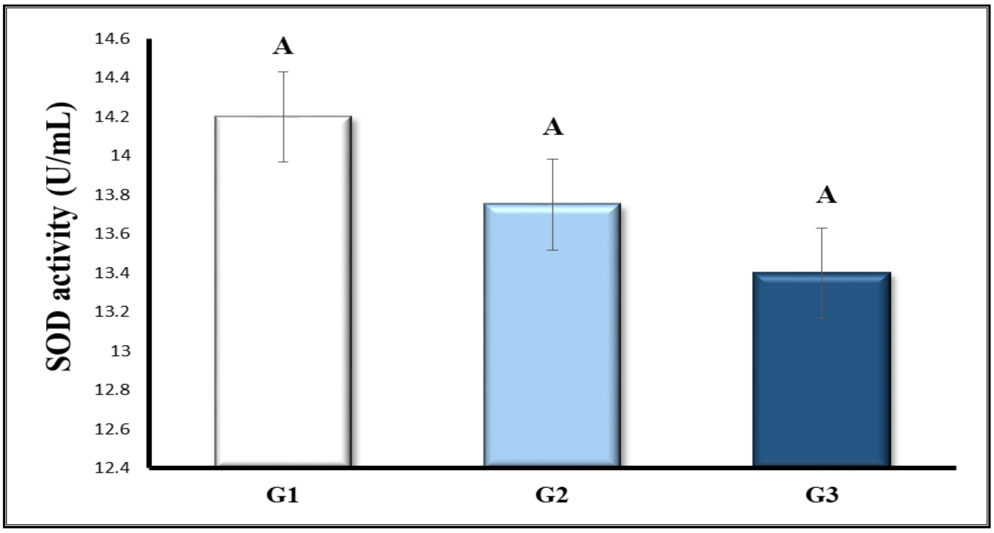


Figure 4.11 Comparison of SOD level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups (P < 0.05)

To evaluate the diagnostic performance of SOD U/mL in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 3.12. Regarding SOD, the cutoff value was < 13 U/mL with 36.3 % sensitivity, 75% specificity, and 0.571 Area.

Table 4.7 Receiver operator characteristic curve for superoxide dismutase

The area under the ROC curve (AUC)	0.571
Standard Error [a]	0.054
95% Confidence interval	0.465 to 0.676
Significance level P (Area=0.5) [b]	0.208
Associated criterion	< 13
Sensitivity %	36.3 %
Specificity %	75 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

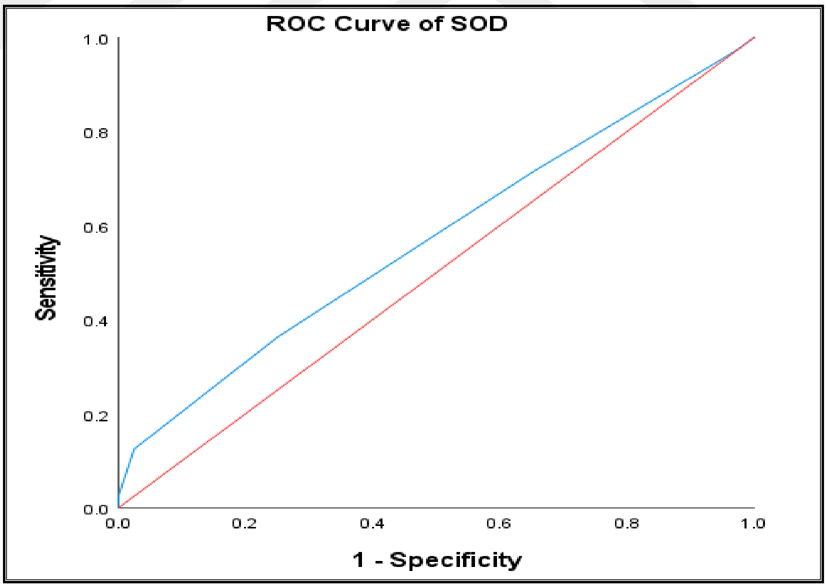


Figure 4.12 Receiver operator characteristic (ROC) curve analysis to find the best serum superoxide dismutase (SOD) cutoff value that can predict a diagnosis of hypothyroidism

4.3.2 Serum catalase activity (CAT)

The results of this study show increased levels of CAT (8.471 ± 2.28) U/mL in patients with Hypothyroidism with treatment (G2) compared to Hypothyroidism without treatment (G3) (7.464 ± 1.25), and control (G1) (5.681 ± 1.25) U/mL. Our study showed a significant difference (p-value < 0.001) in the activity of CAT compared to all patients and control. That agree with (Aleksic *et al.* 2021) study demonstrated that CAT increased in hypothyroidism, but total antioxidant capacity decreased in hypothyroidism patients. This is most likely due to increased metabolism, which leads to increased free radical generation, also Laís Farias Masullo, *et al.* 2018. It is proposed that the clinical problem of hypothyroidism saturates CAT activity and lowers antioxidant defense, and that CAT activity was increased when the patient was treated with levothyroxine.

The measurement of CAT U/mL significant difference was present in mean values between G1 and G2 (p-value < 0.001), the significant difference in mean values between G1 and G3 (p-value < 0.001) the non-significant difference in mean values between G2 and G3 (p-value = 0.017). As shown in Figure 4.13.

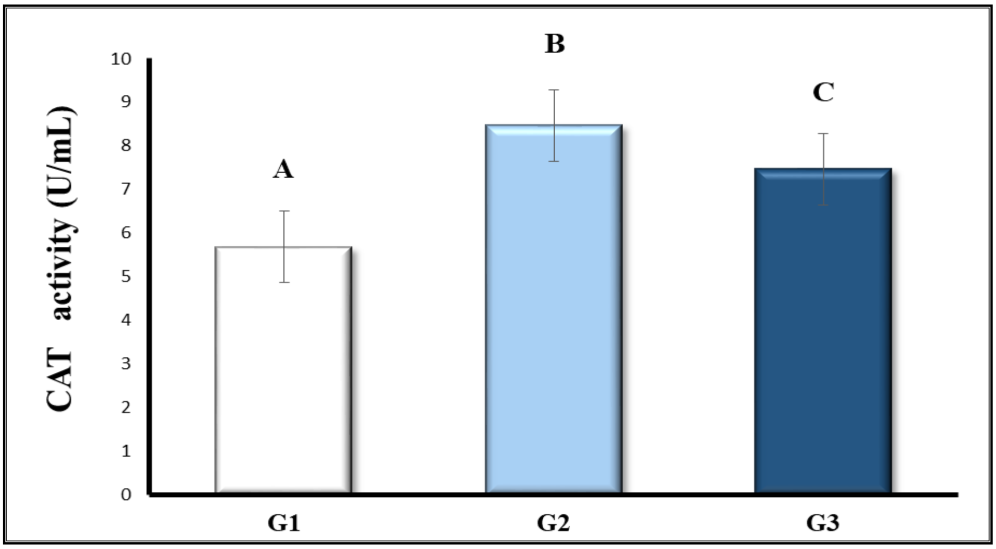


Figure 4.13 Comparison of CAT level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups (P < 0.05)

To evaluate the diagnostic performance of CAT U/mL in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 3.14. Regarding CAT, the cutoff value was > 7.07 U/mL with 71.3 % sensitivity, 92.5% specificity, and 0.858 Area.

Table 4.8 Receiver operator characteristic curve for catalase

The area under the ROC curve (AUC)	0.858
Standard Error [a]	0.036
95% Confidence interval	0.787 to 0.928
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	> 7.07
Sensitivity %	71.3%
Specificity %	92.5 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

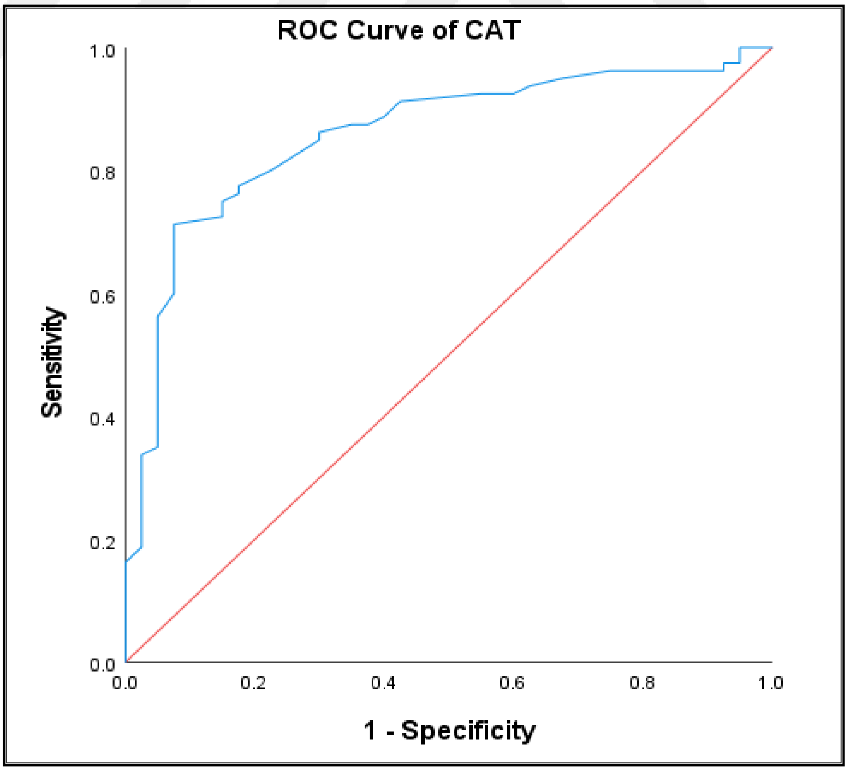


Figure 4.14 Receiver operator characteristic (ROC) curve analysis to find the best serum catalase (CAT) cutoff value that can predict a diagnosis of hypothyroidism

4.4 Markers of Inflammation

4.4.1 Serum tumor necrosis factor alpha level (TNF-α)

The results of this study show increased levels of TNF-α (19.40 ± 4.13) pg/mL in patients with Hypothyroidism without treatment (G3) compared to Hypothyroidism with treatment (G2) (16.97 ± 1.55), and control (G1) (16.06 ± 1.21) pg/mL. Our study showed a significant difference (p-value < 0.001) in the concentrations of TNF-α compared to all patients and control. That agree with (Saraswathi *et al.* 2020) study shown that TNF- in hypothyroidism increase relative to control levels, that raised plasma concentrations for TNF- are displayed by hypothyroid patients, implying the thyroid hormone deficiency is a causal factor for the system production cytokines. This observation might be the result of an intrathyroidal antigen-antibody interaction or a systemic response to illness. ShilPi Goyal *et al.* 2022 showed that hypothyroid individuals treated with levothyroxine had significant changes in their inflammatory profile, including a reduction in pro-inflammatory cytokines such as TNF- That is, the medication has a significant impact on lowering the intensity of inflammation (GOYAL *et al.* 2022).

The measurement of TNF-α (pg/mL) significant difference present in mean values between G1 and G2 (p-value = 0.002), a significant difference in mean values between G1 and G3 (p-value < 0.001) a significant difference in mean values between G2 and G3 (p-value = 0.005). As shown in Figure 4.15.

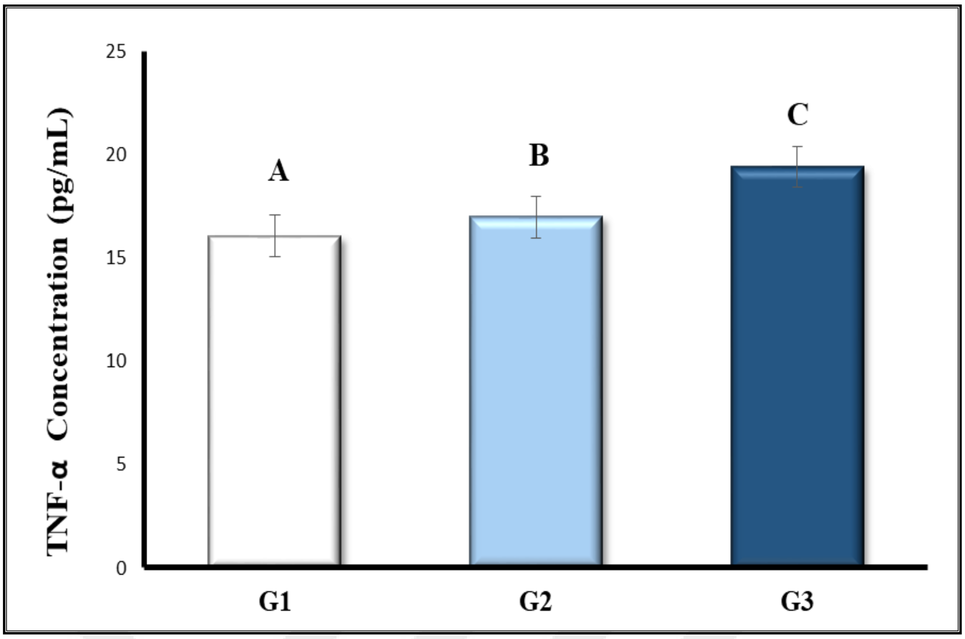


Figure 4.15 Comparison of TNF-α level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups ($P < 0.05$)

To evaluate the diagnostic performance of TNF-α pg/mL in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 3.16. Regarding TNF-α, the cutoff value was > 16.6003 pg/mL with 57.5 % sensitivity, 77.5% specificity, and 0.746Area.

Table 4.9 Receiver operator characteristic curve for tumor necrosis factor alpha

The area under the ROC curve (AUC)	0.746
Standard Error [a]	0.046
95% Confidence interval	0.656 to 0.835
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	> 16.6003
Sensitivity %	57.5 %
Specificity %	77.5 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

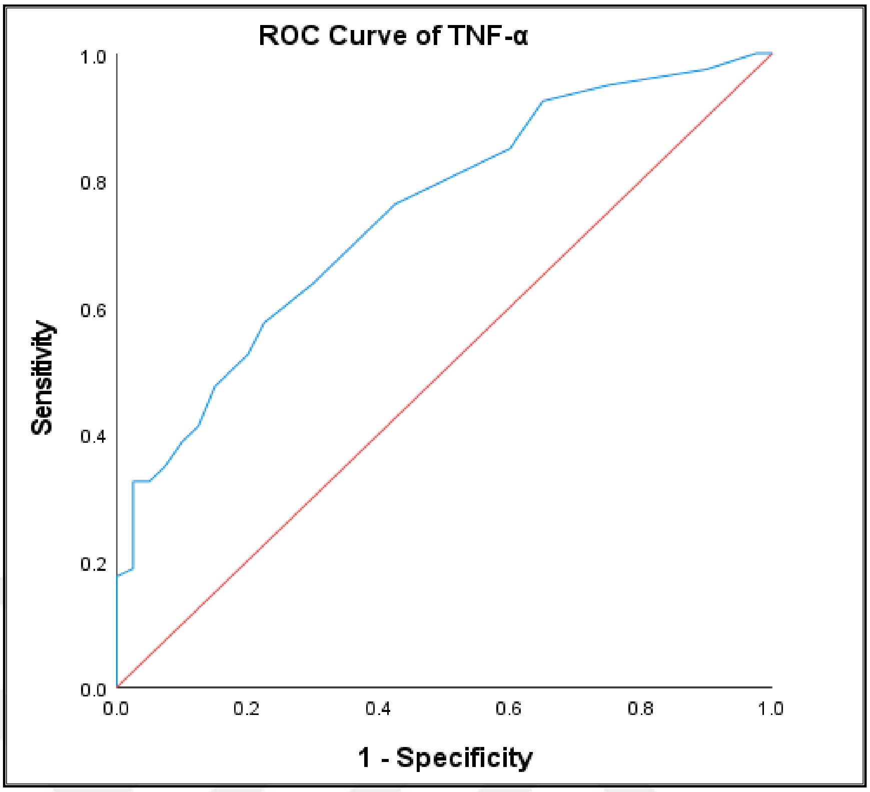


Figure 4.16 Receiver operator characteristic (ROC) curve analysis to find the best serum tumor necrosis factor alpha (TNF-α) cutoff value that can predict a diagnosis of hypothyroidism

4.4.2 Serum C-reactive protein level (CRP)

The results of this study show increased levels of CRP (25.31± 6.44) mg/L in patients with Hypothyroidism without treatment (G3) compared to Hypothyroidism with treatment (G2) (9.397 ± 2.83), and control (G1) (3.92 ± 0.99) mg/L. Our study showed a significant difference (p-value < 0.001) in the concentrations of CRP compared to all patients and control. That agree with (Tang *et al.* 2021) that showed that CRP in hypothyroidism increased relative to the control levels. Changes in lipid levels in the blood and hemodynamic states caused by hypothyroidism may be implicated in the rise in serum CRP, which is regarded as an inflammatory biomarker for hypothyroid individuals.

A prior study found that levothyroxine therapy was beneficial in lowering CRP levels (Stela Vudu *et al.* 2023). This demonstrates that the medication is particularly efficient in lowering inflammation in hypothyroid people (Vudu *et al.* 2023).

The measurement of CRP mg/L significant difference was present in mean values between G1 and G2 (p-value < 0.001), a significant difference in mean values between G1 and G3 (p-value < 0.001) a significant difference in mean values between G2 and G3 (p-value < 0.001). As shown in Figure 4.17.

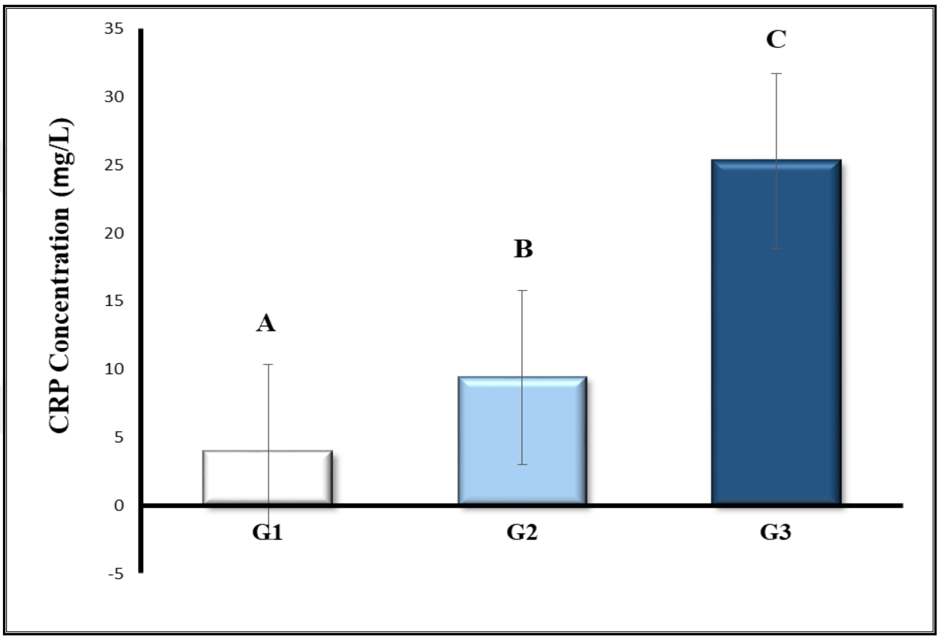


Figure 4.17 Comparison of CRP level in different studied groups Control (G1), Hypothyroidism with treatment (G2), and Hypothyroidism without treatment (G3). Data are expressed as means ± SD, The different letters indicate statistically significant differences between patient groups relative to the control group, as well as between patient groups ($P < 0.05$)

To evaluate the diagnostic performance of CRP mg/L in patients with Hypothyroidism, receiver operator characteristic (ROC) curve analysis was performed, and the results are shown in Figure 3.18. Regarding CRP, the cutoff value was > 5.65 mg/L with 100 % sensitivity, 95% specificity, and 0.998 Area.

Table 4.10 Receiver operator characteristic curve for C-reactive protein

The area under the ROC curve (AUC)	0.998
Standard Error [a]	0.002
95% Confidence interval	0.994 to 1.000
Significance level P (Area=0.5) [b]	0.0001
Associated criterion	> 5.65
Sensitivity %	100 %
Specificity %	95 %

a. Under the nonparametric assumption

b. Null hypothesis: true area = 0.5

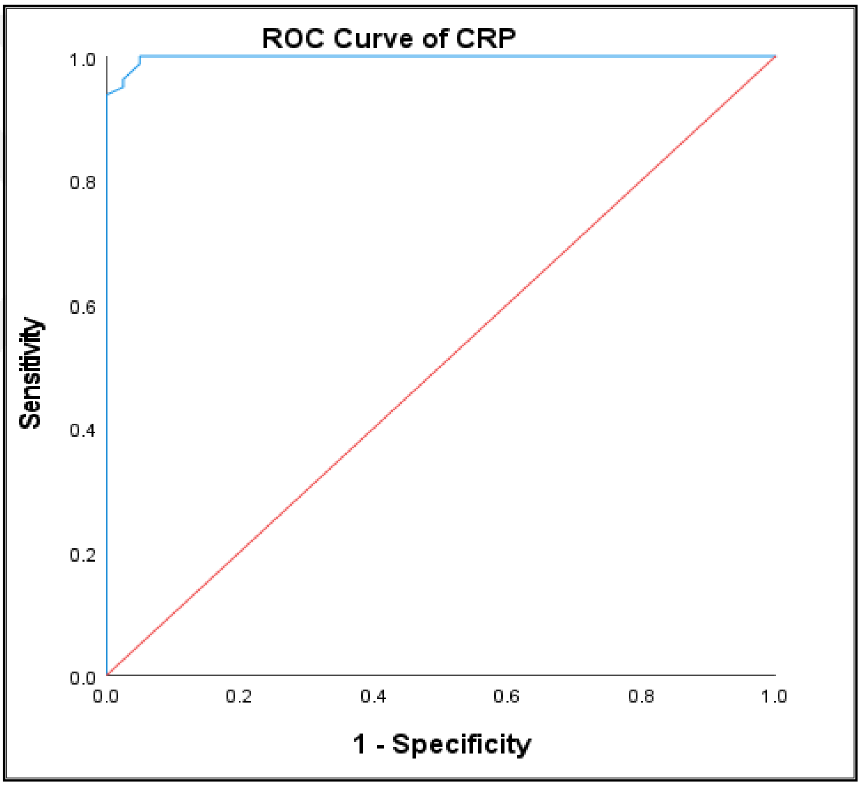


Figure 4.18 Receiver operator characteristic (ROC) curve analysis to find the best serum C-reactive protein (CRP) cutoff value that can predict a diagnosis of hypothyroidism

4.5 Correlations among Biochemical Characteristics

There were strong positive relationships between the T4 level and CAT, while no relationships between the T4 level and SOD. It was found negative correlations with MDA, AOPP, CRP, and in patient groups.

The decrease in the levels of thyroid hormones leads to an increase in oxidative stress; this could be clarified by the direct relationship between T4 and CAT and the inverse relationship between T4 with MDA and AOPPs in hypothyroid women patients.

Negative correlations between T4 and inflammatory markers TNF-α and CRP in hypothyroid patients; prove to have low-grade chronic inflammation because of ineffective functioning of the thyroid gland.

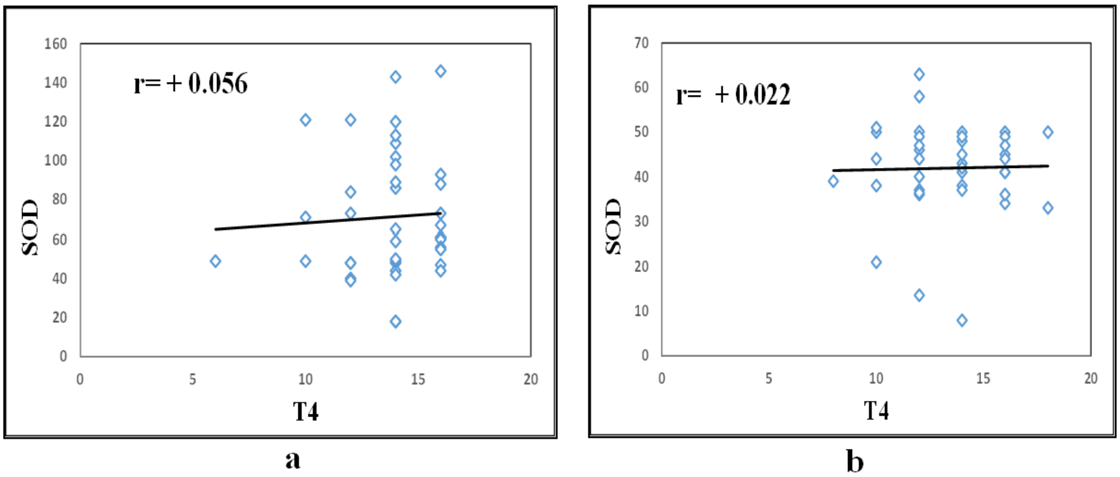


Figure 4.19 Correlation between T4 and SOD in patients with (a) Hypothyroidism with treatment (G2) and (b) Hypothyroidism without treatment (G3)

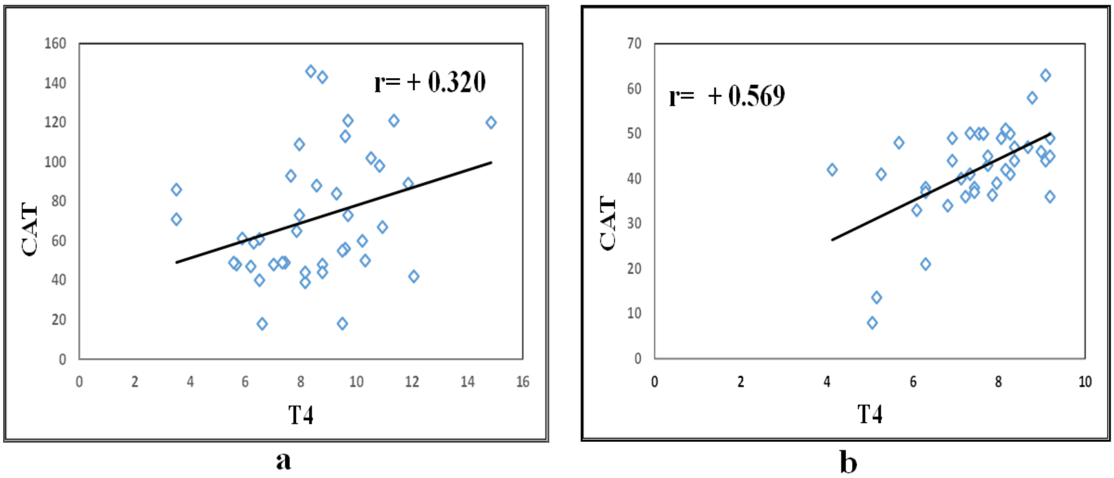


Figure 4.20 Correlation between T4 and CAT in patients with (a) Hypothyroidism with treatment (G2) and (b) Hypothyroidism without treatment (G3)

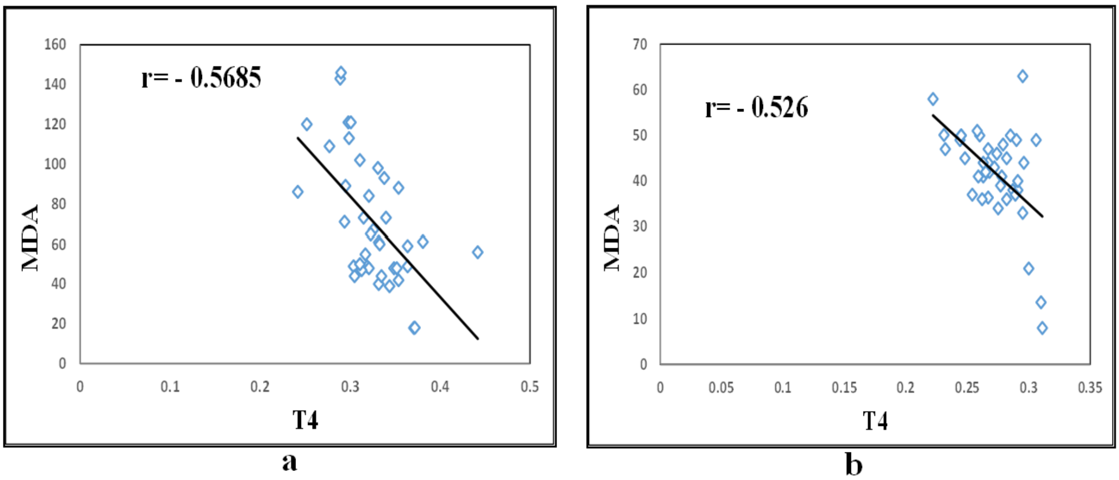
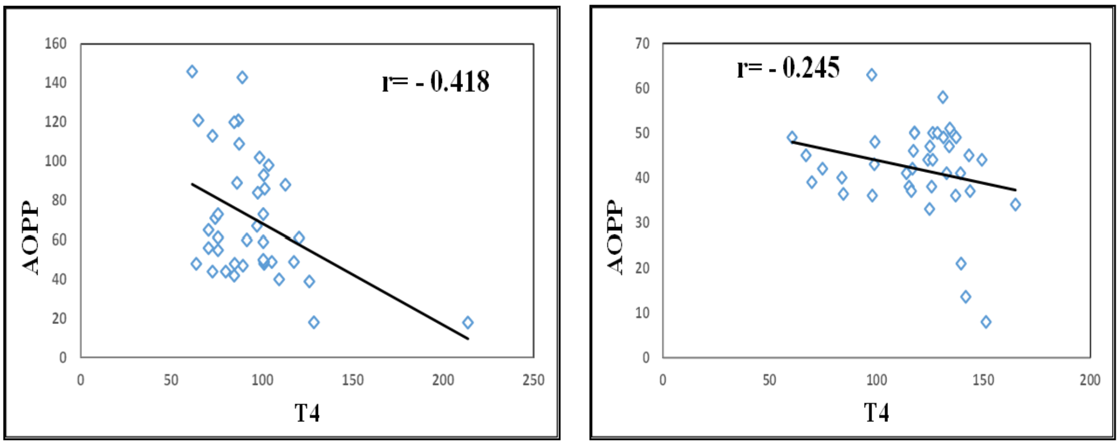
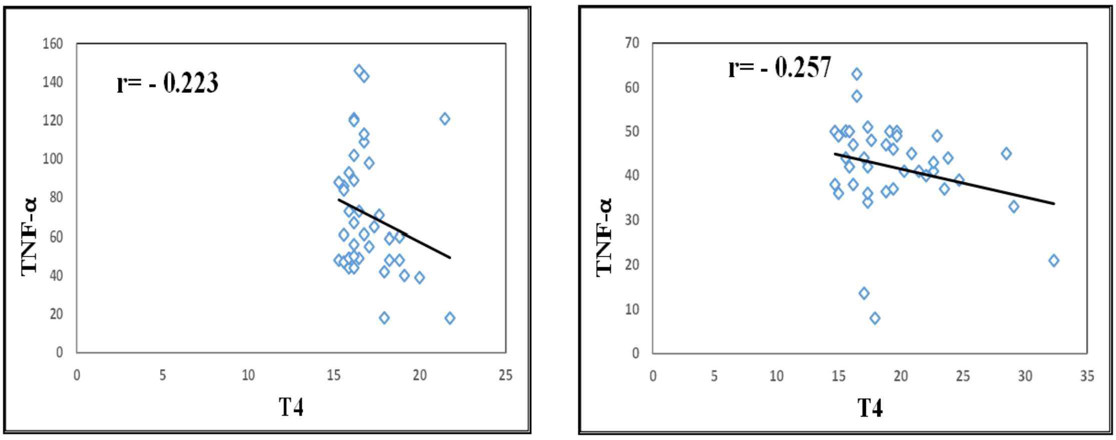


Figure 4.21 Correlation between T4 and MDA in patients with (a) Hypothyroidism with treatment (G2) and (b) Hypothyroidism without treatment (G3)



a b

Figure 4.22 Correlation between T4 and AOPP in patients with (a) Hypothyroidism with treatment (G2) and (b) Hypothyroidism without treatment (G3)



a b

Figure 4.23 Correlation between T4 and TNF-α in patients with (a) Hypothyroidism with treatment (G2) and (b) Hypothyroidism without treatment (G3)

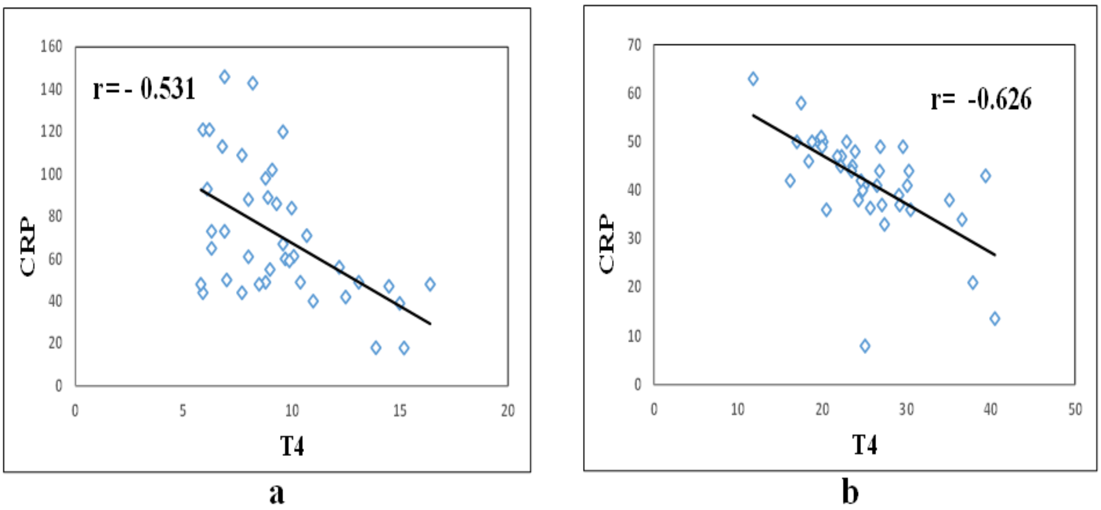


Figure 4.24 Correlation between T4 and CRP in patients with (a) Hypothyroidism with treatment (G2) and (b) Hypothyroidism without treatment (G3)

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

- Thyroid hormones play an important role in regulating of metabolism, oxidative stress, and inflammation.

- The hypothyroid state is associated with elevated oxidative stress and inflammatory markers.

- Levothyroxine replacement treatment has a key role in reversing the state of hypothyroidism by reducing oxidative stress and inflammatory markers by compensating for hormone deficiency. Still, it did not reach the optimal level as in the healthy group, where the secretion of thyroid hormones is regulated according to the body's signals and needs.

- Hypothyroidism may be related with an improved risk of cardiovascular vascular disease because the disorder in lipid metabolism related to dysfunction of thyroid gland

- Treating with levothyroxine in hypothyroidism patients, can be protected from the risk of cardiovascular vascular disease.

5.2 Recommendation

- Oxidative stress appears to be a vital mechanism in hypothyroidism, and MDA and AOPP can be used as useful biomarkers for the quantity and monitoring of oxidative stress. Therefore, adding antioxidant nutritional supplements may be necessary to alleviate the side effects that may result.

- Hypothyroidism is an inflammatory state characterized by elevated cytokines like CRP and TNF-α, Laboratory researches can be performed to determine the feasibility of using anti-TNF-α therapy to prevent the development of severe infections, in addition to thyroxine treatment.

- More studies on other oxidative and inflammatory factors can be conducted to evaluate the health status of hypothyroidism patients of both sexes.

REFERENCES

Aleksic, M., Kalezic, A., Saso, L., Jankovic, A., Korac, B. and Korac, A. 2021. The unity of redox and structural remodeling of brown adipose tissue in hypothyroidism. Antioxidants, 10(4): 591.

Al-Suhaimi, E. A. and Al-Khater, K. 2019. Functions of stem cells of thyroid glands in health and disease. Reviews in Endocrine and Metabolic Disorders, 20(2): 187-195.

Alzahrani, A. S., Al Mourad, M., Hafez, K., Almaghamsy, A. M., Alamri, F. A., Al Juhani, N. R., . . . Alzahrani, M. D. A. 2020. Diagnosis and Management of Hypothyroidism in Gulf Cooperation Council (GCC) Countries. Advances in therapy, 37(7): 3097-3111.

Babić Leko, M., Gunjača, I., Pleić, N. and Zemunik, T. 2021. Environmental factors affecting thyroid-stimulating hormone and thyroid hormone levels. International journal of molecular sciences, 22(12): 6521.

Bigagli, E. and Lodovici, M. 2019. Circulating oxidative stress biomarkers in clinical studies on type 2 diabetes and its complications. Oxidative Medicine and Cellular Longevity, 2019.

Borsò, M., Agretti, P., Zucchi, R. and Saba, A. 2022. Mass spectrometry in the diagnosis of thyroid disease and in the study of thyroid hormone metabolism. Mass Spectrometry Reviews, 41(3): 443-468.

Chakrabarti, S. K., Ghosh, S., Banerjee, S., Mukherjee, S. and Chowdhury, S. 2016. Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. Indian journal of endocrinology and metabolism, 20(5): 674.

Cheignon, C. m., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C. and Collin, F. 2018. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox biology, 14, 450-464.

Chiovato, L., Magri, F. and Carlé, A. 2019. Hypothyroidism in context: where we've been and where we're going. Advances in therapy, 36, 47-58.

Coperchini, F., Chiovato, L. and Rotondi, M. 2021. Interleukin-6, CXCL10 and infiltrating macrophages in COVID-19-related cytokine storm: not one for all but all for one! Frontiers in immunology, 12, 668507.

Czyzewska, K. and Trusek, A. 2018. Encapsulated catalase from Serratia genus for HO decomposition in food applications. Polish Journal of Chemical Technology, 20(4): 39-43.

Di Meo, S. and Venditti, P. 2020. Evolution of the knowledge of free radicals and other oxidants. Oxidative Medicine and Cellular Longevity, 2: 16-20.

Duntas, L. H. and Yen, P. M. 2019. Diagnosis and treatment of hypothyroidism in the elderly. Endocrine, 66, 63-69.

Dworzański, J., Strycharz-Dudziak, M., Kliszczewska, E., Kiełczykowska, M., Dworzańska, A., Drop, B. and Polz-Dacewicz, M. 2020. Glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in patients with diabetes mellitus type 2 infected with Epstein-Barr virus. Plos one, 15(3): e0230374.

Gonul Baltaci, N., Guler, C., Ceylan, H., Kalin, S. N., Adem, S., Kocpinar, E. F. and Budak, H. 2019. In vitro and in vivo effects of iron on the expression and activity of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and glutathione reductase in rat spleen. Journal of Biochemical and Molecular Toxicology, 33(1): e22229.

GOYAL, S., DIXIT, A., VANEY, N. and MADHU, S. 2022. Serum Levels of Inflammatory Markers in Newly Diagnosed Hypothyroid Patients Before and After Levothyroxine Therapy. Journal of Clinical and Diagnostic Research, 16(9): 744-751.

Hadwan, M. H. and kadhum Ali, S. 2018. New spectrophotometric assay for assessments of catalase activity in biological samples. Analytical biochemistry, 542: 29-33.

Hasanuzzaman, M., Bhuyan, M. B., Zulfiqar, F., Raza, A., Mohsin, S. M., Mahmud, J. A. and Fotopoulos, V. 2020. Reactive oxygen species and antioxidant defense in plants under abiotic stress: Revisiting the crucial role of a universal defense regulator. Antioxidants, 9(8): 681.

Hassan, N. A. and Abbas, S. K. 2022. Evaluation Serum levels of Leptin, CRP and Lipid profile in Hypothyroid Women in Kirkuk city/Iraq. Journal of Pharmaceutical Negative Results, 9(11): 2239-2246.

Idrees, T., Palmer, S., Maciel, R. M. and Bianco, A. C. 2020. Liothyronine and desiccated thyroid extract in the treatment of hypothyroidism. Thyroid, 30(10): 1399-1413.

Islam, M. R., Islam, M. R., Ahmed, I., Moktadir, A. A., Nahar, Z., Islam, M. S., . . . Hasnat, A. 2018. Elevated serum levels of malondialdehyde and cortisol are associated with major depressive disorder: a case-control study. SAGE open medicine, 6: 53.

Jain, N. 2021. Non-radical scavenging mechanism of action of os modulators. Sch. J. App. Med. Sci, 2: 47-52.

Jha, J. C., Ho, F., Dan, C. and Jandeleit-Dahm, K. 2018. A causal link between oxidative stress and inflammation in cardiovascular and renal complications of diabetes. Clinical Science, 132(16): 1811-1836.

Jiskra, J., Limanova, Z. and Antosova, M. 2007. Thyroid diseases, dyslipidemia and cardiovascular risk. Vnitrni lekarstvi, 53(4): 382-385.

Kar, K. and Sinha, S. 2014. Correlation between Advanced Oxidation Protein Products (AOPP) and Antioxidant status in Type 2 diabetics in Southern Asian Region. Sch. J. App. Med. Sci, 2(2B): 647-652.

Kim, J. S., Zhang, Y., Chang, Y., Ryu, S., Guallar, E., Shin, Y.-C. and Cho, J. 2018. Subclinical hypothyroidism and incident depression in young and middle-age adults. The Journal of Clinical Endocrinology and Metabolism, 103(5): 1827-1833.

La Vignera, S. and Vita, R. 2018. Thyroid dysfunction and semen quality. International journal of immunopathology and pharmacology, 32: 205.

La Vignera, S., Vita, R., Condorelli, R. A., Mongioì, L. M., Presti, S., Benvenga, S. and Calogero, A. E. 2017. Impact of thyroid disease on testicular function. Endocrine, 58(3): 397-407.

Li, B., Xie, J., Yuan, Z., Jain, P., Lin, X., Wu, K. and Jiang, S. 2018. Mitigation of inflammatory immune responses with hydrophilic nanoparticles. Angewandte Chemie International Edition, 57(17): 4527-4531.

Liu, Z., Yao, X., Jiang, W., Li, W., Zhu, S., Liao, C. and Chen, J. 2020. Advanced oxidation protein products induce microglia-mediated neuroinflammation via

MAPKs-NF-κB signaling pathway and pyroptosis after secondary spinal cord injury. Journal of Neuroinflammation, 17(1): 1-21.

Maenhaut, C., Christophe, D., Vassart, G., Dumont, J., Roger, P. and Opitz, R. 2015. Ontogeny, anatomy, metabolism and physiology of the thyroid. Endotext, 8: 43.

Mancini, A., Di Segni, C., Raimondo, S., Olivieri, G., Silvestrini, A., Meucci, E. and Currò, D. 2016. Thyroid hormones, oxidative stress, and inflammation. Mediators of inflammation, 2: 9-16.

Marchiori, R. C., Pereira, L. A., Naujorks, A. A., Rovaris, D. L., Meinerz, D. F., Duarte, M. M. and Rocha, J. B. 2015. Improvement of blood inflammatory marker levels in patients with hypothyroidism under levothyroxine treatment. BMC endocrine disorders, 15(1): 1-9.

Mas-Bargues, C., Escrivá, C., Dromant, M., Borrás, C. and Viña, J. 2021. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. Archives of Biochemistry and Biophysics, 709, 108941.

Masullo, L. F., Magalhães, R. A., Lemes, R. P. G., de Almeida Filho, T. P., De Castro, M. F., Maia Filho, P. A. and Sun, G. 2018. Levothyroxine replacement improves oxidative status in primary hypothyroidism. Frontiers in endocrinology, 9, 655.

Mateo, R. C. I. and Hennessey, J. V. 2019. Thyroxine and treatment of hypothyroidism: seven decades of experience. Endocrine, 66(1): 10-17.

Mi, C., Teng, Y., Wang, X., Yu, H., Huang, Z., Zong, W. and Zou, L. 2018. Molecular interaction of triclosan with superoxide dismutase (SOD) reveals a potentially toxic mechanism of the antimicrobial agent. Ecotoxicology and environmental safety, 153, 78-83.

Nanda, N. 2016. Oxidative stress in hypothyroidism. International Journal of Clinical and Experimental Physiology, 3(1): 4-9.

Newling, M., Sritharan, L., Baeten, D. and Den Dunnen, J. 2018. P080 C-reactive protein: not only a marker, but also a cause of inflammation through metabolic reprogramming of human macrophages: BMJ Publishing Group Ltd, 9: 32.

Ojima, A., Ishibashi, Y., Matsui, T., Maeda, S., Nishino, Y., Takeuchi, M. and Yamagishi, S.-i. 2013. Glucagon-like peptide-1 receptor agonist inhibits asymmetric dimethylarginine generation in the kidney of streptozotocin-induced diabetic rats by blocking advanced glycation end product–induced protein arginine methyltranferase-1 expression. The American journal of pathology, 182(1): 132-141.

Pan, A., Wang, Y., Yuan, J.-M. and Koh, W.-P. 2017. High-sensitive C-reactive protein and risk of incident type 2 diabetes: a case–control study nested within the Singapore Chinese Health Study. BMC endocrine disorders, 17(1): 1-8.

Pearce, E. N. 2012. Update in lipid alterations in subclinical hypothyroidism. The Journal of Clinical Endocrinology and Metabolism, 97(2): 326-333.

Reddy, V., Gouroju, S., Suchitra, M., Suresh, V., Sachan, A., Rao, P. S. and Bitla, A. 2013. Antioxidant defense in overt and subclinical hypothyroidism. Hormone and Metabolic Research, 45(10): 754-758.

Reier, T., Nong, H. N., Teschner, D., Schlögl, R. and Strasser, P. 2017. Electrocatalytic oxygen evolution reaction in acidic environments–reaction mechanisms and catalysts. Advanced Energy Materials, 7(1): 1601275.

SAH, S. P., BATRA, J., ARORA, M., KUMAR, S. and SAH, S. 2022. Evaluation of Osteopontin and Malondialdehyde Level and its Correlation with Iron Status in Hypothyroidism Patients: A Case-control Study. Journal of Clinical and Diagnostic Research, 16(2).

Saif, A., Mousa, S., Assem, M., Tharwat, N. and Abdelhamid, A. 2018. Endothelial dysfunction and the risk of atherosclerosis in overt and subclinical hypothyroidism. Endocrine connections, 7(10): 1075-1080.

Samuels, M. H., Kolobova, I., Niederhausen, M., Purnell, J. Q. and Schuff, K. G. 2018. Effects of altering levothyroxine dose on energy expenditure and body composition in subjects treated with LT4. The Journal of Clinical Endocrinology and Metabolism, 103(11): 4163-4175.

Saraswathi, I. R., Sadasivan, A., Koshi, E., Vijayakumar, S., Vallabhan, C. G. and Vrinda, S. M. 2020. Effect of nonsurgical periodontal therapy on serum level of interleukin-6 and tumor necrosis factor-α in chronic periodontitis patients with and without hypothyroidism. J Contemp Dent Pract, 21(4): 410-415.

Scarpioni, R., Ricardi, M. and Albertazzi, V. 2016. Secondary amyloidosis in autoinflammatory diseases and the role of inflammation in renal damage. World journal of nephrology, 5(1): 66.

Sencar, M. E., Calapkulu, M., Sakiz, D., Hepsen, S., Kus, A., Akhanli, P. and Ozbek, M. 2019. An evaluation of the results of the steroid and non-steroidal anti-inflammatory drug treatments in subacute thyroiditis in relation to persistent hypothyroidism and recurrence. Scientific Reports, 9(1): 1-8.

Seven, P. T., Yilmaz, S., Seven, I. and Kelestemur, G. T. 2012. The effects of propolis in animals exposed oxidative stress. Oxidative stress-environmental induction and dietary antioxidants, 4(11): 267-288.

Shahid, M. N., Amjad, M., Ashraf, U., Jamal, A. and Wattoo, J. I. 2022. Computational analysis of catalase from different source organisms. Pak. J. Bot, 54(1): 363-369.

Shi, J., Fan, J., Su, Q. and Yang, Z. 2019. Cytokines and abnormal glucose and lipid metabolism. Frontiers in endocrinology, 10: 703.

Sinha, R. A., Singh, B. K. and Yen, P. M. 2018. Direct effects of thyroid hormones on hepatic lipid metabolism. Nature Reviews Endocrinology, 14(5): 259-269.

Snead, Z. S. 2022. Human Thanatomicrobiome Signatures of the Thyroid and Submandibular GLand. Alabama State University, 8: 21.

Suarez, C. C. N. and Anastasopoulou, C. 2023. Endocrine-Related Adverse Events From Immune Checkpoint Inhibitors StatPearls: StatPearls Publishing, 4: 65.

Tang, C., Dong, Y., Lu, L. and Zhang, N. 2021. C-reactive protein and thyroid-stimulating hormone levels as risk factors for hypothyroidism in patients with subacute thyroiditis. Endocrine connections, 10(8): 965-972.

Tayde, P. S., Bhagwat, N. M., Sharma, P., Sharma, B., Dalwadi, P. P., Sonawane, A., and Varthakavi, P. K. 2017. Hypothyroidism and depression: are cytokines the link? Indian journal of endocrinology and metabolism, 21(6): 886.

Tehrani, H. S. and Moosavi-Movahedi, A. A. 2018. Catalase and its mysteries. Progress in Biophysics and Molecular Biology, 140: 5-12.

Thayakaran, R., Adderley, N. J., Sainsbury, C., Torlinska, B., Boelaert, K., Šumilo, D. and Nirantharakumar, K. 2019. Thyroid replacement therapy, thyroid stimulating hormone concentrations, and long term health outcomes in patients with hypothyroidism: longitudinal study. bmj, 3: 66.

Tuttle, R. M., Ahuja, S., Avram, A. M., Bernet, V. J., Bourguet, P., Daniels, G. H. and Führer, D. 2019. Controversies, consensus, and collaboration in the use of 131I therapy in differentiated thyroid cancer: a joint statement from the American

Thyroid Association, the European Association of Nuclear Medicine, the Society of Nuclear Medicine and Molecular Imaging, and the European Thyroid Association: Mary Ann Liebert, Inc., publishers 140 Huguenot Street, 3rd Floor New, 29: 461-470.

Ungati, H., Govindaraj, V., Narayanan, M. and Mugesh, G. 2019. Probing the formation of a seleninic acid in living cells by the fluorescence switching of a glutathione peroxidase mimetic. Angewandte Chemie, 131(24): 8240-8244.

Unluhizarci, K., Kiris, A., Kose, K., Tanrikulu, E., Karaca, Z., Tanriverdi, F. and Kelestimur, F. 2016. Thyroid hormone withdrawal further exacerbates oxidative stress in patients with thyroid carcinoma. Experimental and Clinical Endocrinology and Diabetes, 2(5): 225-229.

Vadgama, P. 2021. Oxidative free radicals and other species: Selective messengers with a reactive capacity for unselective tissue damage. Chemosensors, 9(5): 89.

Vudu, S., Seremet, A. and Vudu, L. 2023. C-reactive protein level in patients with autoimmune hypothyroidism before and after levothyroxine treatment. Paper presented at the Endocrine Abstracts, 4: 86.

Winterbourn, C. C. 2008. Reconciling the chemistry and biology of reactive oxygen species. Nature chemical biology, 4(5): 278-286.

Zhou, Q. G., Peng, X., Hu, L. L., Xie, D., Zhou, M. and Hou, F. F. 2010. Advanced oxidation protein products inhibit differentiation and activate inflammation in 3T3-L1 preadipocytes. Journal of cellular physiology, 225(1): 42-51.

APPENDICES

APPENDIX 1. Biochemical parameters value for the study groups

Characteristic	G1	G2	G3	P-Value
	n=40	n=40	n=40	
The concentration of Serum TSH (µIU/mL)				
Range	0.8- 4	2.54 -21.7	5.2- 31	<0.001
Mean ± SD	1.845 ± 0.838 ^{A}	8.722 ± 4.645 ^{B}	12.28 ± 5.831^{C}	
The concentration of Serum T3 (ng/ml)				
Range	0.8-1.9	0.22 -5.5	0.1- 1	<0.001
Mean ± SD	1.22 ± 0.2877 ^{A}	1.135 ± 0.9031^{B}	0.551 ± 0.195 ^{C}	
The concentration of Serum T4 (ng/ml)				
Range	55-121	17.5-146	8-63	<0.001
Mean ± SD	84.25 ± 17.392 ^{A}	71.17 ± 31.852 ^{B}	41.9 ± 10.28 ^{C}	
The concentration of Serum MDA (µmole/L)				
Range	0.107 - 0.218	0.222- 0.311	0.242- 0.442	<0.001
Mean ± SD	0.137 ± 0.020 ^{A}	0.272 ± 0.021^{B}	0.325 ± 0.036 ^{C}	
The concentration of Serum AOPP (µmole/L)				
Range	55.25-101.25	61.5 -213.775	60.5- 165	<0.001
Mean ± SD	76.7 ± 12.99 ^{A}	94.594 ± 25.782 ^{B}	119.3 ± 24.54 ^{C}	
SOD Activity (U/ml)				
Range	10-18	6-16	8-18	0.24
Mean ± SD	14.2 ± 1.742 ^{A}	13. 75 ± 2.18 ^{A}	13.4 ± 2.362 ^{A}	
CAT Activity (U/ml)				
Range	3.3-9.49	3.5 -14.86	4.12- 9.18	<0.001
Mean ± SD	5.681 ± 1.25 ^{A}	8.471 ± 2.28 ^{B}	7.464 ± 1.25 ^{C}	
The concentration of Serum TNF-α (pg/ml)				
Range	14.39-20.27	15.27- 21.74	14.68- 32.33	<0.001
Mean ± SD	16.06 ± 1.21 ^{A}	16.97 ± 1.55 ^{B}	19.40 ± 4.13 ^{C}	
The concentration of Serum CRP (mg/L)				
Range	1.9-6.2	5.8-16.4	11.8- 40.5	<0.001
Mean ± SD	3.92 ± 0.99 ^{A}	9.397 ± 2.83 ^{B}	25.31± 6.44 ^{C}	

CURRICULUM VITAE

Personal Information

Name and Surname : Murtadha Hadi Jawad ALBAYATI

Education

MSc	Çankırı Karatekin University Graduate School of Natural and Applied Sciences Department of Chemistry	2021-2024
Undergraduate	Al-Qadisiyah University Faculty of Sciences Department of Chemistry	2015-2019