زانکۆی سەلاحەدین - هەولێر
Salahaddin University-Erbil

Association between Thyroid Hormones, Lipids and Oxidative Stress Markers in Hypothyroidism

Research Project Submitted to the department of (Education Chemistry) in partialfulfillment of the requirements for the degree of B.A or BSc. in (Chemistry)

Prepared by

Tara Mahmood Kakamin

Zainab Jalal Ali

Supervised by

Dr. Parween A. Ismail

April-2023

Declaration

I hereby declare that all the information in this document has been obtained and presented in accordance with the academic rules and ethical conduct. I also declare that I have sincerely followed the rules and conduct as required. I assure that I havefully cited and referenced all the material and results that are not original to this work.

Tara Mahmood Kakamin
Zainab Jalal Ali

SUPERVISOR RECOMMENDATION

I am the student's supervisor, **Tara Mahmood Kakamin and Zainab Jalal Ali**

I support that the student has completed all the requirements for submitting the research drawn entitled **Association between Thyroid Hormones, Lipids and Oxidative Stress Markers in Hypothyroidism**-according to the numbered administrative order 3/1/5/1972 on 9th oct. 2022 in accordance with the instructions of Salahaddin university quality assurance and it is ready for discussion.

Dr.Parween Abdulsamad Ismail
Signature with date
7/4/2023

Research project lecturer
Dr. Dler D. Kurda

Acknowledgment

I must be thankful to God, the one who improved me to add extra education to this study. Then I should be thankful to my different people in life, my beloved father who stayed ready to lose everything only to make me win. Besides, my beloved mother who stayed with me was in total pain. My great family such as brothers and sister supported me. Furthermore, all those who were with me from the first step of college down to where I am now. Feel thankful to my respectful supervisor, who is *Dr. Parween Abdulsamad Ismail*. From the first day to the last, who was there for me with a unique study idea in the Kurdistan region. Never forget your support, kindness, andyour level of teaching.

Abstract

Background: Hypothyroidism is a common problem that reduces the functional ability of life. Hypothyroidism is associated with altered lipid levels. Oxidative stress plays a role in the pathogenesis of many chronic diseases. It is recognized in hypothyroidism.

Objective The aim of this study was to determine whether there was increased oxidation of lipids in hypothyroidism, and examine their association with lipids and thyroid hormones., as well as to evaluate the interaction between thyroid hormones and biomarkers of oxidative stress in patients with hypothyroidism.

Materials and Methods: A case -control study was conducted on 48 hypothyroid patients and 35 similar age- and sex-matched controls. Serum total thyroxine(T4), triiodothyronine(T3), thyroid-stimulating hormone (TSH), Total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, thyroid profile, Malonaldehyde (MDA)superoxide dismutase (SOD), and glutathione (GSH) levels were examined in hypothyroid patients.

Results: Serum concentrations of MDA, thyroid-stimulating hormone (TSH), Total cholesterol (TC) low-density lipoprotein (LDL) cholesterol, triglycerides were significantly higher in hypothyroid patients compared with controls. Significant reduction was found in dismutase (SOD), and glutathione (GSH), Serum total thyroxine(T4), triiodothyronine(T3),and high-density lipoprotein (HDL) levels in hypothyroid patients.

Conclusions: hypothyroidism is associated with an increase in oxidative stress, and hypercholesterolemia has a stronger influence on development of oxidative stress in hypothyroid conditions compared with thyroid hormones.

Keywords: Hypothyroidism, Oxidative stress, antioxidant, lipid profile, thyroid profile

LIST OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Symbol	Description
TSH	**Thyroid-Stimulating Hormone**
T3	**Triiodothyronine**
T4	**Thyroxin**
TG	**Thyroglobulin**
TPO	**Thyroid Peroxidase**
OS	**Oxidative Stress**
GPX	**Glutathione Peroxidase**
GR	**Glutathione Reductase**

TAC	**Total Antioxidant Capacity**
TOS	**Total Oxidant Status**
OSI	**Oxidative Stress Index**
TC	**Total Cholesterol**
HDL	**High-Density Lipoprotein**
LDL	**Low-Density Lipoprotein**
MDA	**Malondialdehyde**
SODs	**Superoxide Dismutase**
ROS	**Reactive Oxygen Species**
GSH	**Glutathione**
GPx	**Glutathione Peroxidases**
GR	**Glutathione Reductase**
anti-TPO Ab	**Anti Thyroperoxidase Antibody**
GSTs	**Glutathione S-Transferases**
POX	**Peroxidases**
SeCys	**Selenocysteine**
SePs	**Selenoproteins**
LPO	**Lipid Peroxidation**
SST	**Serum Separator Tubes**
HRP	**Horseradish Peroxidase**
OD	**Optical Density**

1. THYROID GLAND

The thyroid gland is the largest gland located in the neck at about the level of the larynx and upper part of the trachea. Normally weighing 15-20 grams in adults, and is increased in pregnancy. Measuring about (5 centimeters) it consists of two lobes connected by a narrow bridge (isthmus) across the ventral surface of the trachea. The gland is well supplied with blood, has a connective tissue framework and contains many microscopic cavities or vesicles. The functional units of the gland are called follicles. The thyroid of a normal rat contains about 100,000 follicles. These are separated by interfollicular connective tissue containing numerous capillaries. Each follicle consists of single peripheral layer of cuboidal cells surrounding a cavity filled with a colloid (Bianco et al., 2002).

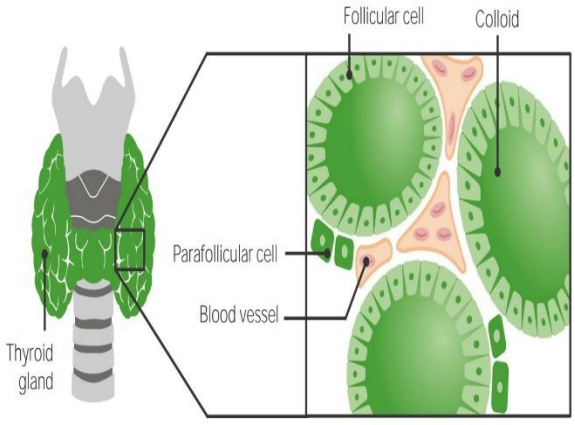


Fig. 1. Histological features of Thyroid (Bianco et al., 2002).

Vesicles are mainly responsible for the production of thyroid hormones whereas colloids are made up of a large complex glycoprotein called thyroglobulin. The hormones remain bound to thyroglobulin until secreted. When they are secreted, colloid is ingested by the thyroid cells, the peptide bonds are hydrolyzed and free T3 and T4 are discharged into the capillaries.

1.1. PHYSIOLOGY OF THYROID GLAND

The primary function of the thyroid is production of the hormones thyroxin (T4), triiodothyronine (T3), and calcitonin. Up to 80% of the T4 is converted to

T3 by peripheral organs such as the liver, kidney and spleen. T3 is about ten times more active than T4. Thyroxin (T4) is synthesized by the follicular cells from free tyrosine and on the tyrosine residues of the protein called thyroglobulin (TG). Iodine is captured with the "iodine trap" by the hydrogen peroxide generated by the enzyme thyroid peroxidase (TPO) (Ekholm and Bjorkman, 1997) and linked to the 3' and 5' sites of the benzene ring of the tyrosine residues on TG, and on free tyrosine. Upon stimulation by the thyroid-stimulating hormone (TSH), the follicular cells reabsorb TG and proteolytically cleave the iodinated tyrosine's from TG, forming T4 and T3 (in T3, one iodine is absent compared to T4), and releasing them into the blood. Deiodinase enzymes convert T4 to T3. Thyroid hormone that is secreted from the gland is about 90% T4 and about 10% T3 (Bianco et al., 2002).

Cells of the brain are a major target for the thyroid hormones T3 and T4. Thyroid hormones play a particularly crucial role in brain maturation during fetal development. A transport protein (OATP1C1) has been identified that seems to be important for T4 transport across the blood brain barrier. A second transport protein (MCT8) is important for T3 transport across brain cell membranes (Jansen et al., 2005).

In the blood, T4 and T3 are partially bound to thyroxin-binding globulin, transthyretin and albumin. Only a very small fraction of the circulating hormone is free (unbound) T4 0.03% and T3 0.3%. Only the free fraction has hormonal activity. As with the steroid hormones and retinoic acid, thyroid hormones cross the cell membrane and bind to intracellular receptors (α1, α2, β1 and β2), which act alone, in pairs or together with the retinoid X-receptor as transcription factors to modulate DNA transcription (Kester et al., 2004).

1.2. HORMONES OF THYROID

THYROID SECRETES TWO HORMONES

The thyroid hormones, thyroxin (T4) and triiodothyronine (T3), are tyrosine-based hormones produced by the thyroid gland. The major form of thyroid hormone in the blood is thyroxin (T4). The ratio of T4 to T3 released in the blood is roughly 20 to 1. Thyroxin is converted to the active T3 (three to four

times more potent than T4) within cells by deiodinases (5'-iodinase). These are further processed by decarboxylation and deiodination to produce iodothyronamine (T1a) and thyronamine (T0a). Most of the thyroid hormone circulating in the blood is bound to transport proteins. Only a very small fraction of the circulating hormone is free (unbound) and biologically active, hence measuring concentrations of free thyroid hormones is of great diagnostic value. When thyroid hormone is bound, it is not active, so the amount of free T3/T4 is an important criterion. For this reason, measuring total thyroxin in the blood can be misleading (Verhaeghe et al., 2008).

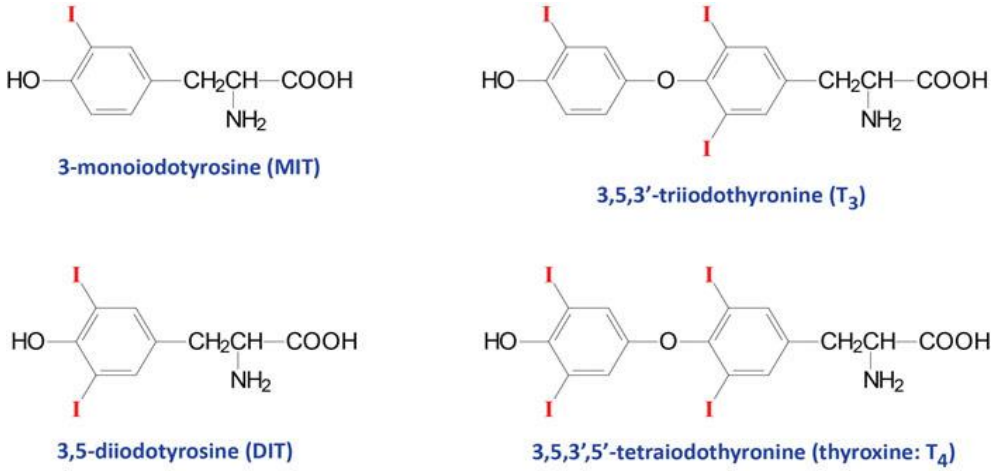


Fig. 2. Thyroid Hormone Structure (Verhaeghe et al., 2008).

THYROCALCITONIN OR CALCITONIN

In human being thyroid also secrets calcitonin which is a large polypeptide with a molecular weight 3400 and a chain of 32 amino acids. Calcitonin is synthesized in parafollicular cells 'C' cells present in the connective tissue around thyroid follicular cells. It is associated with calcium phosphate metabolism. TCT act by causing a transfer of calcium from blood into bone by increased calcification of the bones or by diminished 3 decalcification or by both mechanisms. This function of TCT is opposed by the hyper calcemic hormone produced by the parathyroid.

1.2.1. FUNCTIONS OF THYROID HORMONES

1. Increased metabolic activities- it increases the rate of O2 consumption or oxidative metabolism and resulting heat production of the various cells and tissues of the body. It stimulates many phases of carbohydrate metabolism (Sarkar et al., 2001).

2. Regulatory role- Thyroid hormone has important role in growth and development of cells and tissues. These include the sexual development, maturation of bones, mental development and energy metabolism. In absence of thyroid hormone, growth hormone secretion is also depressed and thyroid hormones potentiate the effect of growth hormone on tissues.

3. Effect on other endocrine glands- Increased thyroid secretion increases the rate of secretion of most other endocrine glands, but it also increases the needs of the tissues for hormones.

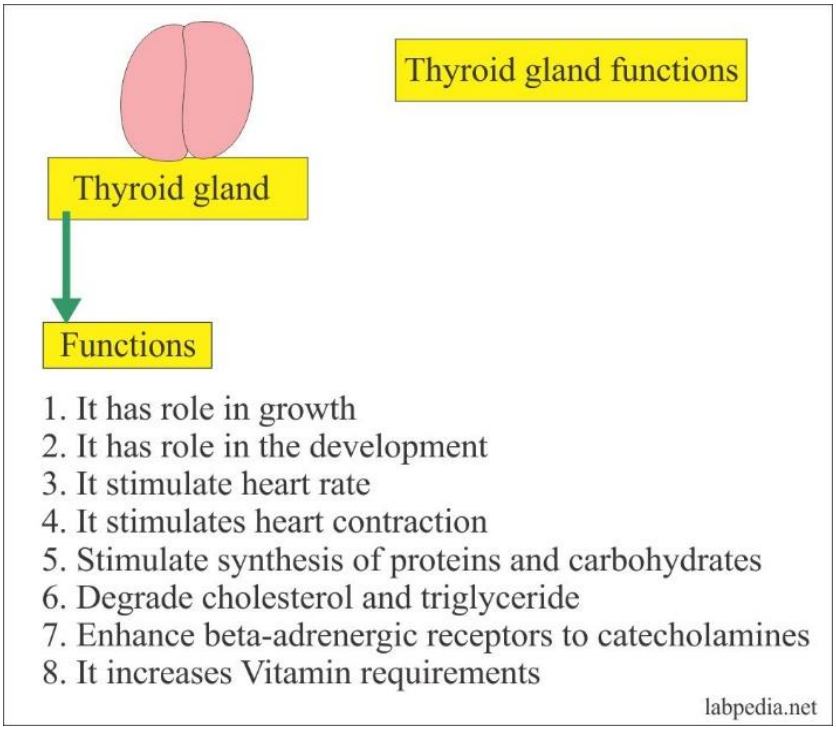


Fig. 3. Thyroid Gland Functions (Sarkar et al., 2001).

4. Effect of calcitonin on blood calcium- Calcitonin reduces the blood calcium ion concentration (an effect, which is opposite to that of parathyroid hormone). But calcitonin mechanism acts only weekly and only as a short-term regulator of calcium iron concentration because it is rapidly overridden by the much more powerful parathyroid mechanism.

5. Other role- Thyroid hormones are essential for hepatic conversion of carotene to vitamin A. The accumulation of carotene in the blood stream (carotenemia) in hypothyroidism is responsible for yellowish tint of the skin.

1.2.2. BIOSYNTHESIS OF THYROID HORMONE

There are six steps in the synthesis of thyroid hormone, and you can remember them using the mnemonic ATE ICE:

- Active transport of Iodide into the follicular cell via the Sodium-Iodide Symporter (NIS). This is actually secondary active transport, and the sodium gradient driving it is maintained by a Sodium-Potassium ATPase.
- Thyroglobulin (Tg), a large protein rich in Tyrosine, is formed in follicular ribosomes and placed into secretory vesicles.
- Exocytosis of Thyroglobulin into the follicle lumen, where it is stored as colloid. Thyroglobulin is the scaffold upon which thyroid hormone is synthesized.
- Iodination of the Thyroglobulin. Iodide is made reactive by the enzyme thyroid peroxidase. Iodide binds to the benzene ring on Tyrosine residues of Thyroglobulin, forming monoiodotyrosine (MIT) then diiodotyrosine (DIT).
- Coupling of MIT and DIT gives the Triiodothyronine (T3) hormone and coupling of DIT and DIT gives the Tetraiodothyronine (T4) hormone, also known as Thyroxine.
- Endocytosis of iodinated thyroglobulin back into the follicular cell. Thyroglobulin undergoes proteolysis in lysosomes to cleave the iodinated tyrosine residues from the larger protein. Free T3 or T4 is then released, and the Thyroglobulin scaffold is recycled.

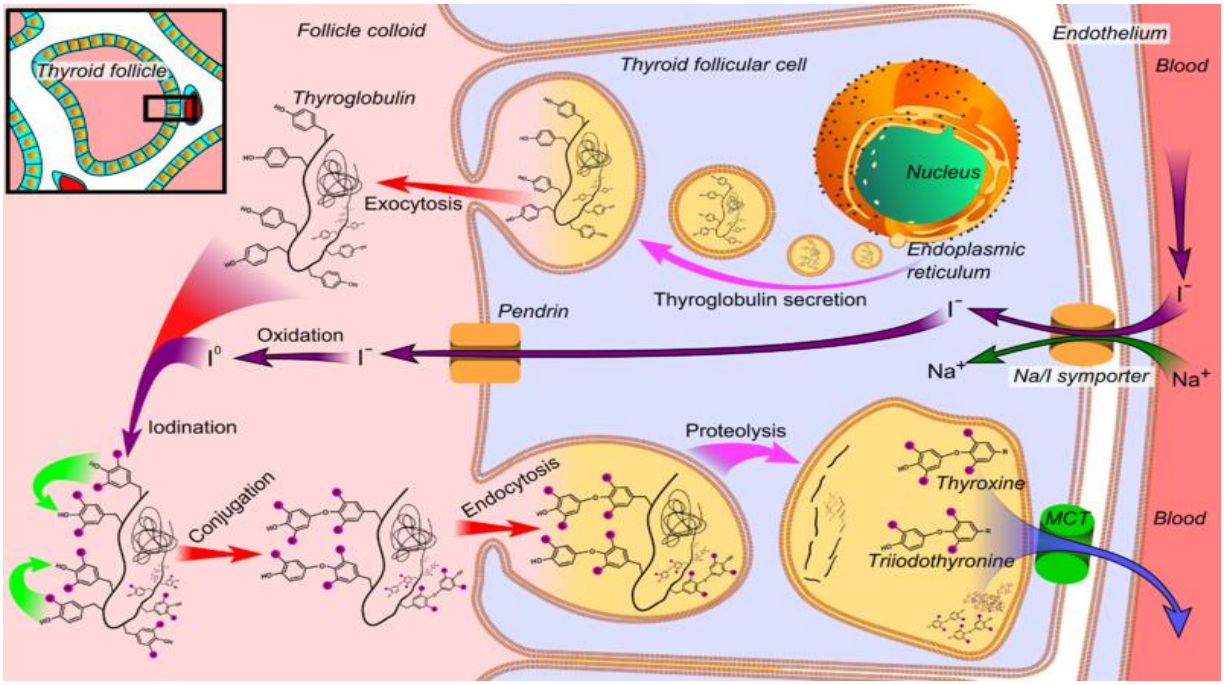


Fig. 4. Biosynthesis of Thyroid Hormone (Ahmed, 2019).

T3 and T4 are the active thyroid hormones. They are fat soluble and mostly carried by plasma proteins – Thyronine Binding Globulin and Albumin.
While T3 is the more potent form, it also has a shorter half-life due to its lower affinity for the binding proteins. Less than 1% of T3 and T4 is unbound free hormone. At the peripheries, T4 is de iodinated to the more active T3.T3 and T4 are deactivated by removing iodine. This happens in the liver and kidney. As T4 has a longer half-life, it is used in the treatment of hypothyroidism over T3 as its plasma concentrations are easier to manage (Ahmed, 2019).

1.3. REGULATION OF THYROID GLAND

The synthesis of thyroid hormones is controlled by feedback regulation. T3 appears to be more actively involved than T4 in the regulation process. The production of (TSH) by pituitary and (TRH) by hypothalamus are inhibited by T3 and, to a lesser degree by T4. The increased of TSH and TRH occurs in response to decreased circulatory levels of T3 and T4., the body has sufficient stores of hormones to last for several weeks. Hence it takes some months to observe thyroid functional deficiency. The recent studies indicate that this process is physiologically regulated (Ziotopoulou et al., 2000). Changes in pituitary

conversion of T4 to T3 are often the opposite of those that accrue in the liver and kidney under similar circumstances. As show in figure (5).

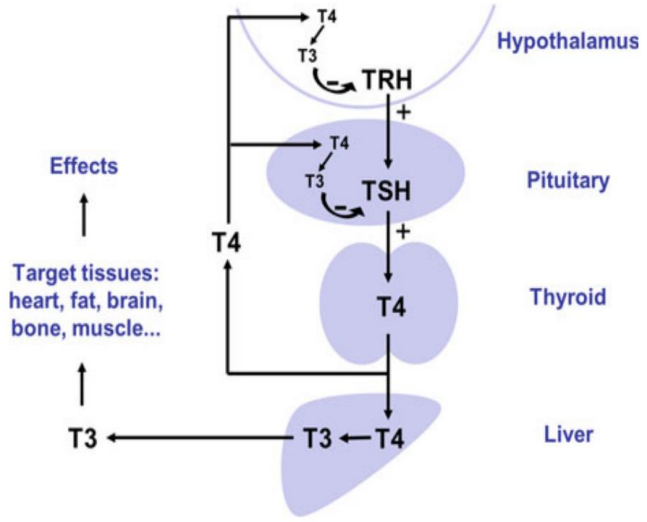


Fig. 5. Regulation of Thyroid Gland (Ziotopoulou et al., 2000).

1.4. THYROID DISORDER

Among the most common types of thyroid disorders are hypothyroidism (underactive thyroid), hyperthyroidism (overactive thyroid), benign nodules, inflammations of the thyroid, and malignant cancers. If there is not enough thyroid hormone in the blood stream, the body's metabolism slows down (hypothyroidism or underactive thyroid).

Complications of hypothyroidism may include an enlarged thyroid gland (goiter), increased risk of heart disease (primarily due to increased levels of LDL cholesterol among people with underactive thyroid), an enlarged heart, severe depression, decreased libido, slowed mental functioning, and rarely, a life-threatening condition known as myxedema (symptoms including intense cold intolerance and drowsiness followed by profound lethargy and unconsciousness). If there is too much thyroid hormone in the bloodstream, the body's metabolism speeds up (hyperthyroidism or overactive thyroid).

Complications of hyperthyroidism may include rapid heart rate, atrial fibrillation and congestive heart failure (a condition in which the heart becomes too weak to

circulate enough blood to meet the needs of the body), osteoporosis (weak, brittle bones because too much thyroid hormone interferes with the body's ability to incorporate calcium), severe eye problems (known as Grave's ophthalmopathy), and rarely severe skin problems causing redness and swelling of the shins and feet, as well as risk for thyrotoxic crisis (condition in which symptoms suddenly intensify, leading to fever, rapid pulse, and delirium) (McMillen et al., 2016).

1.5. HYPOTHYROIDISM

Hypothyroidism is the clinical picture that one sees when the thyroid is unable to produce enough thyroid hormones, triiodothyronine (T3) and thyroxin (T4), to keep blood levels normal and to satisfy the needs of peripheral tissues. Most patients have primary hypothyroidism, a result of disease in the thyroid that destroys its ability to produce adequate thyroid hormones. Hypothyroidism is occasionally secondary, caused by disease in the pituitary gland or hypothalamus resulting in inadequate production of thyroid-stimulating hormone (TSH) (Khandelwal and Tandon, 2012).

1.5.1. TYPES OF HYPOTHYROIDISM

Hypothyroidism can be primary or secondary based on its source of deficiency:

- The thyroid gland hypofunction accounts for more than 99.5% of cases (known as *primary hypothyroidism*) whereas the hypothyroidism resulting from pituitary and hypothalamic dysfunction accounts for the rest 0.5% of the cases. **In primary hypothyroidism**, thyroid hormones are deficient due to defect in the thyroid gland and hence thyroid-stimulating hormone (TSH) levels are higher than normal.
- **In secondary hypothyroidism**, TSH secretion is below normal leading to deficiency of thyroid hormones also.

1.5.2. CAUSES OF HYPOTHYROIDISM

In over 99% of cases, hypothyroidism is caused by a failure of the thyroid gland to produce thyroid hormones (primary hypothyroidism). The remaining 5% of

patients have hypothyroidism from other causes, including secondary hypothyroidism, caused by underproduction of TSH by the pituitary gland, tertiary hypothyroidism, caused by deficiency of thyrotropin-releasing hormone, and peripheral (extra-thyroidal) hypothyroidism. Central hypothyroidism, which includes both secondary and tertiary hypothyroidism, and peripheral hypothyroidism account for less than 1% of cases (Persani, 2012).

- **Primary:** due to problems in the gland itself = most common problem
  - Autoimmune thyroiditis (Hashimoto's thyroiditis)
  - Thyroid nodules or masses

- **Secondary:** due to problems in the pituitary gland and includes:
  - Pituitary tumor
  - post-partum pituitary necrosis (Sheehan's syndrome)
  - Trauma

- **Other:**
  - Due to medications (such as lithium, amiodarone)
  - Due to surgery on the thyroid gland
  - Due to treatment of overactive thyroid with radioactive iodine treatment
  - Infiltrative disease ♣ Fibrous thyroiditis, hemochromatosis,scleroderma, leukemia
  - Iodine deficiency or excess

1.5.3. SIGNS AND SYMPTOMS OF HYPOTHYROIDISM

People with hypothyroidism often have no or only mild symptoms. Numerous symptoms and signs are associated with hypothyroidism and can be related to the underlying cause, or a direct effect of having not enough thyroid hormones. Hashimoto's thyroiditis may present with the mass effect of a goiter (enlarged thyroid gland). In middle-aged women, the symptoms may be mistaken for those of menopause. Delayed relaxation after testing the ankle jerk reflex is a characteristic sign of hypothyroidism and is associated with the severity of the hormone deficit (Khandelwal and Tandon, 2012).

Table (1): Signs and Symptoms of Hypothyroidism (Khandelwal and Tandon, 2012).

Symptoms	Signs
Fatigue	Dry, coarse skin
Feeling cold	Cool extremities
Poor memory and concentration	Myxedema (mucopolysaccharide deposits in the skin)
Constipation, dyspepsia	Hair loss
Weight gain with poor appetite	Slow pulse rate
Shortness of breath	Swelling of the limbs
Hoarse voice	Delayed relaxation of tendon reflexes
In females, heavy menstrual periods (and later light periods)	Carpal tunnel syndrome
Abnormal sensation	Pleural effusion, ascites, pericardial effusion
Poor hearing	

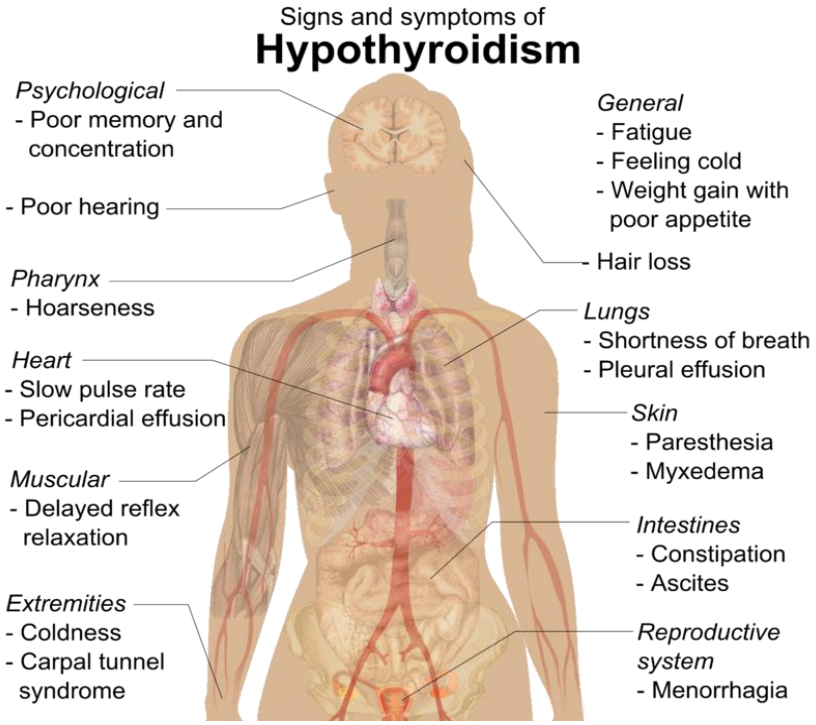


Fig. 6. Symptoms and signs of hypothyroidism (Khandelwal and Tandon, 2012).

1.6. OXIDATIVE STRESS

Oxidative stress (OS), despite the way its name suggested, is a natural phenomenon in the body. However, for the past two decades or so, OS has gained much importance in the field of research. Now, it has been linked to several disorders such as diabetes, cardiovascular diseases, cancer, neurodegeneration, and aging.

These reactions generate harmful molecules and radicals, known as free radicals, which are capable of altering cellular structure in their vicinity affecting cellular function. Therefore, the natural defense system consisting of anti-oxidant system is acting continuously against free radicals. When the natural balance between the rate of generation of free radicals and action of antioxidants is lost, it results in OS. OS in majority of studies has been reported to be consistently associated with hypermetabolic states such as hyperthyroidism, as thyroid hormones promote mitochondrial utilization of oxygen and therefore leads to excess generation of free radicals (Yen, 2001).

Hypothyroidism on the contrary is a hypometabolic state due to deficiency of the thyroid hormones. Therefore, one may assume that OS should not be more in hypothyroidism because of reduced utilization of oxygen. Subsequently, there were studies observing the presence of OS in hypothyroidism.

1.6.1. BIOMARKERS OF OXIDATIVE STRESS IN THYROID DISEASES

Enzymatic mechanisms of antioxidant defence constitute the internal system for maintaining ROS homeostasis (Figure 7). Superoxide dismutase's (SOD1, SOD2, SOD3) are antioxidant enzymes, neutralizing O2 •− (Eleutherio et al., 2021).

The key enzyme responsible for neutralizing hydrogen peroxide is catalase (CAT), which converts it to water and oxygen.

Likewise, glutathione peroxidase (GPX) scavenges and detoxifies H2O2. Glutathione serves as an intracellular buffer against oxidation. In response to excessive ROS release, it forms an oxidized dimer structure by bridging two glutathione molecules. Glutathione reductase (GR) then restores the reduced form of glutathione, lowering its reactivity.

Measurement of antioxidant enzyme activity in serum makes it possible to evaluate the condition of the antioxidant defence system. Lower levels of this activity, compared to the control, may be a sign of inadequate defence against free radicals (Metere et al., 2018).

Biomarkers of oxidative stress also include prooxidant enzymes—NADPH oxidases (NOX), which are an endogenous source of ROS, especially in thyroid tissue. Their increased activity is associated with elevated concentrations of reactive oxygen species in pathological conditions. Direct measurement of ROS concentrations may be a helpful marker in the evaluation of medical conditions, yet its utility may be limited given the short half-life of these molecules (Kim et al., 2017).

Elevated levels are observed in ROS damaged tissues, as the final product of peroxidation, making them markers of oxidative stress in the body (Ruggeri et al., 2021).

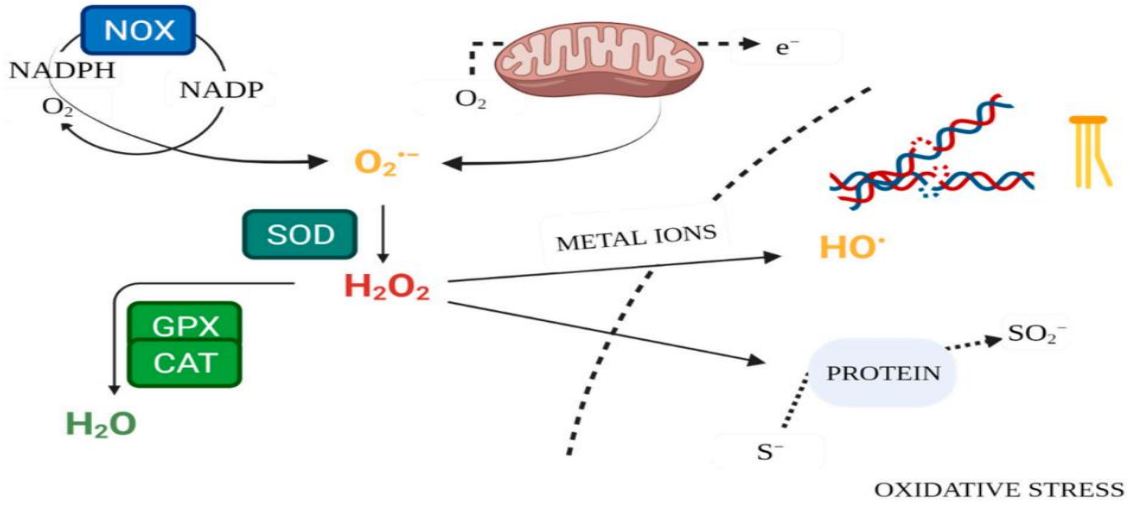


Fig. 7. Biomarkers of Oxidative Stress in Thyroid Diseases (Ruggeri et al., 2021).

Total antioxidant capacity (TAC) is a parameter indicative of the body's overall ability to neutralize oxidants. It takes into account all the antioxidants contained in bodily fluids, including exogenous and endogenous compounds. In turn, total oxidant status (TOS) is based on the oxidation of ferrous ion to ferric ion in the presence of various oxidants. It reflects the oxidation state of bodily fluids, represented by the level of radicals. Oxidative stress index (OSI) is a measure of oxidative stress, calculated as the ratio of total oxidant status to total antioxidant

status and therefore represents the overall oxidation state of the body (Ates et al., 2018).

All the biomarkers employed in the determination of the role of oxidative stress in thyroid diseases in this review are listed in Table 1.

Table 2: List of major reactive oxygen and nitrogen species (Ates et al., 2018).

Reactive oxygen species		Reactive nitrogen species	
Superoxide	O_2^-	Nitric oxide	NO^-
Hydroxyl	HO	Nitrogen dioxide	NO_2
Peroxyl	ROO	Nitrous acid	HNO_2
Perhydroxyl radicals	HO_2	Peroxynitrite	$ONOO^-$
Alkoxy	RO	Alkyl peroxynitrite	ROONO
Hydrogen peroxide	H_2O_2		
Singlet oxygen	$^1O_2$		

1.6.2. RELATIONSHIP BETWEEN OXIDATIVE STRESS, ROS AND THYROID DISEASES

Oxidative Stress (OS) has long been associated with hyperthyroidism in many previous studies because of increased oxygen consumption and increased rate of metabolism leading to generation of free radicals. Hypothyroidism, on the contrary, being a hypometabolic state, would lead to decreased oxygen consumption. Therefore, OS in hypothyroidism deals with entirely a different concept. Moreover, despite few studies reporting the presence of OS in hypothyroidism, its occurrence has been denied by others.

Therefore, OS in hypothyroidism is relatively a controversial topic. Initial studies involving hypothyroidism did not include OS as the main objective. The level of anti-oxidants was mainly assessed in experimental hypothyroidism or in hyperthyroid patients while comparing its levels with euthyroid and hypothyroid individuals. These reports suggested a decreased antioxidant level in hypothyroidism. A lack of rise in antioxidants was implied as a consequence of reduced OS in hypothyroidism.

Many studies were also conducted to assess the effect of hypothyroidism on OS induced by other primary factors such as endotoxin-induced OS in eyes, experimental burn model, (Fahim et al., 2020).

Most reports of OS in hypothyroidism alone were available after 1990. Oxidized low-density lipoprotein (LDL) is a known risk factor for atherosclerosis. (Diekman et al., 1998) showed that LDL from hypothyroid patients is more vulnerable for oxidation, indicating OS.

(Olinescu et al., 1992) showed an increase in malondialdehyde (MDA) level in obese hypothyroid women.(Resch et al., 2002) compared the enzymatic and nonenzymatic anti-oxidants, endogenous peroxides, and antibody titer against oxidized LDL receptor (Saraf-Bank et al., 2019).

Enzymatic antioxidants were higher whereas nonenzymatic antioxidants were lower in hypothyroid patients compared to controls. They illustrated the presence of OS in hypothyroidism by increased anti-oxidized LDL antibody titer, which was higher than controls. However, they did not find any difference in the levels of endogenous peroxides among controls, hypothyroid, and hyperthyroid patients.

Most of the research works conducted in animal models did not reveal the presence of OS. In a study done in female hypothyroid rabbits, an increased resistance to OS was reported suggesting increased protection against OS. In an experimental set up, (El Hassani et al., 2019) showed that both GSH and MDA content in cerebral, hepatic, and cardiac tissues in hypothyroid rats were less than in control rats.

Based on peroxide level, they concluded that there is reduced oxidative damage in hypothyroidism. However, the authors overlooked the corresponding decrease in tissue GSH level compared to control rats that was in their study. A reduced GSH level itself is an indicator of OS, as GSH is the most common anti-oxidant molecule that nullifies the free radicals.

However, there are reports from other animal studies suggesting the presence of OS in hypothyroidism. They not only showed increased MDA content, but also this was decreased by Vitamin E supplementation as an anti-oxidant (Abdalla and Bianco, 2014).

A report by (Dardano et al., 2006) suggested that increased TSH itself may lead to low-grade inflammation and OS. This was an interesting study because raised TSH is a hallmark of primary hypothyroidism. Before entering into a phase of overt hypothyroidism, the body experiences a gradual buildup of TSH and the time period varies among patient to patient depending on genetic-endocrinal-immunological differences.

Hypothyroidism generally is associated with hyperlipidemia which often resembles an atherogenic lipid profile. Ensuing OS if associated with hyperlipidemia provides an excellent platform for lipid peroxidation.

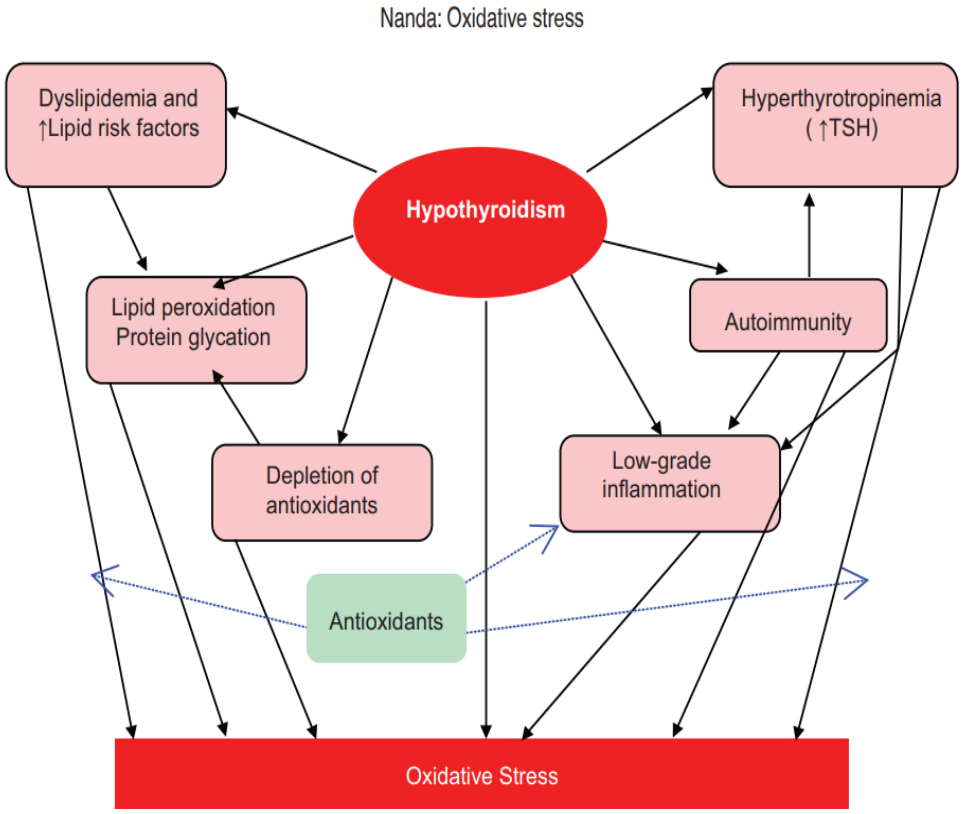


Fig. 8. Multiple pathways operating alone or in concurrence leading to oxidative stress in hypothyroidism. Black arrows show the proposed mechanisms and blue arrows represent various proposed targets of anti-oxidants preventing build-up of oxidative stress. Suggested mechanisms of oxidative stress and its probable solution (Benvenga et al., 2021).

In human study a positive correlation was found between MDA content with various lipid risk factors indicative of atherosclerosis (Benvenga et al., 2021) as well as protein glycation.

Jain and Palmer have suggested that the protein glycation is facilitated by MDA per se by acting as an anchor (Schiff linkage) between sugar and protein moieties. Therefore, the same mechanism can lead to protein glycation in long-term hypothyroidism even at normal glucose level. Though hypothyroidism is a disease more prevalent in women worldwide, the level of lipid peroxidation and protein glycation was higher in male patients suggesting higher OS in male hypothyroid

patients. Likewise, OS is reportedly higher among anti thyroperoxidase antibody (anti-TPO Ab) positive cases compared to anti-TPO Ab-negative cases, suggesting increased OS in autoimmune hypothyroidism.

1.6.3. GLUTATHIONE SYSTEM

The glutathione antioxidant system includes Glutathione (GSH) which is a potent tripeptide antioxidant, Glutathione peroxidases (GPx), glutathione reductase (GR), and glutathione S-transferases (GSTs). This system is a powerful antioxidant defense mechanism found in humans and also animals, plants and microorganisms (Lushchak, 2012).

The major enzyme player in the system is Glutathione peroxidases (GPx) which are the chief thiol-dependent peroxidases (POX) antioxidant metalloenzyme containing four selenium-cofactors that catalyzes the breakdown of hydrogen peroxide (H2O2) and organic hydroperoxides by reduction into water (H2O) and alcohol (ROH). Deficiencies of Selenium (Se) for any reason causes relatively complete catalytic inactivation of Glutathione peroxidases.

Selenium (Se) exists in the human body in the form of selenocysteine (SeCys), a component of selenoproteins (SePs), associated with potent antioxidant activity. The GPx redox reactions occur in the presence of reduced form Glutathione (GSH) that readily converted into oxidized form Glutathione disulfide (GSSG)). There are four isoforms in humans, cytosolic and mitochondrial (GPx1), cytosolic (GPx2), extracellular (GPx3), and the phospholipid peroxide (GPx4).
(Aguilar et al., 2016).

1.6.4. SUPEROXIDE DISMUTASE (SOD)

Superoxide dismutases (SODs) are a group of key metalloenzymes functioning as the first line of antioxidant defense by virtue of the ability to convert highly reactive superoxide radicals (dismutation) into hydrogen peroxide and ordinary molecular oxygen (Figure 8). SODs are metalloproteins that catalyze the disproportionation of superoxide anions into less reactive radical hydrogen peroxide using the redox reaction with their metallic cofactors that could be one or combination of transition metals involves (Cu, Mn, and Fe). Deficiencies and inactivation of those metallic cofactors confer great detrimental effects of their catalytic activity. Three forms of SOD are identified in humans, and also in all

other mammals. Cytosolic SOD (SOD1), Mitochondrial SOD (SOD2), and extracellular SOD (EC-SOD or SOD3) (Younus, 2018).

The cytosolic SOD (SOD1) is a dimer consists of two domains each complexed with copper (Cu) and zinc (Zn) cofactors.

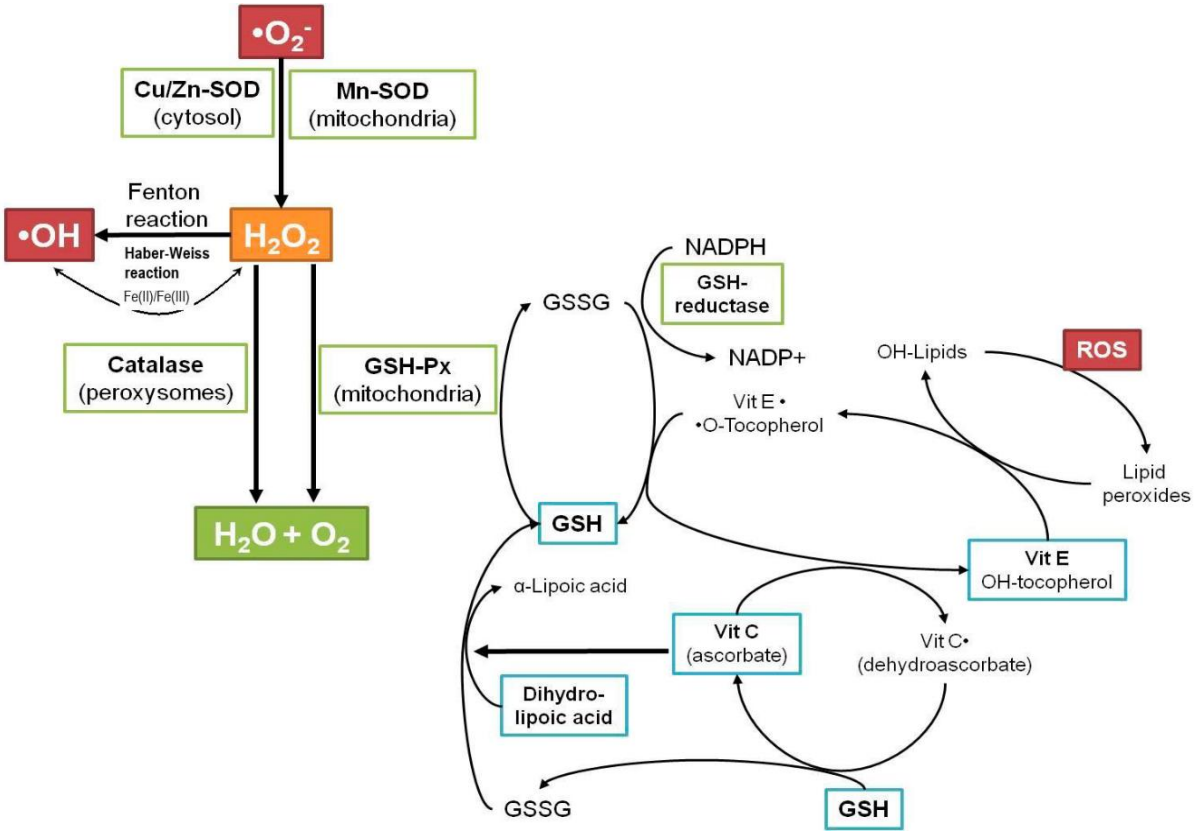


Fig. 9. Major enzymatic and non-enzymatic antioxidant defense mechanisms against ROS (Adapted & Modified from (Fernández-Mejía, 2013)).

SOD2 and SOD3 are tetramers (four subunits). SOD3 contain copper and zinc as a metallic cofactor, whereas SOD2, has manganese (Mg) in its catalytic center. SOD family of metalloenzymes requires transition metal cofactors (Cu, Zn, and Mn) for their biological activity. The loss of those cofactors results in holoenzyme complete inactivation that it may derive multiple diseases in humans as diminished enzymatic SOD activity provokes and amplifies oxidative stress in vivo.

1.6.5. MALONDIALDEHYDE (MDA)

MDA is an end-product generated by decomposition of arachidonic acid and larger PUFAs, through enzymatic or nonenzymatic processes (Figure 10)

Malondialdehyde (MDA) found in the body comes from two sources: food consumed and lipid peroxidation occurring in the tissues. The formation of MDA and the scale and rate of lipid oxidation in the tissues of living organisms is influenced by a number of endo- and exogeneous factors.

The products of lipid peroxidation, in particular MDA, exhibit cytotoxic, mutagenic and carcinogenic properties. They can also inhibit enzymes associated with defending cells against oxidative stress. Not only do the occurring processes contribute to the development of many diseases, but they are also a part of the aging process. The body defends itself to some extent against the effects of free radicals by trapping and neutralizing them.

Lipid peroxidation (LPO) is the primary outcome of the ROS-induced tissue damage. LPO begins with the reaction of ROS with unsaturated fatty acids or lipoproteins of the membrane, altering the structural integrity and function of the cell membrane (Wei et al., 2010). Malondialdehyde (MDA) is one of the main and best products to evaluate unsaturated fatty acid peroxidation reactions (Dame et al., 2015).

MDA is increased during the inflammatory processes and can damage the structure and function of cell membranes in the body if the body's reduction system does not neutralize it. It is produced by ROS and is used as a biomarker of oxidative stress (Khademi et al., 2014).

MDA formation and metabolism. MDA can be generated *in vivo* by decomposition of arachidonic acid (AA) and larger PUFAs as a side product by enzymatic processes during the biosynthesis of thromboxane A_2 (TXA_2) and 12-l-hydroxy-5,8,10-heptadecatrienoic acid (HHT) (blue pathway), or through nonenzymatic processes by bicyclic endoperoxides produced during lipid peroxidation (red pathway). One formed MDA can be enzymatically metabolized (green pathway). Key enzymes involved in the formation and metabolism of MDA: cyclooxygenases (1), prostacyclin hydroperoxidase (2), thromboxane synthase (3), aldehyde dehydrogenase (4), decarboxylase (5), acetyl CoA synthase (6), and tricarboxylic acid cycle (7).

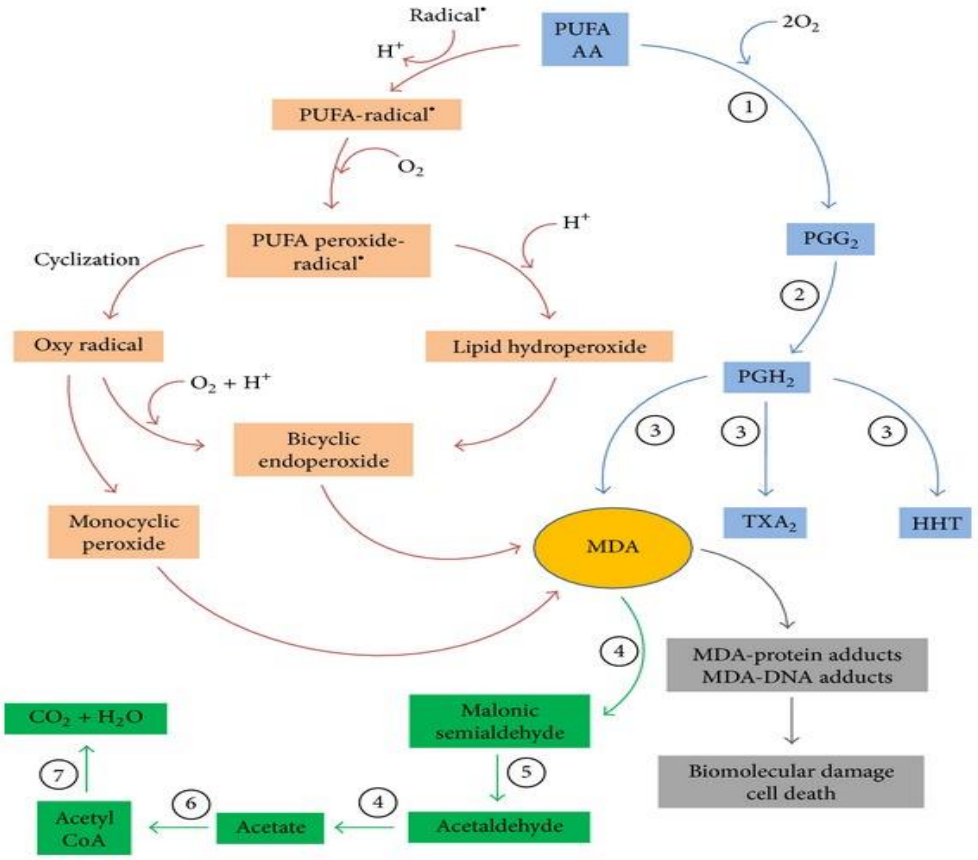


Fig. 10. Generation of Malondialdehyde (MDA) (Wei et al., 2010).

2.1. EXPERIMENTAL DESIGN

In this study 85 samples (were included with age range (27-63) years. Two groups of samples were included in this study. Group (1) contained people with hypothyroidism (n=48) (28 males and 17 females), they were compared to an apparently healthy individual Group (2) (n=35) (25 males and 15 females) control who matched in age and gender. Patients were clinically diagnosed with hypothyroidism. The samples were collected from Razen Medical center in Erbil City. Patients were assessed by full medical history in order to exclude any existing systemic disease.

2.2. COLLECTION OF BLOOD SAMPLES

About 5mL of venous blood was taken from each individual, collected in gold-top serum separator tubes (SST), allowed standing for 15 minutes, separation of serum from blood cell was performed by centrifugation at (3000 rpm) for 15 minutes. The obtained serum was transferred immediately to pre-labeled and coded Eppendorf tubes. These samples were frozen at –20°C for upcoming investigation.

2.3. DETERMINATION OF THYROID PROFILE (T_3, T_4 AND TSH)

Thyroid profile (T3, T4 and TSH) levels were estimated soon after collection of blood sample by cobas E411.

2.4. DETERMINATION OF LIPID PROFILE (TC, TG, LDL, and HDL)

Lipid profile **(**TCHOL, TG, LDL, and HDL**)** levels were estimated soon after collection of blood sample by cobas E411.

2.5. DETERMINATION OF HUMAN MALONDIALDEHYDE) (MDA) CONCENTRATION

The concentration of total **MDA** (Malondialdehyde)) in serum samples was determined by sandwich enzyme- linked immunosorbent assay (ELISA) technique using the kit manufactured by Bio Vision company.

2.5.1. PRINCIPLE OF THE ASSAY

This ELISA kit uses Competitive-ELISA as the method. The microtiter plate provided in this kit has been pre-coated with the antigen. During the reaction, the antigen in the sample or standard competes with a fixed amount of the antigen on the solid phase supporter for sites on the Biotinylated Detection Ab specific to the antigen. Excess conjugate and unbound sample or standard are washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically

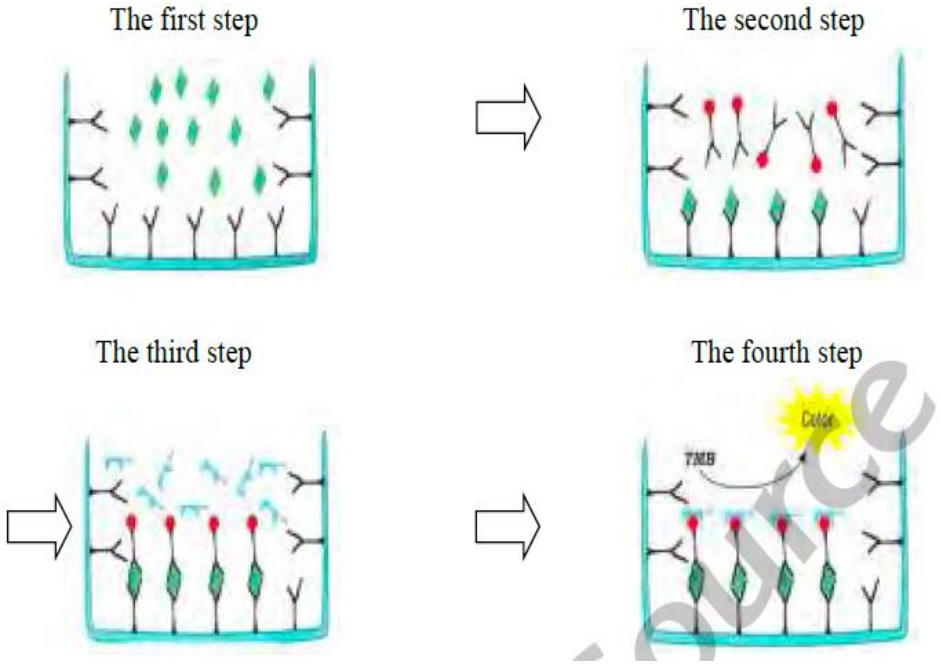


Fig. 11. Principle of The Assay

at a wavelength of 450 nm ± 2 nm. The concentration of the antigen in the samples is then determined by comparing the O.D. of the samples to the standard curve.

2.5.2. ASSAY PROCEDURE

1. Add Sample and Biotinylated Detection Ab: Add 50µl of Standard, Blank, or Sample per well. The blank well is added with sample diluent. Immediately add 50 µl of Biotinylated Detection Ab working solution to each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37°C. (Solutions are added to the bottom of micro-ELISA plate well, avoid inside wall touching and foaming as possible.)

2. Wash: Aspirate each well and wash, repeating the process three times Wash by filling each well with Wash Buffer (approximately 350µl) using a squirt bottle, multi-channel pipette, manifold dispenser or automated washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and pat it against thick clean absorbent paper.

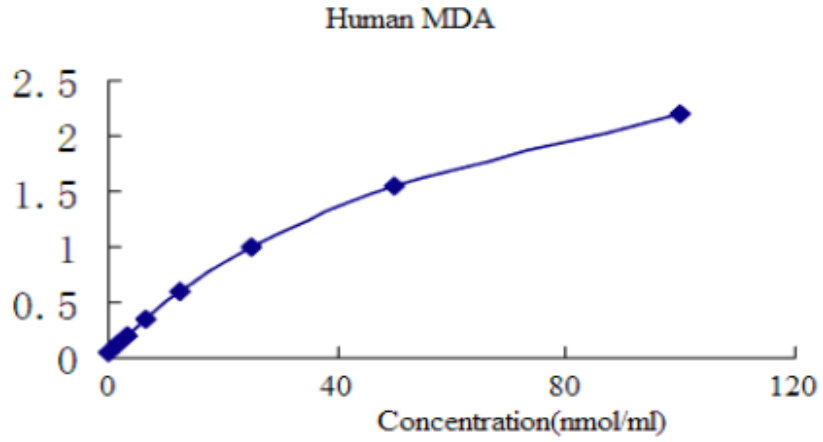
3. HRP Conjugate: Add 100µl of HRP Conjugate working solution to each well. Cover with a new Plate sealer. Incubate for 30 minutes at 37°C.

4. Wash: Repeat the aspiration/wash process for five times as conducted in step 4.

5. Substrate: Add 90µl of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for about 15 minutes at 37°C. Protect from light. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. When apparent gradient appeared in standard wells, you can terminate the reaction.

6. Stop: Add 50µl of Stop Solution to each well. Color turn to yellow immediately. The adding order of stop solution should be as the same as the substrate solution.

7. OD Measurement: Determine the optical density (OD value) of each well at once, using a microplate reader set to 450 nm. You should open the microplate reader ahead, preheat the instrument, and set the testing parameters. 8. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry. Average the duplicate readings for each standard and samples. Create a standard curve by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. It is recommended to use some professional software to do this calculation, such as curve expert 1.3 or 1.4. In the software interface, a best fitting equation of standard curve will be calculated using OD values and concentrations of standard sample. The software will calculate the concentration of samples after entering the OD value of samples.



2.6. DETERMINATION OF HUMAN SUPEROXIDASE DISMUTASE (SOD) CONCENTRATION

The concentration of total SOD in serum samples was determined by sandwich enzyme-linked immunosorbent assay (ELISA) technique using the kit manufactured by Bio Vision company.

2.6.1. PRINCIPLE OF THE ASSAY

Human Superoxide dismutase (SOD) ELISA Kit employs a two-site sandwich ELISA to quantitate SOD in samples. An antibody specific for Human

SOD has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SOD present is bound by the immobilized antibody. After removing any unbound substances, HRP-Conjugated SOD detection antibody is added to the wells. Following a wash to remove any unbound HRP reagent, a Chromogen solution is added to the wells and color develops in proportion to the amount of SOD bound in the initial step. The color development is stopped and the intensity of the color is measured.

2.6.2. ASSAY PROCEDURE

1. Prepare all reagents before starting assay procedure. It is reco mmended that all Standards and Samples be added in duplicate to the microplate.

2. Add standard: Set Standard wells, testing sample wells. Add diluted standard 50 μL to standard well.

3. Add Sample: Add sample diluent 40 μL to testing sample well. Then add sample 10 μL to testing sample well, Blank well doesn't add anything.

4. Cover with a plate cover and incubate for 45 minutes at 37 °C.

5.Aspirate each well and wash, repeating the process four times for a total of five washes, 1-3 minutes per time. Wash by filling each well with Wash buffer (250 μL) using a squirt bottle, manifold dispenser or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

6. Add HRP-Conjugated detection antibody 50 μL to each well, except blank well.

7. Cover with plate cover. Incubate for 30 minutes at 37 °C.

8. Repeat the aspiration/wash process for five times as in step 5.

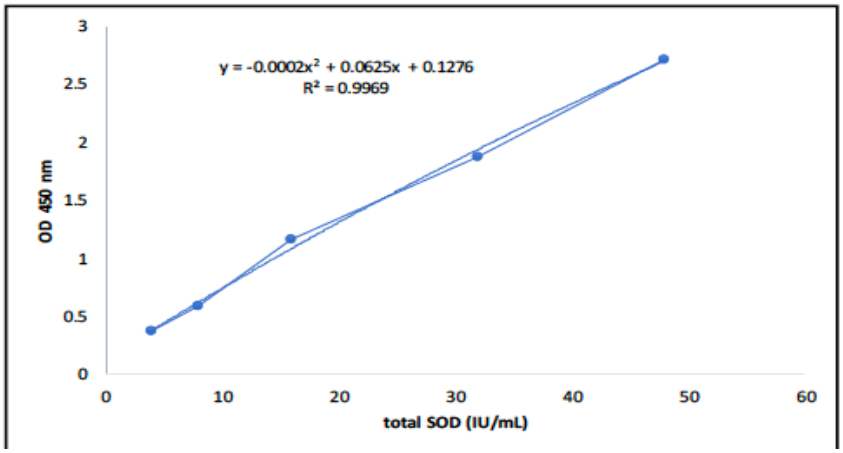
9. Add chromogen solution A 50 µL and chromogen solution B 50 µL to each well. Gently mix and incubate for 15 minutes at 37°C. Protect from light.

10. Add 50 µL Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

2.7. CALCULATION OF RESULTS

Known concentrations of SOD standards and their corresponding OD readings were plotted on the scale (x-axis) and scale (y-axis) respectively. GraphPad Prism and MS Excel computer software were used for data plotting and finding the best curve fitting method. The concentration of human total SODs in samples were determined by plotting their OD values on the Y-axis of the calibration curve. The original concentration was calculated by multiplying it with the dilution factor.



2.7.1. PRINCIPLE OF THE ASSAY

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound

conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

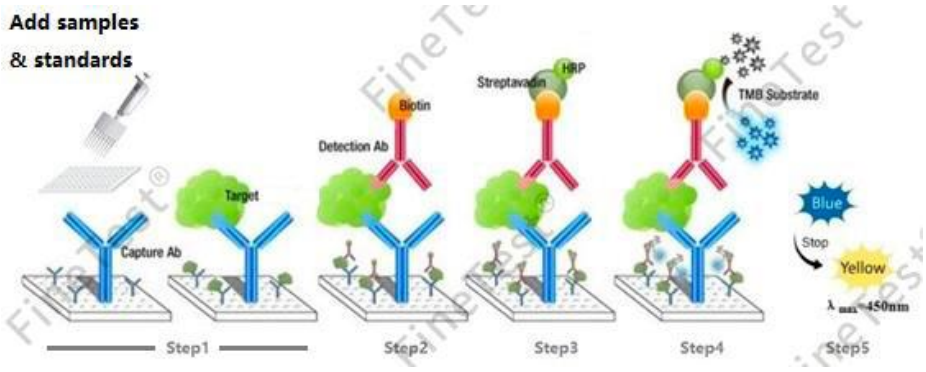


Fig. 12. Principle of the assay

2.7.2. ASSAY PROCEDURE

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, **test samples (diluted at least 1/2 with Sample Dilution Buffer)**, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.

2. Prepare Standards: Aliquot 100ul of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.

3. Add Samples: Add 100ul of properly diluted sample into test sample wells.

4. Incubate: Seal the plate with a cover and incubate at 37°C for 90 minutes.

5. Wash: Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.

6. Biotin-labeled Antibody: Add 100ul Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.

7. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.

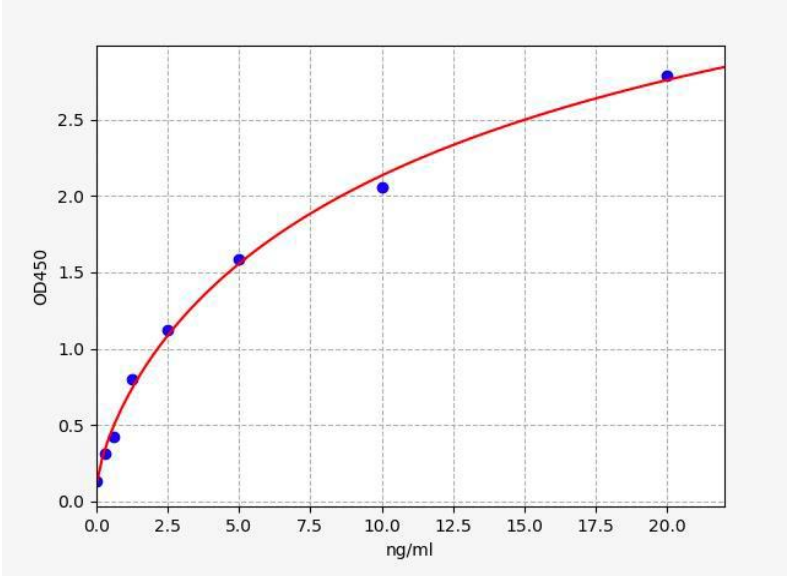
8. HRP-Streptavidin Conjugate (SABC): Add 100ul of SABC Working Solution into each well, cover the plate and incubate at 37°C for 30 minutes.

9. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.

10. TMB Substrate: Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)

11. Stop: Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

12. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.



Step1: Add 100ul standard or sample to each well and incubate for 90 minutes at 37°C.
Wash step: Aspirate and wash plates 2 times.
Step2: Add 100ul Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37°C.
Wash step: Aspirate and wash plates 3 times.
Step3: Add 100ul SABC Working Solution into each well and incubate for 30 minutes at 37°C.
Wash step: Aspirate and wash plates 5 times.
Step4: Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

3.STATISTICAL ANALYSIS

All data are expressed as means ± standard error of means (M ± SD), and statistical analysis was carried out using Graph pad prism 9. The studied parameter means were compared among the patient and control groups using a parametric independent t-test. P value of $\leq$ 0.05 considered statistically significant.

3.1 SERUM LEVELS OF MDA

Mean serum MDA levels in the case and control groups were (4.01 ± 1.13nmol/mL) and (1.02 ± 0.37 nmol/mL) respectively. The results showed significantly higher levels of serum MDA in the hypothyroidism group compared to the healthy control group. Mean and standard deviation of each data are presented in (Table 3) and (Figure13).

Table (3): The Mean Serum Levels of MDA in Patients and Control

parameter	patient Mean±SD	Control Mean±SD	P-Value
MDA (nmol/mL)	4.01 ± 1.13	1.02 ± 0.37	0.012

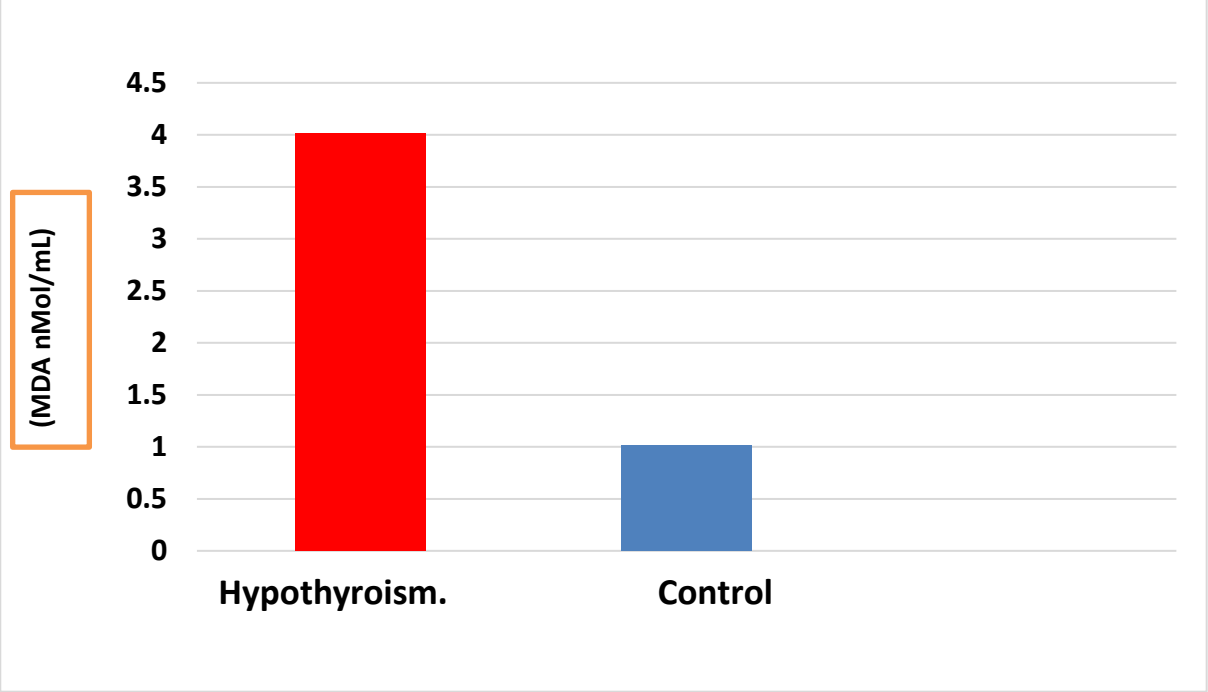


Fig. 13. Comparison of serum Malondialdehyde level between control and hypothyroidism patient.

Malondialdehyde (MDA) is a product of lipid peroxidation by ROS. The marker can be used to evaluate oxidative damage and measure whole-body or tissue-specific oxidative stress (Erdamar et al., 2010).

Plasma MDA is an important biomarker of oxidative damage to lipids. We observed a marked increase in MDA in SCH compared with euthyroid controls which indicates increased oxidative stress.

This increase in MDA was not associated with thyroid hormones but was related to elevated plasma lipids.

Thus, lipid oxidation in SCH may not have been directly caused by low thyroid function, but was enhanced by the presence of elevated plasma cholesterol and LDL secondary to hypothyroidism. This was also observed by (Santi et al., 2012). Prolonged availability of oxidation substrates (mainly LDL) in the plasma increases their susceptibility to oxidative modification (Pearce, 2012).

Oxidized LDL impairs endothelial function leading to atherosclerosis, the first major cardiovascular disease event.

It is plausible that thyroid hormones impacted first on lipids causing hyperlipidemia, and excess lipids acted as substrates for T3. Consequently, accelerated consumption of oxygen by T3 occurred resulting in enhanced generation of ROS, higher consumption of cellular antioxidants and inactivation of antioxidant enzymes (Venditti and Meo, 2006).

On the other hand, many researchers (Giulivi et al., 2003) studied lipid peroxidation in hypothyroidism patients. They observed that the MDA concentrations were significantly increased in hypothyroidism patients that suggest an oxidative damage.

3.2. Serum levels of GSH

(Figure 14) & (Table 4) shows the results of Glutathione levels in (serum a) samples of control and hypothyroidism patients. The results reflect a significant decrease ($P<0.0001$) in the serum level of GSH of hypothyroidism group compared to the healthy control group. GSH is endogenously synthesized in the liver and is the first line of defence against prooxidant stress. This antioxidant molecule is one of the main parts of the cellular endogenous antioxidant systems.

Table (4): The Mean Serum Levels of Antioxidants in Patients and Control

parameter Antioxidants	**patient Mean±SD**	**Control Mean±SD**	**P-Value**
SOD(IU/mL)	87.21±21.99	190.31±24.35	P<0. 001
GSH (ng/mL)	6.04 ±1.75	12.05 ±2.97	P<0.0001

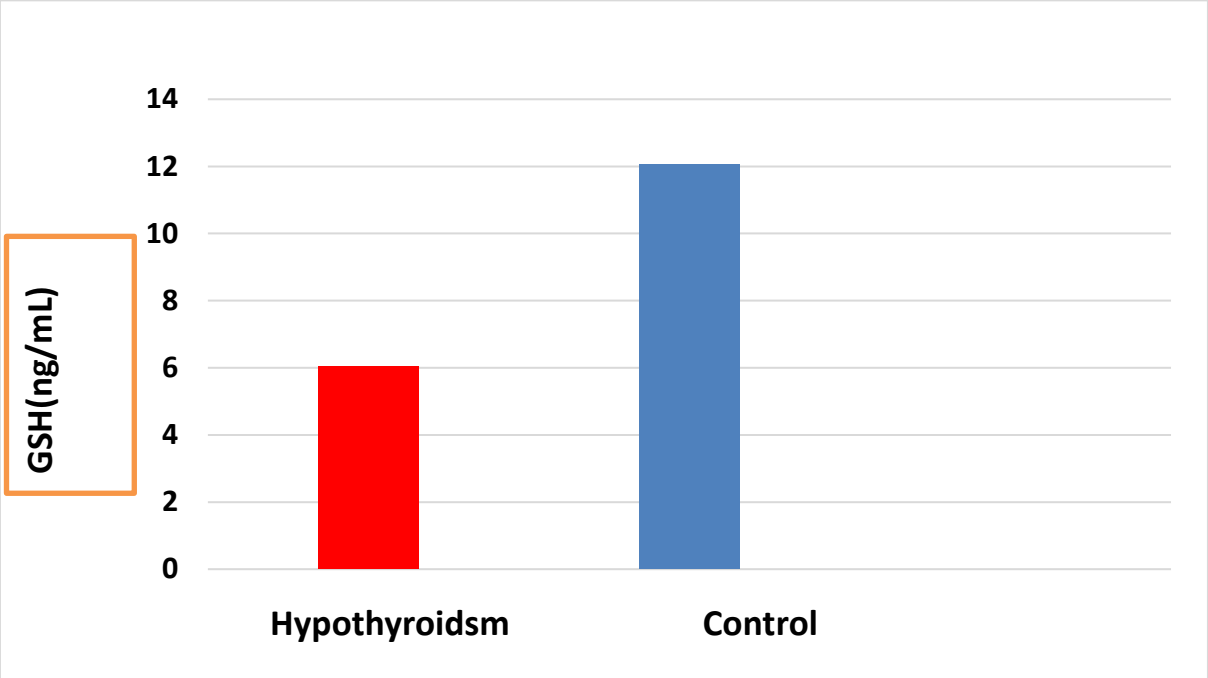


Fig. 14. Comparison of serum Glutathione level between control and hypothyroidism patient

The GSH-dependent defence system plays an important role against lipid peroxidation in cells. Insufficiency of GSH is one of the primary factors that permits lipid peroxidation. It has been reported that GSH plays and important role in the detoxification of hydroperoxides and prevents the effect of lipid peroxidation. Thus, the decreased production of GSH could be due to the overproduction of free radicals and increased lipid peroxidation in hypothyroidism. Thus, it is likely that cells are damaged by prolonged oxidative stress that far exceeds the capacity of the organs to synthesize antioxidant molecules (Komosinska-Vassev et al., 2000).

3.3. Serum Levels of Total Superoxide Dismutase (SOD)

The mean value of sera total SOD activity in control and hypothyroidism patients' groups are presented in (Table 4) and (Figure 15). These results show the presence of significant decrease (P<0. 001) in both hypothyroidism group in comparison to that of the control group.

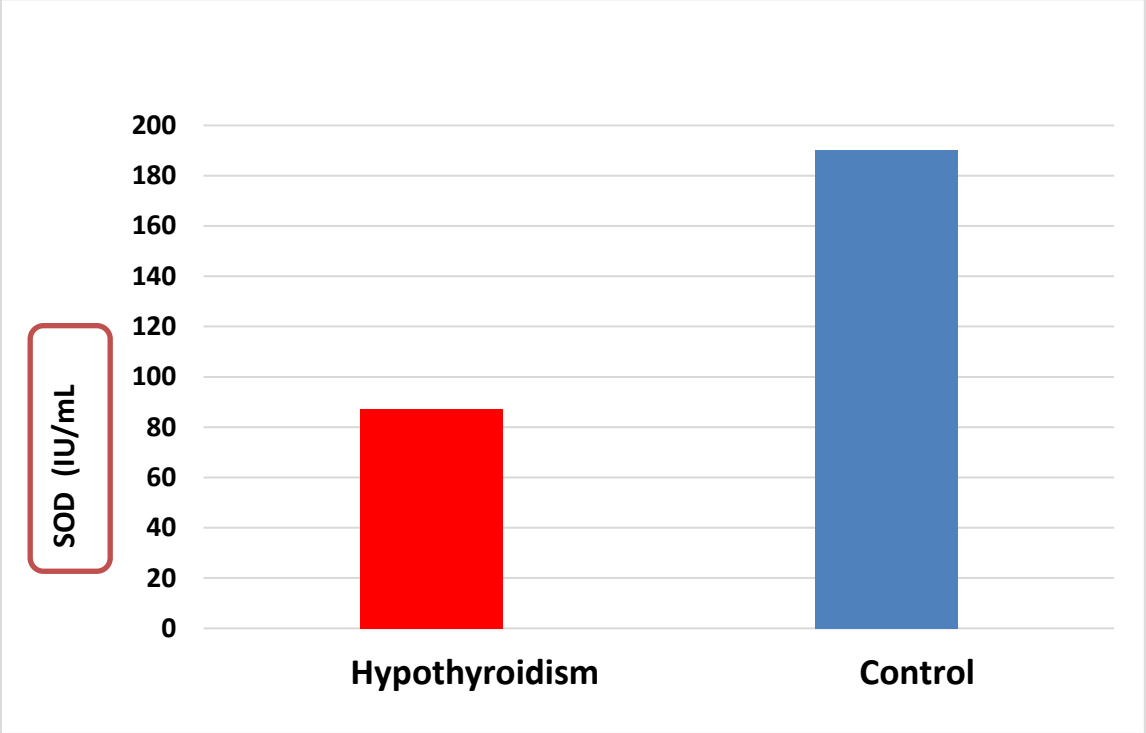


Fig. 15. Comparison of serum Superoxide Dismutase level between control and hypothyroidism patient.

Similar findings of decreased SOD activity were reported by (Reddy et al., 2013). The present finding is inconsistent with (Naazeri et al., 2014) who observed an elevation of SOD activity in hypothyroidism. SOD is the first line of enzymatic defence against intracellular free radicals. It is reported that consequent accumulation of hydrogen peroxide causes inactivation of SOD activity. Decreased SOD activity would expose the cell membrane and other components to oxidative damage.

3.4 SERUM LEVELS OF LIPID PROFILE

The mean value of sera lipid profile in control and hypothyroidism patient's groups are presented in Table (5). These results show the presence of significant increase in TC, LDL cholesterol and TG concentrations and non-significant decrease of HDL in both hypothyroidism group in comparison to that of the control group.

Table (5): The Mean Serum Levels of lipid profile in Patients and Control

parameter lipid profile	**patient Mean±SD**	**Control Mean±SD**	**P-Value**
TC (mg/dl)	241.96±32.24	164.27±18.43	<0.001
LDL (mg/dl)	157.71±20.60	94.87±19.93	<0.001
TG (mg/dl)	157.18±39.22	96.80±32.07	0.0075
HDL (mg/dl)	42.5±12.25	51.01±13.03	0. 146

Our results indicate that hypothyroidism is associated with lipid peroxidation and induction of the antioxidant system. In addition, higher TC, LDL cholesterol and TG concentrations were observed in hypothyroidism patients. These findings are in agreement with results from other recent investigations which showed hyperlipidemia in patients with hypothyroidism (Lee et al., 2004).

Thyroid hormones upregulate LDL receptor expression. Thus, the low concentrations of T3 and fT4, found in hypothyroidism promote a reduction in the catabolism of lipoproteins leading to hypercholesterolemia (Mayer Jr et al., 2006). High serum lipids are associated with lipid peroxidation as well as with oxidative stress damage in hypothyroidism because hypercholesterolemia provides a pool of substrates to be oxidized by free radicals (Nanda et al., 2008).

In the study, thyroid hormones affected lipids resulting in hypercholesterolemia, hypertriglyceridemia and reduced HDL levels in SCH. Hypercholesterolemia is due to decreased fractional clearance of cholesterol from the plasma and the

reduced uptake by cells as a result of reduced number of LDL receptors (Shin and Osborne, 2003).

LDL receptors contain the SREBP-2 gene which is regulated by T3. Hence, LDL receptors could decrease when the SREBP-2 gene expression reduces. Likewise, hypertriglyceridemia in both the SCH and euthyroid group is due to decreased activity of lipoprotein lipase, also stimulated by T3 (Pearce, 2012).

Lipoprotein lipase lowers TG levels through hydrolysis of TG-rich lipoproteins, and augments transfer of cholesterol from these lipoproteins to HDL cholesterol. The reduction in HDL cholesterol is attributed to increased hepatic lipase which hydrolyses phospholipid-rich HDL2 to HDL3 and enhanced cellular uptake.

3.5 Serum levels of Thyroid profile

The mean value of sera thyroid profile in control and hypothyroidism patients groups are presented in Table (6). These results show the presence of significant increase in TSH concentrations and significant decrease of (T3&T4) in both hypothyroidism group in comparison to that of the control group

Table (6): The Mean Serum Levels of thyroid profile in Patients and Control

parameter thyroid **profile**	**patient Mean±SD**	**Control Mean±SD**	**P-Value**
TSH (μIU/ml)	32.83±36.24	2.52±0.895	<0.001
T3 (pg/L)	157.71±20.60	94.87±19.93	<0.001
T4 (pg/L)	157.18±39.22	96.80±32.07	0.0075

As thyroid failure progresses, serum free T4 levels fall, and the combination of elevated TSH and low free T4 concentrations is termed as overt hypothyroidism. Serum total and free T3 levels may not fall until disease is far advanced, because increased TSH levels stimulate T3 release from the thyroid.

4.1. CONCLUSION

The study demonstrated that oxidative stress was increased in hypothyroidism and its association with thyroid hormones as indicated by the elevated lipid peroxidation product, malondialdehyde, our present study suggests that hypothyroid patients accompanied with reduced tT3and tT4 levels showed a very high production of ROS and oxidative stress, characterized by enhanced lipid peroxidation activity (MDA) and concomitant decline in antioxidant defense mechanisms (SOD and GSH).

However, this study indicates a strong influence of TC on development of oxidative stress in hypothyroidism, and the impact of thyroid hormones on biomarkers of oxidative stress.

4.2. RECOMMENDATION

We suggest that monitoring of lipids and thyroid hormones should be considered in the management of hypothyroidism, and lipid lowering drugs may be useful in the reduction of oxidative stress in hypothyroidism. However, further larger clinical studies are required to better understand these associations, as well as the role of oxidative stress on the physiopathology of hypothyroidism.

REFERENCES

ABDALLA, S. M. & BIANCO, A. C. 2014. Defending plasma T3 is a biological priority. *Clinical endocrinology,* 81**,** 633-641.

AGUILAR, T. A. F., NAVARRO, B. C. H. & PÉREZ, J. A. M. 2016. Endogenous antioxidants: a review of their role in oxidative stress. *A master regulator of oxidative stress-the transcription factor nrf2***,** 3-20.

AHMED, H. T. 2019. Biochemical study of Thyroid Hormones in Pregnant Women in Tikrit City. *The Medical Journal of Tikrit University,* 25.

ATES, I., ARIKAN, M. F., ALTAY, M., YILMAZ, F. M., YILMAZ, N., BERKER, D. & GULER, S. 2018. The effect of oxidative stress on the progression of Hashimoto's thyroiditis. *Archives of Physiology and Biochemistry,* 124**,** 351-356.

BENVENGA, S., NORDIO, M., LAGANÀ, A. S. & UNFER, V. 2021. The role of inositol in thyroid physiology and in subclinical hypothyroidism management. *Frontiers in endocrinology,* 12**,** 662582.

BIANCO, A. C., SALVATORE, D., GEREBEN, B., BERRY, M. J. & LARSEN, P. R. 2002. Biochemistry, cellular and molecular biology, and physiological roles of the iodothyronine selenodeiodinases. *Endocrine reviews,* 23**,** 38-89.

DAME, Z. T., AZIAT, F., MANDAL, R., KRISHNAMURTHY, R., BOUATRA, S., BORZOUIE, S., GUO, A. C., SAJED, T., DENG, L. & LIN, H. 2015. The human saliva metabolome. *Metabolomics,* 11**,** 1864-1883.

DARDANO, A., GHIADONI, L., PLANTINGA, Y., CARACCIO, N., BEMI, A., DURANTI, E., TADDEI, S., FERRANNINI, E., SALVETTI, A. & MONZANI, F. 2006. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. *The Journal of Clinical Endocrinology & Metabolism,* 91**,** 4175-4178.

DIEKMAN, T., DEMACKER, P. N., KASTELEIN, J. J., STALENHOEF, A. F. & WIERSINGA, W. M. 1998. Increased oxidizability of low-density lipoproteins in hypothyroidism. *The Journal of Clinical Endocrinology & Metabolism,* 83**,** 1752-1755.

EKHOLM, R. & BJORKMAN, U. 1997. Glutathione peroxidase degrades intracellular hydrogen peroxide and thereby inhibits intracellular protein iodination in thyroid epithelium. *Endocrinology,* 138**,** 2871-2878.

EL HASSANI, R. A., BUFFET, C., LEBOULLEUX, S. & DUPUY, C. 2019. Oxidative stress in thyroid carcinomas: biological and clinical significance. *Endocrine-related cancer,* 26**,** R131-R143.

ELEUTHERIO, E. C. A., MAGALHÃES, R. S. S., DE ARAÚJO BRASIL, A., NETO, J. R. M. & DE HOLANDA PARANHOS, L. 2021. SOD1, more than just an antioxidant. *Archives of Biochemistry and Biophysics,* 697**,** 108701.

ERDAMAR, H., ÇIMEN, B., GÜLCEMAL, H., SARAYMEN, R., YERER, B. & DEMIRCI, H. 2010. Increased lipid peroxidation and impaired enzymatic antioxidant defense mechanism in thyroid tissue with multinodular goiter and papillary carcinoma. *Clinical biochemistry,* 43**,** 650-654.

FAHIM, Y. A., SHARAF, N. E., HASANI, I. W., RAGAB, E. A. & ABDELHAKIM, H. K. 2020. Assessment of thyroid function and oxidative stress state in foundry workers exposed to lead. *Journal of Health and Pollution,* 10.

FERNÁNDEZ-MEJÍA, C. 2013. Oxidative stress in diabetes mellitus and the role of vitamins with antioxidant actions. *Oxidative stress and chronic degenerative diseases-a role for antioxidants,* 209.

GIULIVI, C., TRAASETH, N. & DAVIES, K. 2003. Tyrosine oxidation products: analysis and biological relevance. *Amino acids,* 25**,** 227-232.

JANSEN, J., FRIESEMA, E. C., MILICI, C. & VISSER, T. J. 2005. Thyroid hormone transporters in health and disease. *Thyroid,* 15**,** 757-768.

KESTER, M. H., MARTINEZ DE MENA, R., OBREGON, M. J., MARINKOVIC, D., HOWATSON, A., VISSER, T. J., HUME, R. & MORREALE DE ESCOBAR, G. 2004. Iodothyronine levels in the human developing brain: major regulatory roles of iodothyronine deiodinases in different areas. *The Journal of Clinical Endocrinology & Metabolism,* 89**,** 3117-3128.

KHADEMI, H., KHOZEIMEH, F., TAVANGAR, A., AMINI, S. & GHALAYANI, P. 2014. The Serum and salivary level of malondialdehyde,

vitamins A, E, and C in patient with recurrent aphthous stomatitis. *Advanced Biomedical Research,* 3.

KHANDELWAL, D. & TANDON, N. 2012. Overt and subclinical hypothyroidism: who to treat and how. *Drugs,* 72**,** 17-33.

KIM, Y.-M., KIM, S.-J., TATSUNAMI, R., YAMAMURA, H., FUKAI, T. & USHIO-FUKAI, M. 2017. ROS-induced ROS release orchestrated by Nox4, Nox2, and mitochondria in VEGF signaling and angiogenesis. *American Journal of Physiology-Cell Physiology,* 312**,** C749-C764.

KOMOSINSKA-VASSEV, K., OLCZYK, K., KUCHARZ, E. J., MARCISZ, C., WINSZ-SZCZOTKA, K. & KOTULSKA, A. 2000. Free radical activity and antioxidant defense mechanisms in patients with hyperthyroidism due to Graves' disease during therapy. *Clinica chimica acta,* 300**,** 107-117.

LEE, W.-Y., SUH, J.-Y., RHEE, E.-J., PARK, J.-S., SUNG, K.-C. & KIM, S.-W. 2004. Plasma CRP, apolipoprotein A-1, apolipoprotein B and Lp (a) levels according to thyroid function status. *Archives of medical research,* 35**,** 540-545.

LUSHCHAK, V. I. 2012. Glutathione homeostasis and functions: potential targets for medical interventions. *Journal of amino acids,* 2012.

MAYER JR, O., ŠIMON, J., FILIPOVSKÝ, J., PLÁŠKOVÁ, M. & PIKNER, R. 2006. Hypothyroidism in coronary heart disease and its relation to selected risk factors. *Vascular health and risk management,* 2**,** 499-506.

MCMILLEN, B., DHILLON, M. S. & YONG-YOW, S. 2016. A rare case of thyroid storm. *Case Reports,* 2016**,** bcr2016214603.

METERE, A., FREZZOTTI, F., GRAVES, C. E., VERGINE, M., DE LUCA, A., PIETRAFORTE, D. & GIACOMELLI, L. 2018. A possible role for selenoprotein glutathione peroxidase (GPx1) and thioredoxin reductases (TrxR1) in thyroid cancer: Our experience in thyroid surgery. *Cancer cell international,* 18**,** 1-8.

NAAZERI, S., ROSTAMIAN, M. & HEDAYATI, M. 2014. Impact of thyroid dysfunction on antioxidant capacity, superoxide dismutase and catalase activity.

NANDA, N., BOBBY, Z. & HAMIDE, A. 2008. Oxidative stress and protein glycation in primary hypothyroidism. Male/female difference. *Clinical and experimental medicine,* 8**,** 101-108.

OLINESCU, R., RĂDĂCEANU, V., NIŢĂ, S. & LUPEANU, E. 1992. Age-dependent variations of the plasma peroxides and total antioxidants in women with obesity and hypothyroidism. *Romanian Journal of Internal Medicine= Revue Roumaine de Medecine Interne,* 30**,** 285-290.

PEARCE, E. N. 2012. Update in lipid alterations in subclinical hypothyroidism. *The Journal of Clinical Endocrinology & Metabolism,* 97**,** 326-333.

PERSANI, L. 2012. Central hypothyroidism: pathogenic, diagnostic, and therapeutic challenges. *The Journal of Clinical Endocrinology & Metabolism,* 97**,** 3068-3078.

REDDY, V., GOUROJU, S., SUCHITRA, M., SURESH, V., SACHAN, A., RAO, P. S. & BITLA, A. 2013. Antioxidant defense in overt and subclinical hypothyroidism. *Hormone and Metabolic Research,* 45**,** 754-758.

RESCH, U., HELSEL, G., TATZBER, F. & SINZINGER, H. 2002. Antioxidant status in thyroid dysfunction.

RUGGERI, R. M., GIOVINAZZO, S., BARBALACE, M. C., CRISTANI, M., ALIBRANDI, A., VICCHIO, T. M., GIUFFRIDA, G., AGUENNOUZ, M. H., MALAGUTI, M. & ANGELONI, C. 2021. Influence of dietary habits on oxidative stress markers in Hashimoto's thyroiditis. *Thyroid,* 31**,** 96-105.

SANTI, A., DUARTE, M. M., DE MENEZES, C. C. & LORO, V. L. 2012. Association of lipids with oxidative stress biomarkers in subclinical hypothyroidism. *International Journal of Endocrinology,* 2012.

SARAF-BANK, S., AHMADI, A., PAKNAHAD, Z., MARACY, M. & NOURIAN, M. 2019. Effects of curcumin supplementation on markers of inflammation and oxidative stress among healthy overweight and obese girl adolescents: A randomized placebo-controlled clinical trial. *Phytotherapy Research,* 33**,** 2015-2022.

SARKAR, S. D., AFRIYIE, M. O. & PALESTRO, C. J. 2001. Recombinant human thyroid-stimulating hormone–aided scintigraphy: comparison of imaging at multiple times after I-131 administration. *Clinical nuclear medicine,* 26**,** 392-395.

SHIN, D.-J. & OSBORNE, T. F. 2003. Thyroid hormone regulation and cholesterol metabolism are connected through sterol regulatory element-binding protein-2 (SREBP-2). *Journal of Biological Chemistry,* 278**,** 34114-34118.

VENDITTI, P. & MEO, S. D. 2006. Thyroid hormone-induced oxidative stress. *Cellular and Molecular Life Sciences CMLS,* 63**,** 414-434.

VERHAEGHE, E. F., FRAYSSE, A., GUERQUIN-KERN, J.-L., WU, T.-D., DEVÈS, G., MIOSKOWSKI, C., LEBLANC, C., ORTEGA, R., AMBROISE, Y. & POTIN, P. 2008. Microchemical imaging of iodine distribution in the brown alga Laminaria digitata suggests a new mechanism for its accumulation. *JBIC Journal of Biological Inorganic Chemistry,* 13**,** 257-269.

WEI, D., ZHANG, X. L., WANG, Y. Z., YANG, C. X. & CHEN, G. 2010. Lipid peroxidation levels, total oxidant status and superoxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. *Australian Dental Journal,* 55**,** 70-78.

YEN, P. M. 2001. Physiological and molecular basis of thyroid hormone action. *Physiological reviews,* 81**,** 1097-1142.

YOUNUS, H. 2018. Therapeutic potentials of superoxide dismutase. *International journal of health sciences,* 12**,** 88.

ZIOTOPOULOU, M., MANTZOROS, C. S., HILEMAN, S. M. & FLIER, J. S. 2000. Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice. *American Journal of Physiology-Endocrinology And Metabolism,* 279**,** E838-E845.