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Evaluation of Some Apoptotic Markers and Blood Parameters in Patients with Hypothyroidism

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

إِنَّ اللَّهَ وَمَلَائِكَتَهُ يُصَلُّونَ عَلَى النَّبِيِّ يَا أَيُّهَا الَّذِينَ آمَنُوا صَلُّوا عَلَيْهِ وَسَلِّمُوا تَسْلِيمًا

صدق الله العلي العظيم

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Ezzate

Dedication

To the awaited Imam Al-Mehdi,

The candle of Allah on earth

Peace be upon him

Ezzat

Summary

Hypothyroidism is a common global health condition that can have a significant impact on patients' well-being. There were several indications that apoptosis was implicated in hypothyroidism pathogeneses since it leads to permanent damage to the thyrocytes, therefore, this study focused attention on analyzing soluble apoptotic indicators in hypothyroidism patients. This study comprised 60 hypothyroid patients, 20 of whom were newly diagnosed and 40 of whom had been treated by levothyroxin for different durations. The study was achieved in Al-Sadr medical teaching city in Al- Najaf province from January to July 2020. Also, the study included 30 healthy persons as a control group. Patients and controls were both male and female, with ages ranging from 20 to 70 years. Blood samples were collected from both controls and patients, 3 ml of it were centrifuged to obtain serum for analysis of thyroid profile (TSH, T3 and T4), apoptotic (Fas, FasL, Bcl-2 and Cytochrome c), oxidative stress (TBARS), and antioxidant (SepP1) biomarkers, while the remainder was placed in an anticoagulant tube for measuring complete blood count (CBC).

The study's findings revealed that the majority of patients were females in their forties and fifties, with Hashimoto's thyroiditis and of urban area. Thyroid stimulating hormone (TSH) levels in hypothyroid patients of both groups increased significantly when compared to controls, whereas the levels of thyroid hormones (T3&T4) decreased significantly in hypothyroid patients of without treatment group compared to controls. A significant rise in the levels of apoptotic biomarkers (Fas, Bcl-2, and Cytochrome c) was seen in hypothyroid patients of without treatment group compared to controls, while FasL increased significantly in hypothyroid patients of with treatment group versus to control and patients without treatment groups. The result explained a significant increase in oxidative stress reflected by the levels of TBARS and a significant

decrease in the SepP1 that represent the antioxidant state in patients of without treatment group in comparison to control.

Furthermore, some of the red blood indices (Hb, HCT, MCH and MCHC) were decreased significantly in both patient groups compared to control. On other hand, there was a significant increase in MCV of patients with treatment compared to control. Also, there was a significant decrease in the number of both lymphocytes and monocytes in both patient groups versus to control. With regard to platelets count, plateletcrit (PCT) and platelets distribution width (PDW) were decreased significantly in hypothyroid patients when compared to control. According to this study, females had sex-specific variations in thyroid profile, apoptotic, oxidative stress, and antioxidant biomarkers. There was a significant positive correlation between TBARS & Fas in patients of without treatment group while the correlation between FasL & hemoglobin and TBARS& red blood cells count were significant negative correlation in patients of with treatment group. The area under curve (AUC) of Fas apoptotic biomarker was (0.864) reflecting the possibility of using it in the determination of the prognosis of the disease while the other studied biomarkers had a small value of AUC. Finally, when compared to the other biomarkers investigated, the Fas biomarker exhibited a significant relative risk of (2.9).

In conclusion, patients with hypothyroidism have increased apoptosis in the blood, this is reflects by elevating the levels of apoptotic biomarkers in both groups of patients compared to control. Soluble FAS may be used for predicting the prognosis of the disease.

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Abbreviations

APAF-1	Apoptotic protease activating factor
BAX	BCL2 associated X protein
BAK	BCL2 antagonist killer 1
Bcl-2	B-cell lymphoma protein 2
BFU-E	Burst forming unit erythroid
BH3	Bcl-2 Homology 3
CBC	Complete blood count
CFU-E	Colony-forming-units of erythroid
DAMPs	Damage-associated molecular patterns
DD	Death domain
DED	Death effector domain
DISC	Death-inducing signaling complex
FADD	Fas-associated death domain
Fas	Fatty acid synthetase
FasL	Fatty acid synthetase ligand
GWASs	Human genome-wide association studies
Hb	Hemoglobin
HCT	Hematocrit
HIF-1	hypoxia inducible factor1
HLA	human leukocyte antigen
HPCs	Hematopoietic progenitor cells
HT	Hashimoto's thyroiditis
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean cell volume

MOM	Mitochondrial outer membrane
MOMP	Mitochondrial outer membrane permeabilization
MPV	mean platelets volume
NIS	sodium/iodine symporter
PB	Peripheral blood
PCT	plateletcrit
PDW	platelets distribution width
proNGF	Precursor of Nerve growth factor
RDW	Red cell distribution width
RIP	Receptor-interacting protein
SCH	Subclinical hypothyroidism
SepP1	Selenoprotein P
sFas	Soluble Fatty acid synthetase
SMAC	Second mitochondrial activator of caspases
SMAC/DIABLO	Second mitochondrial activator of caspases/direct
T3	Triiodothyronin
T4	Tetraiodothyronin
TBARS	Thiobarbituric Acid Reactive Substances
TH	thyroid hormones
TNF	Tumor necrosis factor
TRADD	TNF receptor-associated death domain
TRAIL	TNF-related apoptosis-inducing ligand
TRH	Thyroid releasing hormone
TRs	Thyroid receptors
TSH	Thyroid stimulating hormone

CHAPTER ONE

INTRODUCTION

1.1 Introduction

The endocrine system is a highly sophisticated hierarchical system that dictates efficiency as well as dynamic control of different processes in the body such as metabolism, growth, and sexual and emotional development. The thyroid gland is unique among the endocrine organs as it maintains a large store of hormone. It is a brown-red highly, vascular gland located anteriorly in the lower neck extending from the fifth cervical vertebra down to the first thoracic vertebra (Standring, 2016). It weighs 15–20 g in adult and its weighs in a new borne is 1 g that increases by about 1 g/year until age 15 (Braun *et al*., 2007).

The thyroid gland and its hormones play multifaceted roles in organ development and in the homeostatic control of fundamental physiological mechanisms in all vertebrates (Maenhaut *et al*., 2015). Thyroid dysfunction is one of the most common endocrine disorders seen in clinical practice. The prevalence of thyroid dysfunction varies by age, sex, race/ethnicity, and geographically through variations in dietary iodine intake (Ittermann *et al*., 2015) .

Abnormal thyroid function has important ramifications on health outcomes pertinent to older adults, including cardiovascular arrhythmia. Globally, millions of people suffer from thyroid-related problems (Alipourzamani *et al*., 2011). Several previous studies had reported that the most common type of thyroid disease is goiter (Vanderpump, 2005). Hypothyroidism and hyperthyroidism are also the prevalent types of thyroid disease (Vanderpump, 2010).

Hypothyroidism is a highly prevalent global health problem that can substantially affect patients' wellbeing (Taylor *et al*., 2018). Lifelong treatment with thyroid hormone (replacement therapy) is needed when

the diagnosis of persistent thyroid hormone deficiency is confirmed (Biondi and Wartofsky, 2014) . It has been reported that changing in life styles in Arab world has led to the emerging of double burden of diseases, including the thyroid related diseases (Sawka *et al*., 2008).

Apoptosis (or programmed cell death) is an active process of cell self-destruction requiring the activation of a genetic program, leading to changes in morphology, DNA fragmentation, and protein cross-linking (Vitale *et al*., 2000). Physiological cell death is an essential mechanism which contributes for the growth and permanent maintenance of the human body (Obulesu & Lakshmi, 2014). Many evidence suggests that programmed cell death is also involved in the pathogenesis of diseases of the endocrine system such as Hashimoto's thyroiditis (HT), Graves' disease (GD) and diabetes mellitus type 1 which have been considered to be autoimmune in origin(Andrikoula & Tsatsoulis, 2001).

Thyroid hormones (T3&T4) have critical roles in regulating production of red blood cells. This function is regulated by attachment of the active form of thyroid hormone T3 to specific members of the nuclear receptors family (TRα and TRβ). Other effects of thyroid hormones include involvement in hemoglobin production in adult and maturation of Hb in fetus (Saranac *et al*., 2011). Thyroid hormones often have important effect on erythropoiesis. They enhance erythropoiesis through hyper proliferation of immature erythroid progenitors and increase secretion of erythropoietin (EPO) by inducing erythropoietin gene expression (Kawa *et al*., 2010).

With regard to lymphocytes, T3 is a precursor substance for normal B cell formation in bone marrow through its mediation of pro-B cell proliferation. Therefore, thyroid disorders can induce different effects on various blood cell lineages (Drews, 2003).

A slightly depressed total leucocyte count, neutropenia and thrombocytopenia have been observed in hypothyroid patients (Lima *et al*., 2006). There are significantly decreased levels of RBCs in hypothyroid patients. Similarly, low Hb concentrations were recorded which clinically indicates anemia (Miłosz *et al*., 2010).

The previous studies indicated that patients with hypothyroidism due to Hashimoto's thyroiditis suffer from macrocytic anemia that can be related to autoimmune processes in peripheral tissues (Weetman, 2005). The action of THs strongly influences the apoptotic process in haematopoietic progenitor cells (HPCs) by affecting the expressions of pro- and anti-apoptotic genes, such as BCL-2, BCL-xL and BAX, which reflects the very complex nature of this process in humans *in vivo* (Miłosz *et al*., 2010). Incubation with non-physiological concentrations of T3 induced programmed cell death in human cord blood, peripheral blood and bone marrow-derived CD34-enriched Hematopoietic progenitor cells HPCs (Grymuła *et al*., 2007).

Globally, the studies deal with hypothyroidism from cellular and molecular aspects and those that explain the effects of apoptosis in the blood of hypothyroid patients are still very few. Up to our knowledge, there was any published local studies dealing with apoptosis in people with hypothyroidism. This study conducted to focus highlight on the importance of apoptosis in patients with hypothyroidism in Iraq generally and in Al-Najaf province especially.

1.2 Aim of the study

This study is designed to investigate the physiological changes in blood of patients with hypothyroidism due to apoptosis in Iraqi patients. This aim was achieved by the following:

1. Determination thyroid profile of the patients which include (TSH,T3,T4).

2. Evaluation the role of soluble (Fas), (FasL), (Bcl-2) and Cytochrome C as biomarkers of apoptosis in hypothyroidism .

3. Evaluation of TBARS as a biomarker of oxidative stress in hypothyroidism patients.

4. Evaluation of SepP1 as a biomarker of antioxidant in hypothyroidism patients.

5. Investigate the changing in complete blood count (CBC) in patients with hypothyroidism.

6. Study the correlation among studied biomarkers in patients groups.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Hypothyroidism

Hypothyroidism is the most common endocrine disease after diabetes, whose incidence increase with age. This disease is mainly caused by disorders in thyroid gland that lead to decreases in triiodothyronine (T3) and thyroxin (T4) production and secretion resulting in primary hypothyroidism (Guyton&Sepehhri, 2011). The share of hypothyroidism among other endocrine diseases is gradually increasing. It is encountered in females more than in males. The idiopathic form of hypothyroidism occurs mainly in females older than 40 years. Hypothyroidism is usually progressive and irreversible (Roberts&Ladenson, 2004).

Hypothyroidism refers to the common pathological condition of thyroid hormone deficiency. If untreated, it can lead to serious adverse health effects and ultimately death. Because of the large variation in clinical presentation and general absence of symptom specificity, the definition of hypothyroidism is biochemical pre-dominantly (Cooper&Biondi, 2012).

2.1.1 Classification of hypothyroidism.

Hypothyroidism can be classified into three types, depending on the type of thyroid axis dysfunction:

1- Primary

It refers to a type of hypothyroidism when the dysfunction of the regulating system occurs in the thyroid gland in form of a low concentration of thyroid hormones (TH). This is because the thyroid gland presents morphogenetic errors that modify the normal components of the thyroid gland during gestation as TSH receptors (quantity or/and affinity) and the enzymes involved in (TH) synthesis as thyroperoxidase (Budenhofer *et al*., 2013).

Also, there are environmental factors that can cause primary hypothyroidism, such as the sodium/iodine symporter (NIS) inhibitors, perchlorate, thiocyanate, and nitrate, which pollute the surrounding areas (Pearce & Braverman, 2009).The two most common forms of primary hypothyroidism are:

(A) Hashimoto's thyroiditis which is an autoimmune condition.

(B) Overtreatment of hyperthyroidism (an overactive thyroid) (Elizabeth & Agabegi, 2008).

2- Secondary or central

Central hypothyroidism is defined as hypothyroidism due to insufficient stimulation by thyroid stimulating hormone (TSH) of an otherwise normal thyroid gland (Yamada & Mori, 2008). It is roughly equal in both sexes and can arise from a number of pathogenic mechanisms involving: The pituitary (secondary hypothyroidism) or hypothalamus (tertiary hypothyroidism). In children, this is usually cause by craniopharyngiomas or previous cranial irradiation for brain tumors or hematological malignancies (Schmiegelow *et al*., 2003). In adults, it is more commonly due to pituitary macroadenomas, pituitary surgeries or irradiation (Rose, 2001).

3- Tertiary

This type of hypothyroidism is characterized by low serum concentrations of TRH, due to a tumor in the hypothalamus. In addition, TRH-target cell response may be modulated by the rate of degradation of the hormone at specific target sites. This mechanism could effectively control the intensity of stimulation and/or the duration of TRH action (Schomburg& Bauer, 1997).

Hypothyroidism can also be classified according the severity of disease into:

1- Subclinical hypothyroidism (SCH) .

Subclinical hypothyroidism exists when serum thyroid hormone levels are within the reference range, but serum thyrotropin levels are elevated outside the reference range (Cooper&Biondi, 2012). The diagnosis of subclinical hypothyroidism is a biochemical diagnosis solely based on thyroid function testing. In iodine-sufficient populations it affects up to10% of the population, with the highest prevalence among women and elderly individuals (Hollowell *et al*., 2002). However, subclinical hypothyroidism frequently reverts to euthyroidism (Somwaru *et al*., 2012) .

The etiology of subclinical hypothyroidism is extremely broad due to variety of factors that can induce it. One cause may be via chronic lymphocytic thyroiditis, an autoimmune disorder of the thyroid gland (Surks *et al*., 2004). Alternatively, it may occur following a sub-acute, postpartum, painless thyroiditis, or after a partial thyroidectomy (Biondi & Cooper, 2008).

2- Overt hypothyroidism.

Overt hypothyroidism, which is characterized by low levels of free T4 and, due to reduced negative feedback, increased TSH. This form of hypothyroidism is associated with full onset of the symptoms and doctors will treat this condition with levothyroxine. Overt hypothyroidism is often associated with Hashimoto's disease, also known as autoimmune thyroid disease, in which the immune system attacks the thyroid gland (Stagnaro-Green & Pearce, 2012). This often occurs following a bout of thyroiditis, the inflammation of the thyroid gland (Pearce *et al*.,2003).

The modest fraction of patients with subclinical hypothyroidism (approximately 3 to 5% per year, depending on age and magnitude of TSH elevation) have progression to overt hypothyroidism with a decline

in serum free thyroxine concentration (Huber *et al*.,2002). The progression to overt thyroid failure is thought to be a gradual process (Helfand, 2004).

2.1.2 Pathophysiology of Hypothyroidism .

The commonest cause of hypothyroidism in developed countries is autoimmune thyroiditis, which may be associated with a goiter (Hashimoto's thyroiditis, HT) or, with equal frequency, thyroid atrophy (Harris & Pass, 2007) as explain in figure (2-1). Classically, HT is considered to be a T helper1 (Th1) mediated disease, this classification has altered due to the description of new T helper cell subsets including Th17 cells (Nanba *et al*.,2009). In HT, as a consequence, chronic inflammatory cell infiltrates into the thyroid gland, which includes predominantly thyroid-specific B and T cells. In result, goiter may initially be caused (Pearce *et al*.,2003).

Subsequently, hypothyroidism, the characteristic hallmark of thyroiditis, can develop when sufficient numbers of follicular cells responsible for the production and secretion of thyroid hormones thyroxine (T4) and triiodothyronine (T3) are destroyed (Caturegli *et al.*, 2014). Radioiodine ablation or surgical thyroidectomy as treatment for hyperthyroidism or thyroid cancer are also responsible for important numbers of patients with hypothyroidism. (Harris & Pass, 2007).

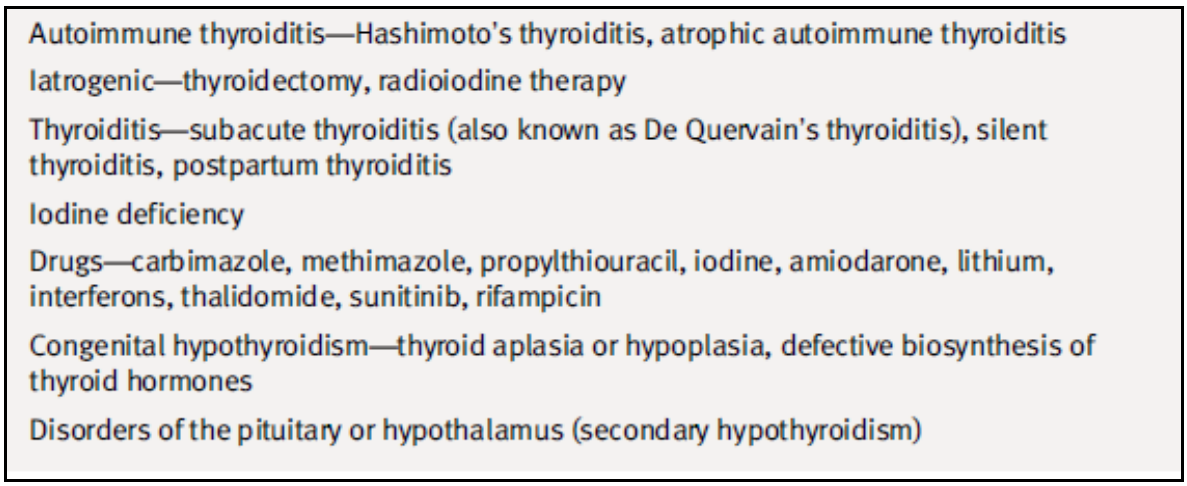

Figure (2-1): The important causes of hypothyroidism (Bijay & Simon, 2008).

2.1.3 Epidemiology of Hypothyroidism.

Hypothyroidism is common throughout the world and is particularly common in the UK. Iodine deficiency and autoimmune disease (known as Hashimoto thyroiditis) account for the vast majority of cases of primary hypothyroidism (Chaker *et al.*, 2017). A third of the world's population lives in iodine-deficient areas (Zimmermann, 2009). In iodine-sufficient countries, the prevalence of hypothyroidism ranges from 1% to 2% (Vanderpump, 2010), rising to 7% in individuals aged between 85 and 89 years (Gussekloo *et al.*, 2004). In the absence of age-specific reference ranges for TSH, an ageing population is likely to result in a higher prevalence of hypothyroidism. Hypothyroidism is approximately ten times more prevalent in women than men (Vanderpump, 2010).

Data on the incidence of hypothyroidism in Middle Eastern countries are limited. One systematic review evaluated 21 studies that addressed thyroid disease prevalence across ten Middle Eastern countries; however, there was wide heterogeneity in the populations studied, and most of the available studies were convenience, all of which include

patients who are at high risk of thyroid dysfunction (Al Shahrani *et al.*, 2016).

2.1.4 Diagnosis criteria

The common clinical features associated with hypothyroidism are tiredness, weight gain, dry skin, cold intolerance, constipation, muscle weakness, puffiness around the eyes, hoarse voice, and poor memory. These symptoms are nonspecific and common in the euthyroid population. Therefore, the diagnosis of hypothyroidism must be made biochemically (Canaris *et al.*, 2000).

Primary hypothyroidism is diagnosis by measuring TSH concentrations which appear to be above the reference range (most commonly used (0.4- 4 mIU/L) and free thyroxine concentrations below the reference range, which is dependent on the type of assay used and the population studied (LeFevre *et al.*,2015) as shown in figure (2-2).

The measurement of plasma TSH is the commonly accepted and most sensitive screening test for primary thyroid disorders, because the pituitary gland responds with great changes in its secretion, even to slight changes in the levels of free thyroid hormones. The sensitivity of its measurement in the diagnostics of tissue hormone excess is estimated to be higher than 95%, and the specificity – approximately 90%, while its daily fluctuations are very small and are of no importance for the interpretation of results (Gietka-Czernel, 2008).

Thyroid stimulating hormone (TSH) has circadian fluctuations, with higher concentrations towards the evening. Patients with severe hypothyroidism show irregularity of TSH secretion (Roelfsema *et al.*, 2010). Seasonal variations have also been described, with higher TSH concentrations in winter and spring than in autumn and summer.

Measurement of thyroid peroxidase antibody is not strictly necessary to diagnose hypothyroidism but is useful to affirm the diagnosis of autoimmune primary hypothyroidism (Kim *et al.*, 2013).

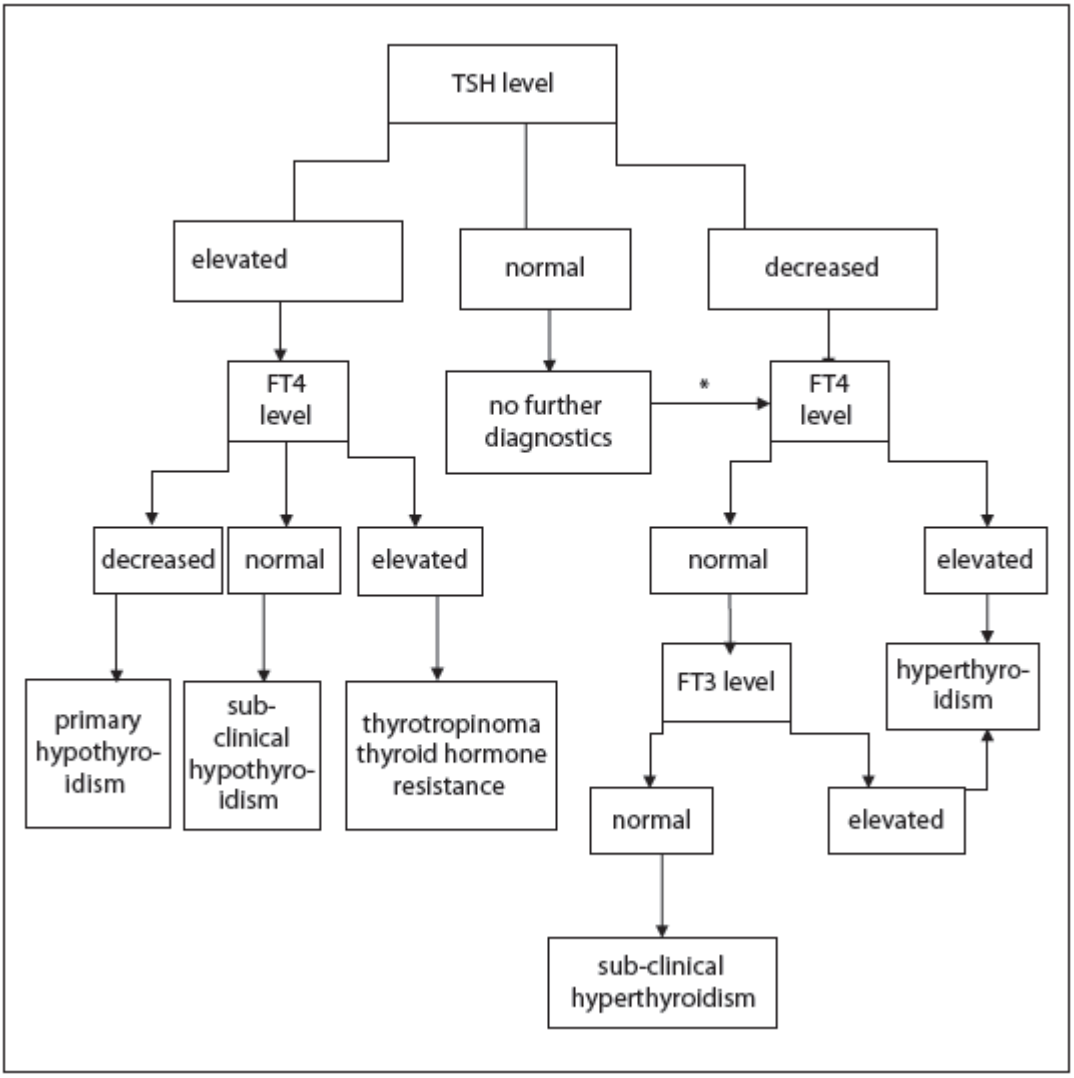

Figure (2-2): Algorithm for diagnostic procedure in thyroid diseases (Gietka-Czernel, 2008).

2.1.5 Conditions that interfere with diagnosis

Several conditions can interfere with the laboratory measurements of thyroid analyses. Interference should be suspected when thyroid function tests do not match the clinical presentation. Human anti-animal antibodies in patient's serum can cause falsely high TSH concentrations and can interfere with free thyroxine equilibrium dialysis platform assays.

Heparin, including low-molecular-weight heparin, can lead to falsely elevated concentrations of free thyroxine (Jaume *et al.*,1996). High intake of biotin, a popular over-the-counter supplement, can interfere with biotin-based hormone assays, leading to false results of thyroid function tests (van Deventer *et al.*, 2011).

2.1.6 Risk factors of Hypothyroidism

In epidemiology, a risk factor is a variable associated with an increased risk of disease or infection (Parritz&Troy, 2018). The high prevalence of hypothyroidism maybe because of traditional risk factors such as:

1- Female sex

Sex hormones and the skewed inactivation of the X chromosome are suspected to be triggers for hypothyroidism and hyperthyroidism (Garmendia *et al.*, 2014).

2- Iodine deficiency

Severe iodine deficiency can cause hypothyroidism (Vanderpump, 2005). Differences in iodine status affect the prevalence of hypothyroidism, which occurs more frequently both in populations with a relatively high iodine intake and in severely iodine-deficient populations (Laurberg *et al.*, 2010).

3- Transition from iodine deficiency to sufficiency

Transition from iodine deficiency to sufficiency was associated with an increase in thyroperoxidase antibodies; one study reported this transition by increasing in the concentration of the former antibodies from 14.3% to 23.8% (Bulow *et al.*,2011). As a result, the incidence of overt hypothyroidism increased almost 20% from 38.3 per 100,000 per year at baseline to 47.2 per 100,000 per year (Pedersen *et al.*, 2007).

4- Other autoimmune conditions

One study reported that another autoimmune disease was present in almost 15% of patients with Hashimoto's thyroiditis such as rheumatoid arthritis (Boelaert *et al.,* 2010). Hypothyroidism associated with sclerodema (SSc) in 23% of the cases, rheumatoid arthritis (RA) in 21%, systemic lupus erythematosus (SLE) in 17.9%, and multiple sclerosis (MS) in 9.1% as reported in a study conducted by (Rojas-Villarraga *et al*, 2012).

5- Genetic risk factors

Both Graves' disease and Hashimoto thyroiditis have genetic predispositions. Genome-wide association data have identified four novel genomic regions (1 including the *RERE* gene and 3 in the extended *HLA* region) that contain variants associated with thyroperoxidase antibody positivity and thyroid disease (Schultheiss *et al.,* 2015) .

6- Selenium deficiency

One study reported that patients with newly diagnosed hypothyroidism had lower selenium levels than the normal population (Bulow *et al.,* 2013). It has been proposed that Se-deficiency in Hashimoto thyroiditis patients is associated with an array of adverse effects, such as compromised antioxidant state, increased expression of harmful epitopes and apoptosis. However, the absence of adequate laboratory evidence has hampered the clarification of the underlying molecular mechanisms (Zimmermann *et al.*, 2002).

7- Drugs

Examples of drugs that can cause hypothyroidism include amiodarone (Bartalena *et al.,* 2004), lithium and IFN-γ (Shine *et al.,* 2015).

8- Syndromic conditions

Almost 25% of patients in a large registry of patients with Down syndrome had thyroid disease, the most common being primary hypothyroidism (Pierce *et al.*,2017). The prevalence of hypothyroidism in Turner syndrome is approximately 13%, but the incidence increases substantially by the third decade of life (Marinò *et al.,* 2015).

2.2 Physiological effects of thyroid hormones on blood cells.

A growing number of studies have demonstrated a direct role of THs in normal human and animal erythropoiesis (Leberbauer *et al.*, 2005). Human genome-wide association studies (GWASs) have identified genetic variances in the thyroid receptor beta (TRβ) locus associated with abnormal hematological traits (van der Harst *et al.,* 2012). Thyroid hormones (TH) are required for terminal erythroid differentiation. Targeting thyroid hormone signaling by TH receptor agonists enhances the differentiation of erythroid progenitors (Xiaofei *et al.,* 2017).

Thyroid hormone receptors are present on hematopoietic stem cells and serum levels of thyroid hormone may modulate the production of blood cells including platelets (Chute *et al.,* 2010). There is a relationship between platelet indices and thyroid hormones which are vary depending on the hormone and whether it is binding in serum (Ijaz *et al.,* 2018).

As thyroid hormones act in the nucleus by modulating gene expression, these hormones can only affect platelets indirectly through megakaryocytes (Choksi *et al.*, 2003).

There was a study on underlying molecular mechanisms of thyroid hormones associated with changes in hematopoiesis revealed that modifying in gene expressions of TRs affect hematopoietic progenitors *in vivo* (Kawa *et al.,* 2010). Also, thyroid hormones can modulate cell production in the bone marrow (Kendrick *et al.*, 2008).

Thyroid hormones augment repletion of hypoxia inducible factor1 (HIF-1) and then motivate growth of erythroid colonies (BFU-E, CFU-E).These hormones also intensify erythrocyte 2,3 Diphosphoglyceric acid (DPG) compactness, which enhances the delivery of oxygen to tissues (Kawa *et al.*,2010). It has been shown that T3 is required for normal B cell production in the bone marrow through regulation of pro-B cell proliferation (Arpin *et al.*, 2000).

2.2.1 The impact of hypothyroidism on blood cells count and red blood cells indices.

Hypothyroidism could cause certain forms of anemia in humans (Chandel *et al.,* 2015). The prevalence of anemia in patients with hypothyroidism has been shown to be 20-60% (Kosenli *et al.*, 2009) and by affecting hematopoietic process, hypothyroidism results in anemia through slowing the oxygen process (Lippi *et al.,* 2008). The anemia is usually macrocytic hypochromic and/ or normocytic anemia with an increased MCV, and hypothyroidism with moderate severity (Ibrahim *et al.*, 2012).

Alteration in other hematological parameters such as hemoglobin (Hb), hematocrit (HCT), mean corpuscular hemoglobin (MCH), white blood cell (WBC) count and platelet count is associated with thyroid dysfunction is observed as well , but all changes return to normal if an euthyroid (normal) state is obtained (Kawa *et al.*,2010).

Previously reported investigations demonstrated that exposure to a higher and a lower than normal concentration of TH significantly influenced clonogenicity and induced apoptosis in human HPCs from normal bone marrow, cord and peripheral blood (PB) (Grymuła *et al.*, 2007). A previous study demonstrates that TRs are sensitive to the pathological conditions present in hypo- and hyperthyroidism regarding

their expression and revealed that hypo- and hyperthyroidism modify *TR* gene expression in haematopoietic progenitors *in vivo* (Miłosz *et al.*,2010).

Nejar Bruno *et al.* (2005) reported that 5'-nucleotidase activity in platelets which enzyme caused degradation of adenosine monophosphate to adenosine were increased in the hypothyroid and decreased in the hyperthyroid rats. Furthermore, the altered platelet 5'-nucleotidase activity during hypo- and hyperthyroidism could lead to changes in adenosine levels and consequently influenced the platelet aggregation.

2.3 Apoptosis

Apoptosis can be simply defined as a set of biochemical cytoplasmic and mitochondrial events that may lead to the execution phase of nuclear events (Radosevich, 2018). Apoptosis is a mechanism to regulate cell number and is vital throughout the life of all animals. Although several different types of biochemical events have been recognized as important in apoptosis, perhaps the most fundamental is the participation of the caspases (Reed & Green, 2011).

The convergence of the apoptotic signal is considered the activation of a family of cysteine aspartyl- specific proteases (caspases) ,composed of 12 proteins strictly involved in the apoptotic cell death process. A wide array of stress stimuli can trigger the apoptotic process, and the biochemical signal can then be amplified in the cytoplasm and mitochondria by both extrinsic and intrinsic pathways (Radosevich, 2018).

2.3.1 Apoptosis pathways.

There are two different pathways that lead to apoptosis: the intrinsic and extrinsic pathways that correlate with the signal type. They are also referred to as the mitochondrial and death receptor pathways,

respectively The pathways converge at the executioner pathway (Zaman *et al.*, 2014).

1- The intrinsic pathway

The intrinsic mechanism of apoptosis invoves the mitochondria and mitochondrial proteins explained in figure (2-3). Cells with damaged DNA or up regulated oncogenes can stimulate this pathway (Xu *et al.*, 2015). Additional stimuli for this pathway includes growth factor deprivation, surplus Ca2+, DNA-damaging molecules, oxidants and microtubule targeting drugs (Hassan *et al.*, 2014). The overall pathway is regulated by the BCL-2 family of proteins (Zaman *et al.*, 2014).

Various apoptotic stimuli result in the up regulation of BH3-only proteins, which then activate both BAX and BAK (Lomonosova & Chinnadurai , 2008). Once activated, BAX and BAK oligomerize, which leads to mitochondrial outer membrane permeabilization (MOMP) which is the defining event of intrinsic apoptosis and is considered the point of no return (Lopez & Tait, 2015).

The permeabilization allows the release of intermembrane proteins like cytochrome c, second mitochondria-derived activator of caspase (SMAC) and Omi. Upon the release of cytochrome c, the apoptosome is formed from cytochrome c, apoptotic protease-activating factor-1 (APAF-1), dATP and procaspase-9 (Hassan *et al.*, 2014). Within the apoptosome, procaspase-9 is converted into caspase-9 which activates the executioner caspases-3 and -7. The executioner caspases quickly begin to break down proteins leading to cell death (Green&Llambi, 2015).

2- Extrinsic pathway.

The extrinsic pathway uses extracellular signals to induce apoptosis explained in figure (2-3). Cell death signals, also known as death ligands, bind to tumor necrosis factor (TNF) family death receptors

(Zaman *et al.*, 2014). Some death ligands include Fas ligand (Fas-L), TNF-related apoptosis-inducing ligand (TRAIL) and tumor necrosis factor (TNF) (Goldar *et al.*, 2015). The binding of Fas ligand to Fas receptor results in the binding of the adapter protein FADD and the binding of TNF ligand to TNF receptor results in the binding of the adapter protein TRADD with recruitment of FADD and RIP (Wajant, 2002).

Initiator procaspases-8 and -10 bind to the adaptor protein, forming the death-inducing signaling complex (DISC). The procaspases have a death effector domain (DED) that binds to the adaptor protein at its DED. Procaspases-8 and -10 are activated by DISC. Executioner caspases-3,-6 and -7 are then activated and begin the cleavage of proteins and the cytoskeleton leading to cell death (Goldar *et al.*,2015).

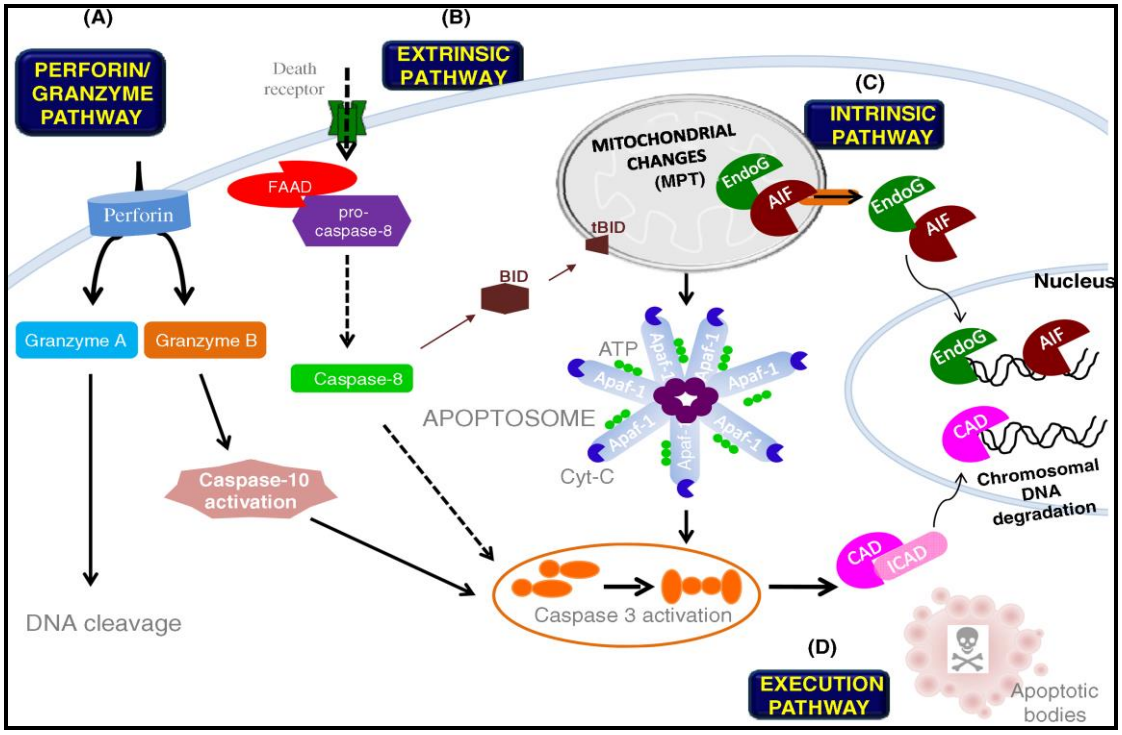

Figure (2-3): Pathways of apoptosis (Radosevich, 2018).

2.3.2 Fas and Fas ligand

Human mature CD95 (known as Fas or APO-1) is a 45–48 kDa (319 amino acids) single spanning transmembrane receptor binds to its

cognate ligand FasL/CD95L; a 17-amino acid transmembrane domain, and a C-terminal cytoplasmic domain of 145 amino acids that harbors a "death domain" (DD) required to transmit apoptotic signals (Gajate & Mollinedo, 2015).

CD95 (Fas/APO-1/TNFRSF6), a cell surface protein that mediate apoptosis when bound to its natural ligand, CD95L (CD178/TNFSF6) or stimulated with agonistic antibodies. It is ubiquitously expressed in the body, but is particularly abundant in the thymus, liver, heart, and kidney (Peter *et al.*, 2015).

Fas ligand expression has been reported to occur only in activated cytotoxic T lymphocytes and natural killer cells and tissues such as the eye and testis, which are regarded as `immune privileged'. Cells from these tissues are protected from cytotoxic T cells by FasL expression which binds to Fas antigen on lymphocytes and induces apoptosis (Andrikoula&Tsatsoulis, 2001).

Fas/CD95 does not possess enzymatic activity, but acts by binding to other proteins through its DD, which is a protein– protein interaction domain that enables Fas/CD95 and other DD-containing death receptors to interact by homotypic binding with the bipartite DD adapter protein Fas-associated death domain protein (FADD) (Dickens *et al.*, 2012).

2.3.3 Soluble Fas (sFas) and soluble Fas ligand (sFasL).

The Fas/FasL system contains both membrane-bound versions of (mFas and mFasL) and soluble versions (sFas and sFasL), sFas is mainly expressed on epithelial cells and activated lymphocytes, it regulates T cell homeostasis by mediates proliferation and death of T lymphocytes (Jaleco *et al.*, 2003) as explained in figure (2-4).

The membrane-bound form (mFasL) can be cleaved from the cell surface by metalloproteinases to produce a truncated soluble product

(sFasL) of 26 kDa derived from the extracellular domain (Tanaka *et al.*, 1995). However, it is not clear what triggers sFasL release, but it is plausible that abnormal or excessive activation of T cells causes the production of sFasL, with deleterious systemic effects (Suda *et al.*, 1997).

Soluble FasL does not activate Fas, and it competes with mFasL reducing its cytotoxic activity (Schneider *et al.*, 1998). This is due to the fact that sFasL binds Fas, but it is unable to induce its oligomerization, thus preventing activation of the proapoptotic signaling pathway by mFasL (Jang *et al.*, 2003). High sFas levels have been associated with several autoimmune diseases including multiple sclerosis, autoimmune lymphoproliferation syndrome, and autoimmune thyroiditis (Hashimoto's thyroiditis) (Rieux-Laucat *et al.*, 2003).

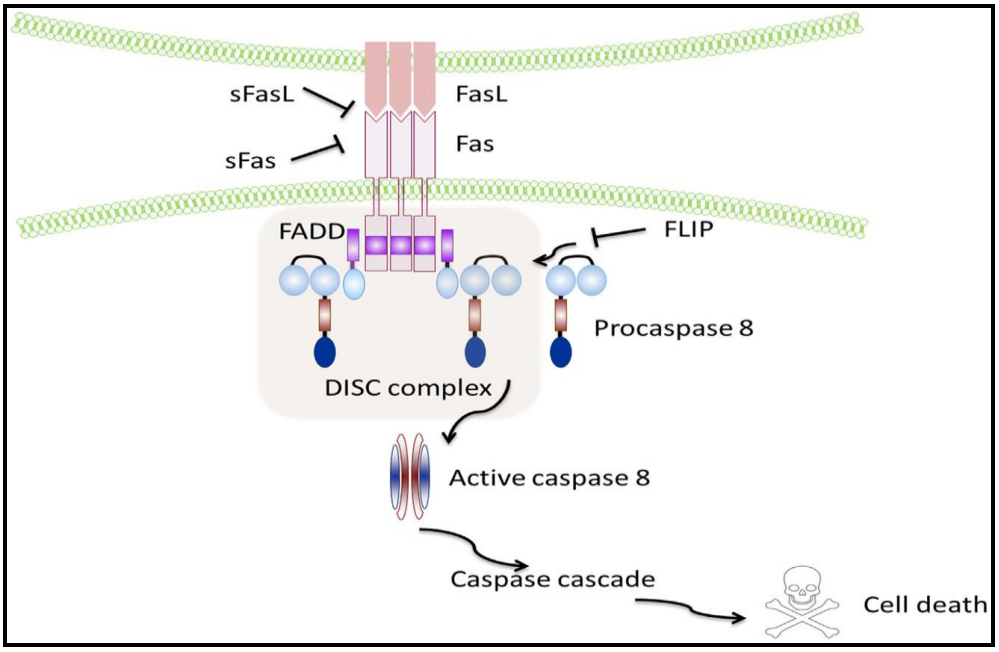

Figure (2-4): Fas and FasL pathway of apoptosis (Volpe *et al.*, 2016)

2.3.4 B-cell lymphoma 2 (Bcl-2 protein)

Mammalian Bcl-2 family proteins interact with each other to regulate apoptotic cell death. An interesting feature of this protein family is the semblance of "competition" between individual family members that act by countering the functions of other Bcl-2 family members to

either induce or inhibit apoptosis. Bcl-2 was first discovered at the chromosome translocation breakpoints diagnostic of follicular lymphoma and leukemic cells, and was the first oncogene of its kind—one that inhibits apoptosis rather than promoting cell growth (Gavathiotis, 2019). Bcl-2 is a tail-anchored protein; namely, it contains a C-terminal hydrophobic helix (α8) that functions as a transmembrane (TM domain). The structure of full-length Bcl-2 protein has not been determined (Ku *et al.,* 2011) as explained in figure (2-5). The Bcl-2 was previously shown to inhibit cell death by preventing the binding of the BH3-only (or pro-apoptotic) proteins to the mitochondrial outer membrane (MOM). Thus, overexpression of Bcl-2 can suppress MOM permeabilization (MOMP) in numerous cell death pathways, preventing the release of apoptogenic factors such as cytochrome c, and SMAC/DIABLO from mitochondria (Adams & Cory, 2018).

The anti-apoptotic Bcl-2 family proteins exert their pro-survival function by binding and inhibiting the pro-apoptotic proteins, the sensors of cellular stress (the BH3-only proteins) and the effectors of apoptosis (Bax and Bak) (Kale *et al.*,2018). The BH4 domain of Bcl-2 and Bcl-xL is able to bind other proteins that do not belong to Bcl-2 protein family, allowing them to play a role beyond their classical role in inhibiting apoptosis, in other important cellular functions such as proliferation, autophagy, differentiation, DNA repair, tumor progression and angiogenesis (Gabellini *et al.,* 2017). The bcl2 protooncogene has been reported a conferring resistance to apoptosis in the thyroid. It may be speculate that increased sBcl-2 accompanied with decreased sFas level in HT patients in euthyroid state due to levothyroxine may be connected with “protective” influence of exogenes thyroid hormone administration in HT (Myśliwiec *et al.,* 2006).

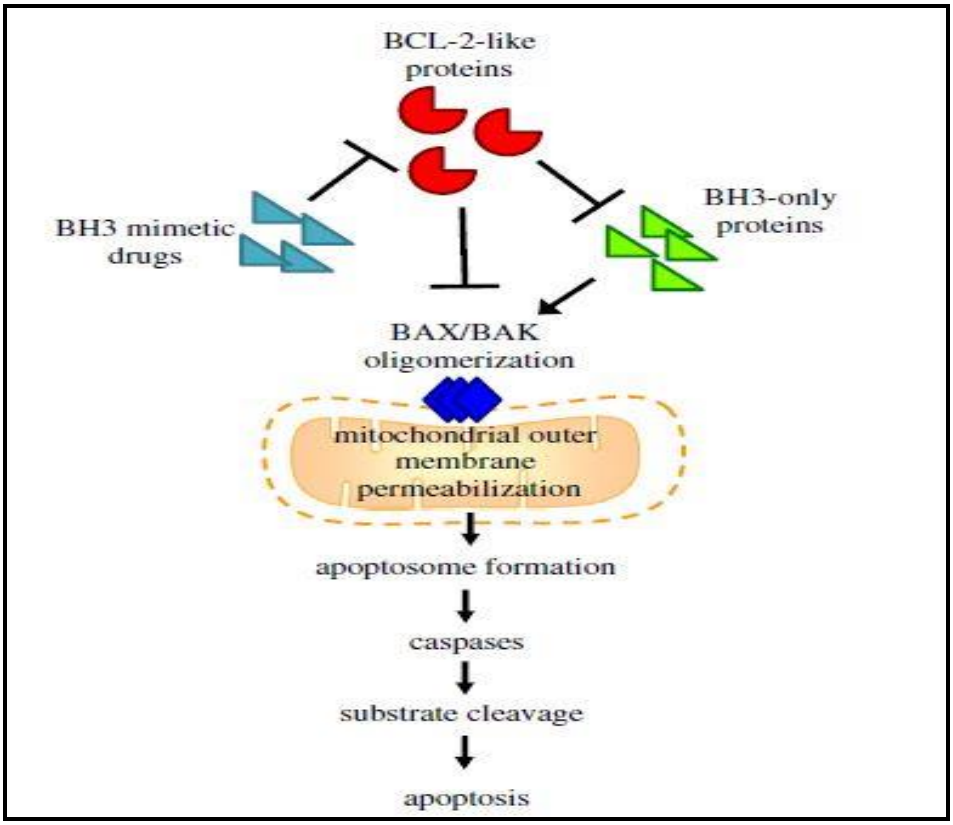

Figure (2-5): The role of Bcl-2 in apoptosis (Campbell & Tait, 2018)

2.3.5 Cytochrome C.

Cytochrome c (Cc) is a small soluble electron carrier hemeprotein located in large amounts in the inner mitochondrial membrane. By transferring electrons from complex III to complex IV، cytochrome c facilitates cell energy production (Kuhlbrandt, 2015). Although is not encoded by mtDNA but by a gene located at the short arm of chromosome 7, maintenance of the cytochrome c inside the mitochondrion is imperative, since its release into the cytosol results in cell apoptosis (Hillier *et al.*,2003). Cytochrome c plays an essential role in homeostasis and apoptosis. In apoptosis, Cc is released from mitochondria into the cytoplasm. During this process, cytosolic Cc and dATP bind to apoptosis protease-activating factor-1 (Apaf-1), forming

the apoptosome, a macromolecular plat form that in turn leads to the activation of initiator caspases (Yu *et al.*, 2005).

Cytochrome c release into the extracellular space and ultimately into the blood in various conditions characterized by cell death. Evidently, the more the cellular or tissue damage, the higher the serum cytochrome c level. Thus, cytochrome c may be a useful clinical marker for diagnosing and assessing the severity of such pathological entities. Reasonably, detection of high cytochrome c level into the circulation concurs with release of various other molecules that serve as damage associated molecular patterns (DAMPs) when found extracellularly, such as mtDNA and formyl peptides (FPs). Finally, the release of this universal compound into the extracellular space makes cytochrome c an ideal molecule to act as a DAMP (Eleftheriadis *et al.*, 2016). Thyroid hormones have profound impact upon mitochondrial biogenesis and activity (Weitzel *et al.*, 2003). Hypothyroidism is known to diminish oxygen consumption and promote low metabolism causing disturbances in hemodynamic, cardiac، and renal functions (Franco *et al.*, 2011).

2.3.6 Role of thyroid hormones in apoptosis of blood cells.

Thyroid hormones play an important role in many physiological processes, such as differentiation, growth, development, and the physiology of all cells. Apoptosis plays a critical role in the development and homeostasis of tissues, especially those with high cell turnover such as the lymphoid system (Cano-Europa *et al.*, 2012).

There are data indicating that excess thyroid hormones cause apoptosis, due to enhance the expression of several death receptors and their ligands, such as TNF and FasL resulting in activation of apical caspase-8, which is further amplified through the activation of the further caspases which ultimately lead to apoptosis (Kumar *et al.*,2007).

Mihara *et al.* (1999) found that T lymphocytes, cultured with T3 and T4 in vitro, showed enhanced apoptosis, evidenced by DNA ladder formation and characteristic morphological changes. Furthermore, the treatment with thyroid hormones of T lymphocytes induced reduction of mitochondrial transmembrane potential and production of reactive oxygen species, both of which are intimately associated with apoptotic cell death. These findings suggest that thyroid hormones have the potential to induce apoptotic cell death of human lymphocytes *in vitro*.

Thus, enhanced apoptosis of thyroid hormone-treated lymphocytes may be due to the enhanced ROS production and/ or the reduction of antioxidant effects by decreasing Bcl-2 protein expression (Katyare *et al.*, 2005).

2.3.7 Apoptosis and Hypothyroidism

Apoptosis that mediated by Fas/FaL plays an important role in the active stage of the autoimmune process of both Hashimoto's thyroiditis and Graves' disease, however, in Hashimoto's thyroiditis they contribute to irreversible damage of thyrocytes (Myśliwiec *et al.*, 2006).

In autoimmune process combination of inflammatory cytokines: TNF, interferon γ and interleukin 1 may activate expression of FasL on T cells and may sensitized thyroid follicular cells (Wang *et al.*, 2002).

Thyroid gland immunohistochemycal analysis in HT have shown enlarge number of apoptotic follicular cells, inmost in periphery of lymphocyte infiltrates (Stojanović *et al.*, 2009), furthermore in HT caspase-3 and caspase-8 are up-regulated and activated (Stassi & De Maria, 2002). Some evidence suggest thyroid cell destruction in autoimmune hypothyroidism is dependent on T cell-mediated cytotoxicity with the likely additional effect of death receptor-mediated apoptosis (Stojanović *et al.*, 2009) .

Thyroid follicular cells in tissue samples from HT exhibited strong staining for Fas and FasL and high apoptotic rate (30.3%), while normal control follicles exhibited moderate Fas, minimal or no FasL and a low percentage of apoptosis (Hammond *et al.,* 1997).

Thyroid follicular cells in Hashimoto thyroiditis (HT) undergo apoptosis by up-regulation of Fas and FasL and down-regulation of Bcl-2 protein. Infiltrating lymphocytes do not seem to be directly involved in this process with their own FasL but they are likely to provide the appropriate cytokine milieu that results in the up-regulation of Fas and FasL and subsequently the activation of the apoptotic machinery (Mitsiades *et al.,* 1998).

2.4 Oxidative stress and antioxidant system

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Durackova, 2010).

The excess amounts of ROS are either produced in the organism on call for pathogenic defenses or generated at normal levels but not neutralized due to the insufficient anti-oxidant capacity of the redox homeostasis system. Reactive oxygen species (ROS) are formed as intermediates and by-products in the energy production cycle in mitochondria in the electron transfer chain reactions and in endoplasmic reticulum (Alfadda & Sallam, 2012).

Antioxidant is any substance that can prevent, reduce, or repair the ROS-induced damage of a target biomolecule. Antioxidants manage ROS

and other related reactive species induced damage by three general mechanisms, which include:

1- Inhibition of ROS generation,

2- Scavenging of ROS already formed,

3- Repair of ROS-induced damage (Halliwell, 2007).

2.4.1 Role of oxidative stress and antioxidant system in hypothyroidism .

Thyroid hormones can be one of the main physiological modulators of *in vivo* cellular oxidative stress due to their known effects on mitochondrial respiration. In particular, it has been suggested that the increase in reactive oxygen species induced by a deficiency of thyroid hormones can lead to an oxidative stress condition in the liver and in the heart and some skeletal muscle (Yilmaz *et al.*, 2003).

In physiological conditions, ROS (hydrogen peroxide H_2O_2) are molecules that are necessary for the thyroid hormone synthesis within thyroid epithelial cells. However, there may be an excessive increase in H_2O_2 levels in the presence of some stimulants such as inflammation (T and B lymphocyte activation), radiation, chemical materials, excessive iodine intake and drugs. In this milieu of excessively increased H_2O_2, thyrocytes become apoptotic, necrotic and consequently destructed (Poncin *et al.,* 2008).

Hypothyroidism-associated oxidative stress is the consequence of both increased production of free radicals and reduced capacity of the antioxidative defense (Sarandol *et al.,* 2005). Hypothyroidism-induced dysfunction of the respiratory chain in the mitochondria leads to accelerated production of free radicals (i.e., superoxide anion, hydrogen peroxide, and hydroxyl radical as well as lipid peroxides), which consequently leads to oxidative stress (OS) (Yilmaz *et al.,* 2003).

Metabolic disorder from autoimmune-based hypothyroidism can also increase oxidative stress (Carmeli *et al.,* 2008). Excess TSH in hypothyroidism patients is known to directly produce oxidative stress (Dardano *et al.,* 2006). Reports of higher oxidative stress markers in hypothyroid patients with antithyroid peroxidase (anti-TPO) positivity compared with those without (anti-TPO (–) (Nanda *et al.,* 2012).

2.4.2 Role of oxidative stress and antioxidant system in apoptosis

Cell apoptosis initiation originates from extracellular or intracellular signals via the death receptors and the mitochondria-mediated pathways. Upon cell apoptosis initiation, the ROS increases through disrupting intracellular redox homeostasis, and irreversible oxidative modifications of lipid, protein, or DNA, which in turn can activates oxidative stress-induced apoptotic signaling (Song *et al.,* 2011). In addition, ROS-induced activation of c-Jun N-terminal kinase (JNK) which can also induce the apoptotic signaling (Chang *et al.,* 2006). This enzyme is a member of the mitogen-activated protein kinase (MAPK) family. Increasing evidence indicates a crucial role of JNK in mitochondrial dysfunction with subsequent initiation of apoptosis (Mao *et al.,* 2008).

The mitogen activated protein kinase (MAPK) pathways is activated by ROS, which are important mediators of signal transduction and play a key role in regulating many cellular processes (Navarro *et al.,* 2006). Many kinds of drugs can induce ROS-mediated apoptosis in a series of cancer cells has been reported in succession (Duan *et al.,* 2014).

An excess of H_2O_2 induces an imbalance in ROS generation, alters cellular antioxidant defenses, induces oxidative damage to membrane lipids, cellular proteins and DNA, and eventually triggers cell death by apoptosis (Hamdi *et al.,* 2015). Finally Oxidant-induced apoptosis is not

the simple result of the biochemical interactions of ROS and cellular macromolecules but rather is actively regulated by cell signaling cascades (Singh & Czaja, 2007).

2.4.3 Biomarkers of oxidative stress

Biomarkers of oxidative stress can be classified as molecules that are modified by interactions with ROS in the microenvironment; and molecules of the antioxidant system that change in response to increased redox stress. These molecules can be modified by excessive ROS *in vivo*. Of these modifications, some are known to have direct effects on function of the molecule (e.g. inhibit enzyme function), but others merely reflect the degree of oxidative stress in the local environment. There are factors influencing the clinical applicability of a ROS bio-marker include the ease of obtaining an appropriate biological specimen; the stability of the biomarker throughout various storage conditions, specimen preparation steps, the specificity, sensitivity and reproducibility of the assay used to measure the modification (Dalle-Donne *et al.,* 2005). One of biomarker that reflects lipid peroxidation is:

2.4.4 Thiobarbituric Acid Reactive Substances (TBARS).

Lipid peroxidation is a process in which free radicals, such as reactive oxygen species and reactive nitrogen species, attack carbon-carbon double bonds in lipids, a process that involves the abstraction of hydrogen from a carbon and insertion of an oxygen molecule. This process leads to a mixture of complex products including, lipid peroxyl radicals, and hydroperoxides as the primary products, as well as malondialdehyde (MDA) and 4-hydroxynonenal as predominant secondary products (Tsikas, 2017). Lipid peroxidation has been implicated by over-production of ROS, causing altered structural integrity of the cell membrane, DNA, proteins and lipids (Valko *et al.,* 2007).

Lipid peroxidation generates a complex variety of products that are commonly used to determine cell damage. One by-product of this cascade is malondialdehyde, which is known to attack DNA causing mutative damage (Fogarty *et al.,* 2011).

Thiobarbituric acid (TBA) is reacted with MDA, which is resulting in a color compound, which can be determined spectrophotometrically, chromatographically, or through image processing techniques (Xiong *et al.*,2015). Due to the reactivity of TBA with several reactive substances in the biological sample, a more widely accepted terminology called thiobarbituric acid reactive substances is now commonly used (TBARS) (Sun *et al.,* 2001).

Assay of TBARS concentration is used as a marker of oxidative stress or lipid peroxidation (Rodríguez-Gutiérrez *et al.,* 2019). Generally, TBARS is an indirect marker of oxidative stress, but it is a direct marker of lipid damage caused by increased oxygen consumption during exercise (Fogarty *et al.*, 2011). Formation of TBARS results from degradation of polyunsaturated fatty acids by free radicals (Bober *et al.,* 2006). Erdamar *et al.* (2008) demonstrated that TBARS is a sensitive marker of lipid peroxidation, once it is a specific degradation product. There are higher TBARS levels in hypothyroidism patients as confirmed by (Öztürk *et al.*, 2012). The elevation of TBARS was shown in both overt hypothyroidism and subclinical hypothyroidism (Nanda *et al.,* 2007).

2.4.5 Selenoprotein P

Selenoprotein P is a major selenium (Se)-containing protein in human plasma, and the "P" denotes its presence in plasma (Saito *et al.*, 2012) and synthesized mainly in the liver and secreted to extracellular fluid. Moreover, SeP contains the essential trace element Se in the form of selenocysteine (Sec), which is an analog of cysteine that contains Se

instead of sulfur (Ma *et al.*, 2002) and contains ten Sec residues per polypeptide, which are encoded by the UGA stop codon. SeP is a unique selenoprotein with multiple Sec residues, while other selenoproteins only have one or two Sec residues (Shetty *et al.*, 2014).

Selenoprotein P is made up of 2 domains. The larger N-terminal domain contains 1 selenocysteine residue in a redox motif and the smaller C-terminal domain contains the other 9 selenocysteines (Raymond *et al.*, 2009). Hepatic synthesis of Sepp1 affects whole-body selenium content and the liver is the source of most plasma Sepp1. ApoER2, a member of the lipoprotein receptor family, binds Sepp1 and facilitates its uptake into testis and retention of its selenium by the brain. Megalin, anotherlipoprotein receptor, facilitates uptake of filtered Sepp1 into proximal tubule cells of the kidney. Thus, Sepp1 serves in homeostasis and distribution of selenium (Olson *et al.*, 2007).

Seleno protein P is a multifunctional protein, possessing GPx-like enzyme activity to reduce phospholipid hydroperoxide in the presence of glutathione and Se-transport activity to effectively supply Se to cells (Rock *et al.*, 2010). SeP is also a major methylmercury-binding protein in plasma, suggesting that it plays a role in the detoxication of heavy metals (Liu *et al.*, 2018). Selenoprotein P, one of the most highly up-regulated proteins, regulates the cellular lipid redox state and maintains cell viability (Yukihito *et al.*, 2007).

Seleno protein P (SepP) has an antioxidant activity (Muzembo *et al.*, 2013). It can reduce hydroperoxides, protecting plasma proteins and endothelial cells against oxidative damage (Cominetti *et al.*, 2011). SepP is found in almost all body tissues, regulating energy metabolism (Misu *et al.*, 2012). Selenoprotein P has been postulated to defend against oxidative injury in the extracellular space. Its phospholipid hydroperoxide

thiol peroxidase activity and its ability to bind to cells are properties that might support such a function. In addition, in vitro incubation experiments showed that adding selenoprotein P delayed free radical–induced oxidative damage to low-density lipoproteins (Traulsen *et al.*, 2004).

Thyroid glands contain several selenoproteins including iodothyronine 5_-deiodinase, glutathione peroxidase (GPx) which is part of the antioxidant defense mechanism against oxidative stress,thioredoxin reductase type 1, and selenoprotein P (SePP) (Combs *et al.,* 2009).

Selenium (Se) is crucial for thyroid gland functioning and thyroid hormone biosynthesis and metabolism (Duntas & Benvenga, 2015). On the other hand, thyroid hormone affects Se metabolism directly or indirectly and affects the serum selenium status and regulates the expression of several selenoproteins (Mittag *et al.,* 2010). Selenium deficiency reduces the plasma concentrations of SePP and selenium (Burk *et al.,* 2003). Severe and persistent selenium deficiency impairs thyroid hormone biosynthesis and also exaggerates destruction of follicular structures and their replacement by fibrotic tissue (Kohrle& Gartner, 2009). It has been hypothesized that nutritional selenium deficiency may promote the initiation or progression of thyroid autoimmunity (Effraimidis *et al.,* 2014).

Selenoprotein P is an important protein in selenium homeostasis and since its concentration falls in selenium deficiency, it can be used as an index of selenium nutritional status (Mitra *et al.,* 2016).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

3.1.1 Equipments:-

The equipment's used to perform this study are shown in Table (3-1).

Table (3-1) The equipment's of the current study

Equipment	Company and origin
Centrifuge	Digi system laboratory instruments/ Taiwan
ELISA	Biotek /USA
Hematology analyzer (Automated blood cells counting)	Mindary/ China
Automatic micropipette (100-1000 µl)	DRAGON LAB/ China
Automatic micropipette (10-100 µl)	DIAMOND/Germany

3.1.2 Kits and Reagents

All reagents which including kits and their origin used throughout the study are mentioned in Table (3-2) :

Table (3-2) Kits used in the current study

Kit	Company and origin
Human soluble Fatty acid synthetase (sFas)	Meslon medical / Chinese by French distinction
Human soluble Fatty acid synthetase ligand (sFasL)	Meslon medical / Chinese by French distinction
Human Bcl-2 (B- cell Leukemia/Lymphoma 2)	Elabscience/ China
Human Cytochrome C	Elabscience/ China
Human Thiobarbituric Acid Reactive Substances (hTBARS)	BT-science / China
Human Selenoprotein P1(hSepP1)	BT-science / China
Thyroid stimulation hormone (TSH)	CALBIOTECH/ USA
Triiodothyronin (T3)	CALBIOTECH/ USA
Tetraiodothyronin (T4)	CALBIOTECH/ USA

3.2 Methods

3.2.1 Study setting.

In Al-Najaf governorate, this study was carried out in a specialized center for diabetes and endocrine center due to more feasibility of cases of hypothyroidism at that center. This specialized center is situated in the out patients department of Al-Sadr Medical Teaching City. Some cases were also collected from the private laboratories in Al-Najaf Province. The collection of samples was conducted during the period from January to July 2020.

3.2.2 Sampling of cases.

The diagnosis of hypothyroidism was carried out by a physician who specialized in thyroid disorders. The groups included in the current study were as follow:

(a) Control group : Thirty subjects of normal persons who were apparently healthy and do not undergo any thyroid diseases or cardiovascular diseases or renal diseases. Control group was age-matched with patients group of both genders.

(b) Without-treatment group: Twenty cases of patients that newly diagnosed with hypothyroidism are included in this study, their ages ranging from 20 to 70 years of both genders.

(c) With-treatment group: forty cases of patients with hypothyroidism disease diagnosed clinically and by ultra sound examination of the thyroid gland by a physician, were included in this study, all of them were receiving hormonal drugs which include mainly Levothyroxine.

The history as well as personal information for each patient had been obtained through a questionnaire which was designed to meet the aim of this study as seen in appendix A.

3.2.3 Collection of blood samples.

Blood samples were collected from control persons and patients with hypothyroidism. Approximately 5ml of blood were collected from each subject using standard procedures. Blood sample was divided into two parts: 2ml of blood were put into tri-potassium ethylenediamine tetra-acetic acid (K3EDTA) anticoagulant bottle which was well mixed by gentle inversion for complete blood count (CBC) analysis. Three (3) ml allowed to stand at room temperature for at least one-half hour or until it was thoroughly clotted and then refrigerated

within 2 hours of collection ,blood was centrifuged and serum was separated and put into sterile apendroff tubes ,the letters were labeled and stored at -70C (Dorgan *et al.*, 2010).

3.2.4 Exclusion criteria

Patients who have hyperthyroidism, thyroid tumors (benign and malignant), any other autoimmune diseases, were excluded from this study.

3.2.5 Study Design

To achieve the aim of the present study, a cohort study design was adopted (Figure 3.1)

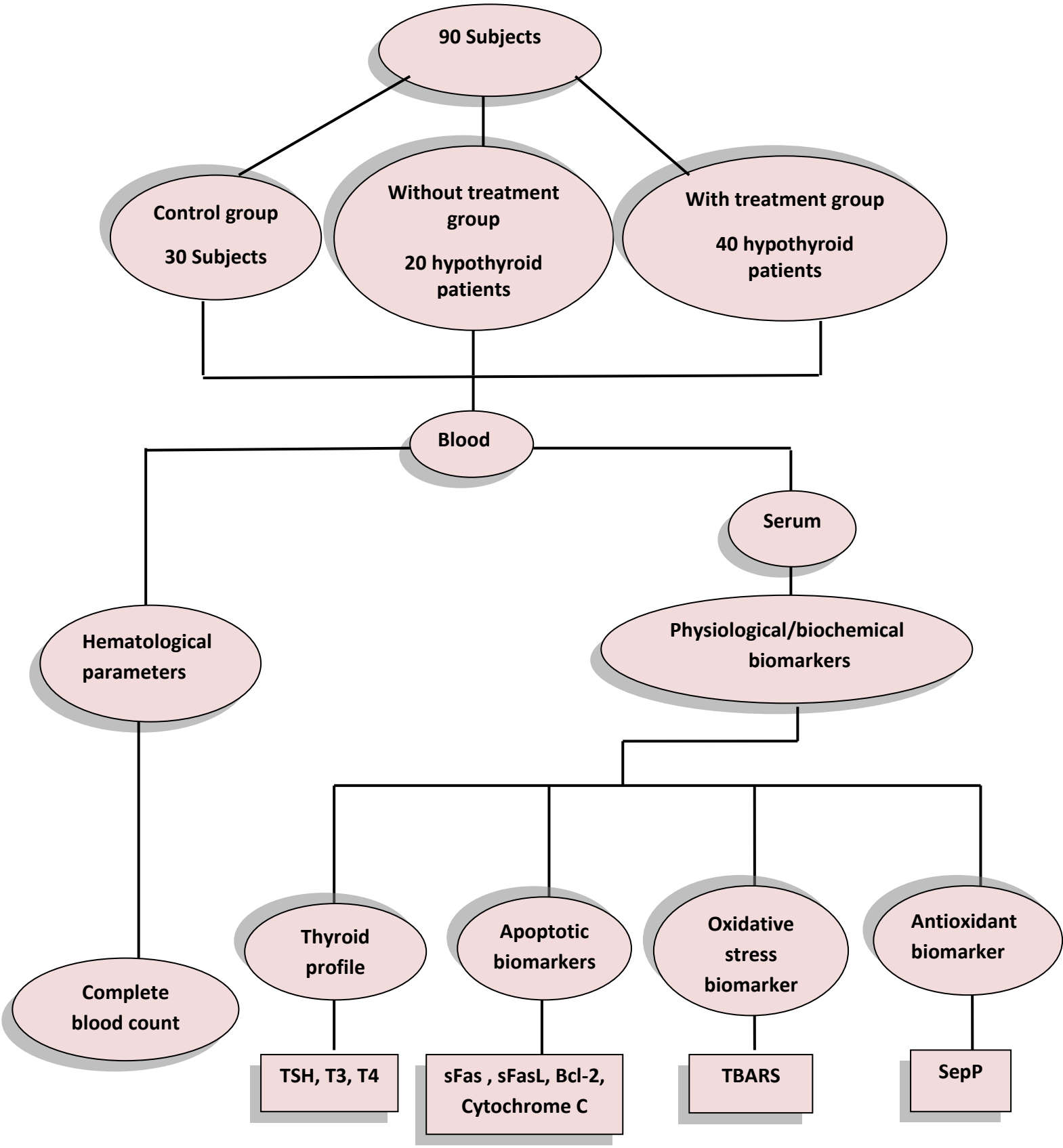

Figure (3-1): Experimental Design

3.3 Measurement of complete blood count (CBC)

3.3.1 Principle

The mindary machine was used in this test which is a hematology analyzer (fully automatic machine) used for measuring RBC count, hematocrit and platelet count by direct current sheathed flow. Moreover; measuring leukocyte differential count, by using fluorescent flow cytometry with a semiconductor laser and fluorescent dyes. In addition; the differential leukocyte counts were analyzed by using optical information, this machine detected three types of optical information; which include; forward-scattered light, side-scattered light and side fluorescence light. There is a separate channel called immature myeloid information (IMI) that provides additional information of WBC on the presence of immature granulocytes, hematopoietic progenitor cells and blasts (Polzar *et al.*, 2014).

3.3.2 Procedure

A blood sample was withdrawn from the patient placed in a special tube container EDTA; the solution used in this machine was three types; Isotonic diluent, lyse solution, cleaning agent. Data were entered with the name of the patient and sample number inside the device by the keyboard related to the computer unit built with the device. The results were printed out on special paper.

3.4 Measurement thyroid profile hormones (TSH, T3, T4).

3.4.1 Measurement of Thyroid Stimulating Hormone (TSH)

The Calbiotech,Inc.(CBI) TSH ELISA kit is intended for quantitative measurement of TSH in human serum.

A/ Principle of the test

The (CBI) TSH was a solid phase sandwich ELISA method. The samples and anti-TSH-HRP/Biotin conjugate were added to the wells coated with streptavidin. TSH in the patients sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate were washed off by wash buffer. Upon the addition of the substrate, the intensity of color was proportional to the concentration of TSH in the sample. A standard curve was prepared relating color intensity to the concentration of the TSH.

B/ Assay Procedure

Prior to assay, reagents were allowed to stand at room temperature (20-25 C°) and all reagents were gently mixed before use.

1. The desired number of coated strips was placed into the holder.

2. Fifty (50 µl) of TSH standards, control and patient specimens were pipetted into designated wells.

3. One hundred (100 µl) of ready use conjugate reagent were added to all wells and shook for (10-30) seconds.

4. The plate was covered and incubated for 60 minutes at room temperature (20-25 C°)

5. The liquid was removed from all wells and washed 4 times with 300 µl of 1X wash buffer then blotted on absorbent paper towels.

6. One hundred (100 µl) of TMB substrate were added to all wells then incubated for 15 minutes at temperature (20-25 C°).

7. Fifty (50 µl) of stop solution were added to all wells and the plate was gently shacked to mix the solution.

8. The absorbance was read on ELISA reader at 450 nm within 15 minutes after adding the stopping solution.

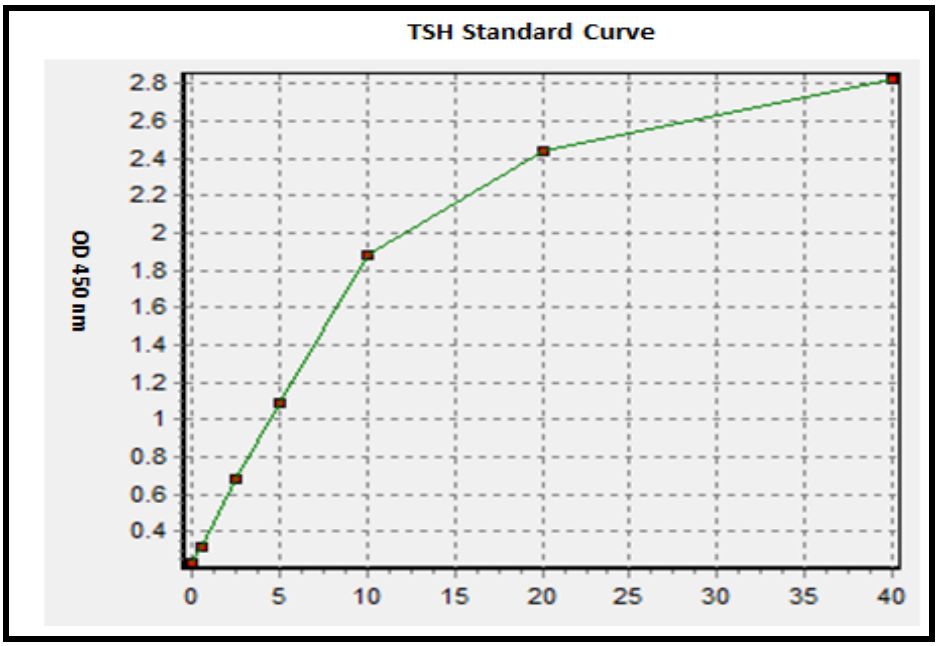

Figure (3-2): Standard curve of thyroid stimulating hormone (TSH).

3.4.2 Measurement Triiodothyronin (T3) hormone .

A/ Assay Principle

The T3 is a solid phase sandwich ELISA method. The samples and assay buffer and T3 enzyme conjugate are added to the wells coated with anti-T3 monoclonal antibody. T3 in the patients serum compete with a T3 enzyme conjugate for binding site. Unbound T3 and T3 enzyme conjugate are washed off by wash buffer. Upon the addition of the substrate , the intensity of color is inversely proportional to the concentration of T3 in the sample. A standard curve is prepared relating color intensity to the concentration of the T3.

B/ Assay Procedure

Before proceeding with the assay, reagents were allowed to stand at room temperature (20-25 C°) and all reagents were gently mixed before use.

1. The microplate's wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate. Any unused microwell strip was backed into the aluminum bag sealed and stored at (2-8 C°).

2. Fifty (50µl) of the appropriate serum reference , control or specimen into the assigned well.

3. One hundred (100µl) of T3-enzyme conjugate solution were added to all wells.

4. The plate was swirled gently for 20-30 second for mixing then covered and incubated for 60 minutes at room temperature (20-25 C°)

5. The liquid was removed from all wells and washed 3 times with 300 µl of 1X wash buffer then blotted on absorbent paper towels.

6. One hundred (100µl) of TMB substrate were added to all wells then incubated for 15 minutes at temperature (20-25 C°).

7. Fifty (50µl) of stop solution were added to all wells and the plate gently shook to mix the solution.

8. The absorbance was read on ELISA reader at 450 nm within 15 minutes after adding the stopping solution.

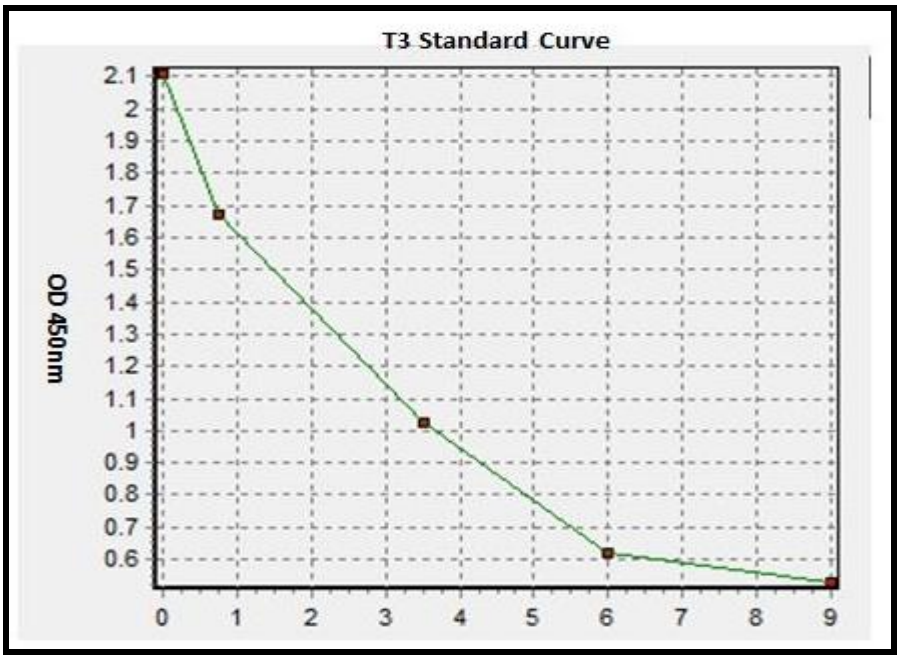

Figure (3-3): Standard curve of Triiodothyronin T3 hormone

3.4.3 Measurement Thyroxin (T4) hormone.

A/ Principle of the test

The (CBI) T4 is a solid phase competitive ELISA . The samples, working T4-HRP conjugate and anti-T4-biotin solution are added to the wells coated with streptavidin. T4 in the patients serum compete with a T4 enzyme (HRP) conjugate for binding sites. Unbound T4 and T4 enzyme conjugate are washed off by wash buffer. Upon the addition of the substrate , the intensity of color is inversely proportional to the concentration of T4 in the sample. A standard curve is prepared relating color intensity to the concentration of the T4.

B/ Assay Procedure

Before proceeding with the assay, reagents were allowed to stand at room temperature (20-25 C°) and all reagents were gently mixed before use.

1. The microplate's wells were formatted for each serum reference, control and patient specimen to be assayed in duplicate. Any unused microwell strip was backed into the aluminum bag sealed and stored at (2-8 C°).

2. Twenty five (25µl) of the appropriate serum reference, control or specimen into the assigned well.

3. Fifty (50µl) of the working T4-enzyme conjugate solution were added to all wells.

4. Fifty (50µl) of T4-antibody-biotin solution were added to all wells.

5. The plate was swirled gently for 20-30 second for mixing then covered and incubated for 60 minutes at room temperature (20-25 C°)

5. The liquid was removed from all wells and washed 3 times with 300 µl of 1X wash buffer then blotted on absorbent paper towels.

6. One hundred (100µl) of TMB substrate were added to all wells then incubated for 15 minutes at temperature (20-25 C°).

7. Fifty (50µl) of stop solution was added to all wells and the plate was gently mixed for 15-20 second.

8. The absorbance was read on ELISA reader at 450 nm within 15 minutes after adding the stopping solution.

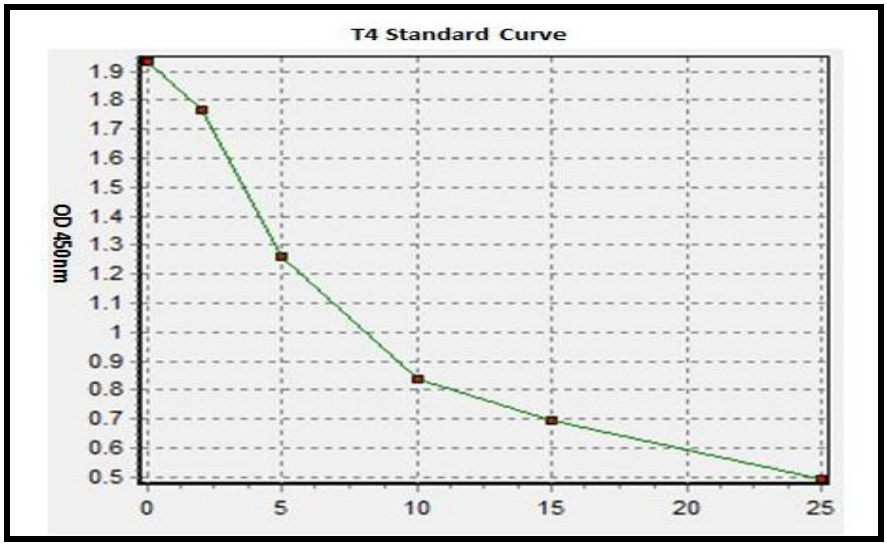

Figure (3-4): Standard curve of thyroxin hormone T4.

3.5 Measurement of Apoptotic biomarkers

3.5.1 Measurements of sFAS and sFAS ligand.

ELISA kit was be applied to in vitro-quantitative determination of human soluble FAS and soluble FAS ligand concentration in serum.

A/ Principle of the test of sFAS and sFASL.

These sFAS and sFAS ligand ELISA kit is intended laboratory for research use only and is not for use in diagnostic or therapeutic procedure. The stop solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm using a spectrophotometer. In order to measure the concentration of both sFAS and sFAS ligand in the sample. These kits that previously mentioned include a set of calibration standards. The calibration standards are assayed at the same time as the samples and allow the operator to produce a standard curve of optical density versus sFAS and sFAS ligand concentration. The concentration of the apoptotic biomarkers in the samples is then determined by comparing the O.D of the sample to standard curve.

B/ Assay Procedure

1- All reagents were prepared before starting assay procedure. It is recommended that all standards and samples be added in duplicate to microelisa stripplate.

2- The standards and testing sample wells were set then 50 µl of the standards were added to each well.

3- Ten (10) µl of testing sample were added firstly then 40 µl of sample diluent were added to testing sample wells; anything doesn't add to blank well.

4- One hundred (100) µl of HRP- conjugate reagent was added to each well, covered with an adhesive strip and incubated for 60 minutes at 37C°.

5- Each well was aspirated and washed. This process was repeated 4 times for a total of five washes. Each well was filled with wash solution (400µl) using squirt bottle, manifold dispenser or autowasher. The removal of liquid completely at each step is essential to good performance. After the last wash, any remaining of wash solution was removed by aspirating or decanting. The plate was inverted and blotted against clean paper towels.

6- Fifty (50µl) of chromogen solution A and 50µl of chromogen solution B

was added to each well. Gently were mixed and incubated for 15 minutes at 37C°.

8- Fifty (50µl) of stop solution was added to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or color change doesn't appear uniform. The plate was gently tapped to ensure thorough mixing.

9- The optical density (O.D) at 450nm was read using a microtiter plate reader within 15 minutes.

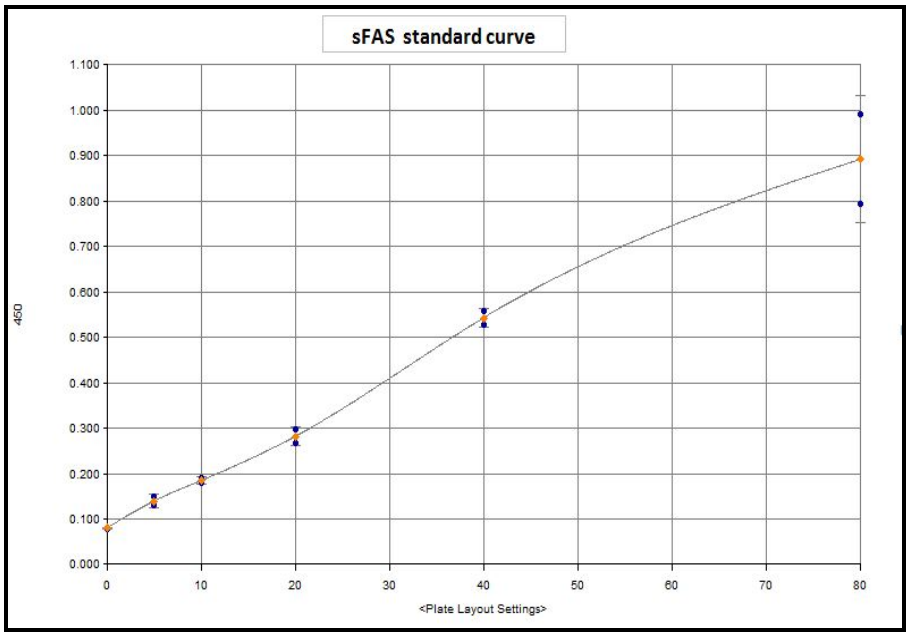

Figure (3-5): Standard curve of soluble FAS.

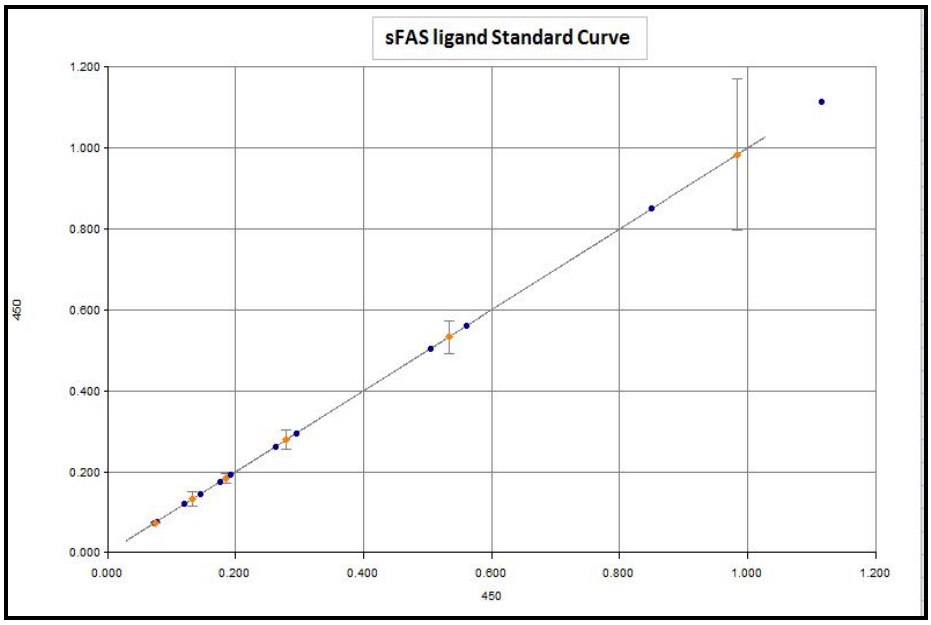

Figure (3-6): Standard curve of soluble FAS Ligand.

3.5.2 Measurements of Bcl-2 and Cytochrome C.

A/ Principle

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plates provided in these kits has been pre-coated with an antibody specific to human Bcl-2 and cytochrome C. Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain human Bcl-2 and cytochrome C biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm±2nm. The OD value is proportional to the concentration of human Bcl-2 and cytochrome C. The calculation of concentrations of Bcl-2 and cytochrome C occur by comparing the OD of the samples to the standard curve.

B/ Assay Procedure

1- The wells were determined for diluted standard, blank and sample. 100µl was added to each dilution of standard, blank and sample into appropriate wells (It was recommended that all samples and standards be assayed in duplicate). The plate was covered with sealer provided in the kit. The plate was incubated for 90 min at 37°C. solutions were added to the bottom of micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible

2- The solution was decanted from each well and didn't wash. Immediately 100 µl of Biotinylated detection Ab working solution was added to each well. The plate was covered with the plate sealer. The mixed gently and incubated for 1 hour at 37°C.

3- The solution was decanted from each well. 350µl of wash buffer were added to each well. Soaked for 1-2 minutes and the solution was aspirated or decanted from each well and patted it dry against clean absorbent paper. This wash step was repeated 3 times in total. Note: a microplate washer can be used in this step and other wash steps. The tested strips was made in use immediately after the wash step. The wells didn't allowed to be dry.

4- One-hundred (100µl) of HRP Conjugate working solution were added to each well. The plate was covered with sealer and incubated for 30 minutes at 37°C.

5- The solution was decanted from each well. The wash process was repeated for 5 times as conducted in step3.

6- Ninety (90µl) of substrate reagent were added to each well. The plate was covered with sealer and incubated for 15 minutes at 37°C. The plate was protected from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. The microplate reader was preheated for about 15 min before OD measurement.

7- Fifty (50µl) of stop solution were added to each well. Note: adding stop solution should be done in the same order as the substrate solution.

8- The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450nm.

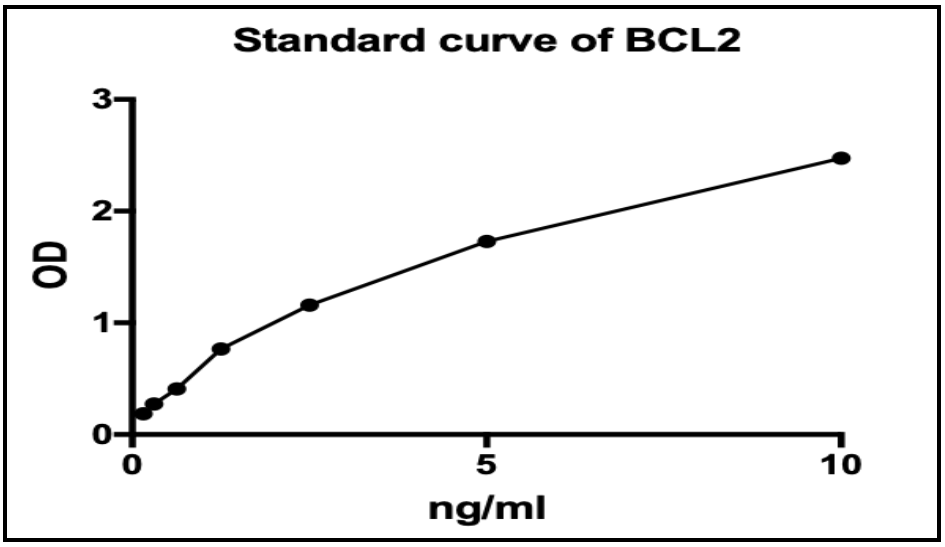

Figure (3-7): Standard curve of human B- cell Leukemia/Lymphoma 2 (Bcl-2)

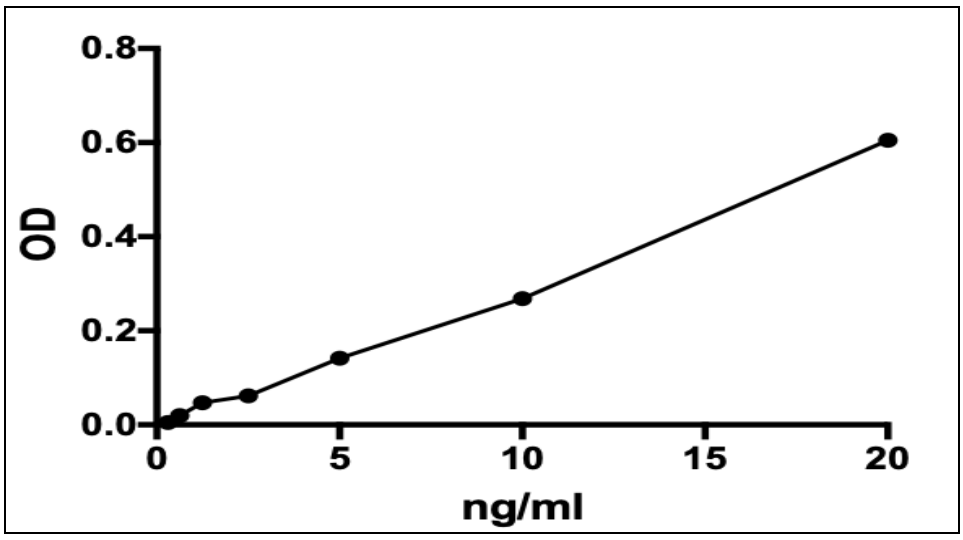

Figure (3-8): Standard curve of cytochrome C .

3.6 Measurement of oxidative stress biomarker TBARS.

This sandwich kit is for the accurate quantitative detection of human Thiobarbituric Acid Reactive Substance (also known TBARS) in the serum.

A/ Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human TBARS antibody. TBARS present in the sample is added and bound to antibodies coated on the wells and then biotinylated human TBARS antibody is added and bind to TBARS in the sample, then Streptavidin-HRP is added and bound to the Biotinylated TBARS antibody. After incubation unbound Streptavidin-HRP has washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human TBARS. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B/ Assay procedure

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use . the assay is performed at room temperature.

2. The number of strips was determined which required for the assay and the strips were inserted in the frames for use. The unused strips should be stored at 2-8 C°.

3. Fifty (50 µl) of standard were added to standard wells. The antibody didn't add to standard wells because the standard solution contain biotinylated antibody.

4. Forty (40 µl) of sample were added to sample wells and then 10 µl of anti-TBARS antibody were added to sample wells. Then 50 µl of streptavidin-HRP

were added to sample and standard wells (not blank control well). Then mixing well and the plate was covered with sealer and incubated 60 minutes at 37 C°.

5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minutes for each wash. For automated washing all wells were aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer. The plate was blotted on to paper towels or the absorbent material.

6. Fifty (50 µl) of substrate solution A and B were added to each well then the plate was incubated after being covered with new sealer for 10 minutes at 37 C° in the dark.

7. Fifty (50 µl) of stop solution were added to each well, the blue color will change in to yellow immediately .

8. The optical density (O.D) value of each well was determined using a microplate reader set to 450 nm within 10 minutes after adding stop solution .

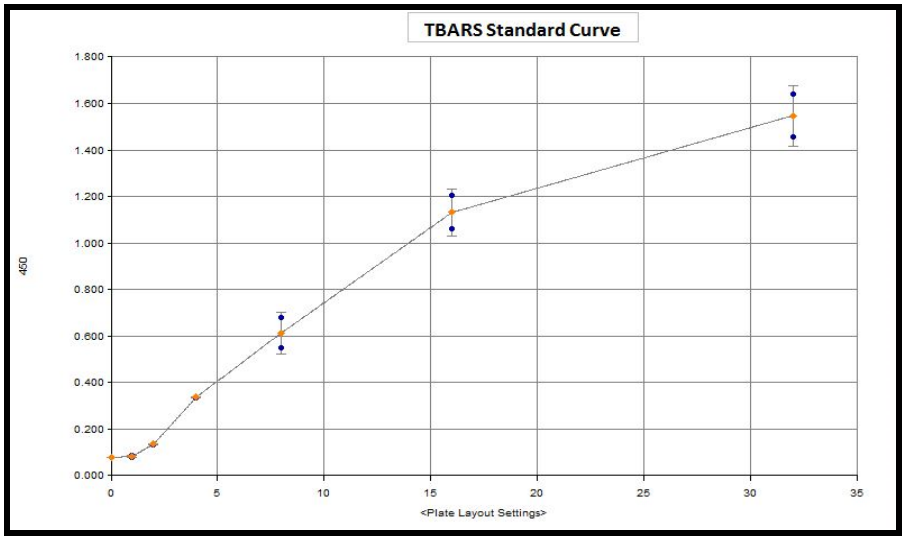

Figure (3-9): Standard curve of Thiobarbituric Acid Reactive Substance (TBARS) .

3.7 Measurement of antioxidant biomarker SepP1.

This sandwich kit is for the accurate quantitative detection of human Selenoprotein P1(also known SEPP1) in the serum.

A/ Assay Principle

This kit is an Enzyme-Linked Immunosorbent Assay (ELISA). The plate has been pre-coated with human SEPP1 antibody. SEPP1 present in the sample is added and bind to antibodies coated on the wells and then biotinylated human SEPP1 antibody is added and bind to SEPP1 in the sample, then Streptavidin-HRP is added and bind to the Biotinylated SEPP1 antibody. After incubation unbound Streptavidin-HRP is washed away during a washing step. Substrate solution is then added and color develops in proportion to the amount of human SEPP1. The reaction is terminated by addition of acidic stop solution and absorbance is measured at 450 nm.

B/ Assay procedure

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were bring to room temperature before use . the assay is performed at room temperature.

2. The number of strips was determined which required for the assay and the strips were inserted in the frames for use. The unused strips should be stored at 2-8 C°.

3. Fifty (50 µl) of standard were added to standard wells. The antibody didn't add to standard wells because the standard solution contain biotinylated antibody.

4. Forty (40µl) of sample were added to sample wells and then 10 µl of anti-TBARS antibody were added to sample wells. Then 50 µl of streptavidin-HRP

were added to sample and standard wells (not blank control well). Then mixing well and the plate was covered with sealer and incubated 60 minutes at 37 C°.

5. The sealer was removed and the plate was washed 5 times with wash buffer. The wells were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minutes for each wash. For automated washing all wells were aspirated and washed 5 times with wash buffer, overfilling wells with wash buffer. The plate was blotted on to paper towels or the absorbent material.

6. Fifty (50 µl) of substrate solution A and B were added to each well then the plate incubated after covered with new sealer for 10 minutes at 37 C° in the dark.

7. Fifty (50 µl) of stop solution were added to each well, the blue color will change in to yellow immediately .

8. The optical density (O.D) value of each well was determined using a microplate reader set to 450 nm within 10 minutes after adding stop solution .

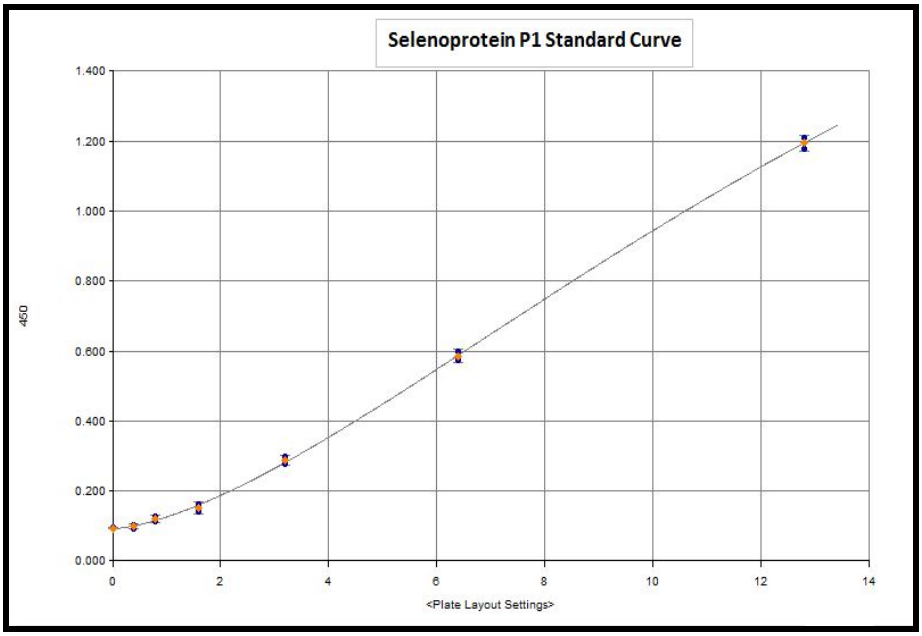

Figure (3-10): Standard curve of Selenoprotein P1 (SepP1)

3.8 Statistical Analysis

Using the SPSS edition 23, statistical analysis is carried out for the acquisition of mean, standard error, Chi square, one way ANOVA , sensitivity, specificity and ROC curve. Correlation is accomplished among studied biomarkers by the Pearson coefficient. The results were significant at $P<0.05$.

CHAPTER FOUR

RESULTS

4.1 Demographic and characteristics of study population

During six months period spent for data collection, total samples of 90 subjects were collected divided into: 30 person as a control, 20 patients had hypothyroidism without treatment and 40 hypothyroid patients with treatment.

Table (4.1) demonstrates characteristics of the study population related to age distribution, gender, smoking, types of hypothyroidism and residence. The sample was grouped into four age strata that covered the study population ages. There was increased in the incidence of hypothyroidism at interval age (40-49) in both patients groups (with and without treatment) by(25% and 35% respectively) while the increasing of the disease occur only in with treatment group of 50% at ≥50 of age.

The same table showed that the majority of cases and controls were females (90% and 85%) in both patients groups. There were about (60% and 57%) of cases in without and with treatment groups respectively had Hashimoto's thyroiditis while (40% &42%) of cases had thyroidectomy. Furthermore, the table showed that (65%) of patients (without and with treatment groups) were from urban area whereas only (35%) of them from rural area.

Table (4.1) Demographic and characteristics of patients and control groups.

Studied groups / Study variable	Control Group NO.(%)	Patient Groups		X^2	P value
		without treatment NO.(%)	With Treatment NO.(%)		
Age				10.879	0.071
20-29	3(10)	3(15)	6(15)		
30-39	10(33.3)	6(30)	4(10)		
40-49	7(23.3)	7(35)	10(25)		
≥50	10(33.3)	4(20)	20(50)		
Total	30(100)	20(100)	40(100)		
Gender				0.450	0.804
Male	5(16.7)	2(10)	6(15)		
Female	25(83.3)	18(90)	34(85)		
Total	30(100)	20(100)	40(100)		
Type of hypothyroidism				0.034	0.853
Hashimoto's thyroiditis	0(0)	12(60)	23(57.5)		
Thyroidectomy	0(0)	8(40)	17(42.5)		
Total	30(100)	20(100)	40(100)		
Residence				0.225	0.897
Rural	9(30)	7(35)	14(35)		
Urban	21(70)	13(65)	26(65)		
Total	30(100)	20(100)	40(100)		

* significant difference P≤ 0.05 among studied groups.

4.2 Thyroid profile

The results of table (4.2) was demonstrated that the level of thyroid stimulating hormone (TSH) increased significantly in patients of without treatment group (12.29±1.56) in compared to control group (1.13±0.17) then tend to decrease in patients of with treatment group (9.73±0.98) in compared to those of without treatment. Triiodothyronin T3 and thyroxin T4 levels were

decreased significantly in patients without treatment (0.57±0.09, 3.29±0.19) respectively when compared to control (1.07± 0.16 , 4.10±0.10) respectively.

Table (4.2) Comparison of serum TSH and thyroid hormones levels between control and patients groups.

Studied groups / Thyroid hormones	Control group No. (30)	Patients group		P value
		Without treatment No. (20)	With treatment No. (40)	
	Means ± SE	Mean± SE	Mean± SE	
TSH (µIU/ml)	1.13±0.17 a	12.29±1.56 b	9.73±0.98 b	0.001
T3 (pg/ml)	1.07±0.16 a	0.57±0.09 b	0.86±0.10 ab	0.016
T4 (mg/dl)	4.10±0.10 a	3.29±0.19 b	4.02±0.14 a	0.001

- Similar letters refer to non-significant difference among studied groups.
- Different letters refer to significant difference among studied groups.

4.3 Apoptotic biomarkers

The levels of Fas and FasL as biomarkers for apoptosis recorded an increasing in without treatment group (6.77±0.61, 63.97±8.11) respectively in comparison with control group (4.86±0.31, 56.52±3.32). After treatment the levels of Fas and FasL appeared to be continued in their elevation may be due to insufficient the dose of the levothyroxine to restore the normal function of the body. The levels were (7.74±0.51, 80.58±4.94) of Fas and FasL respectively in compared to control group as explained in table (4.3).

The same table was explained the levels of two opposite indicators of apoptosis one had anti-apoptotic characteristics which is Bcl-2 and the other which is Cytochrome C, its release from mitochondria initiate the apoptosis.

Both markers were increased significantly in patients of without treatment group (Bcl-2 = 0.49±0.13, Cytochrome C = 9.62±1.24) in comparison with control group (0.25±0.04, 5.43±0.46) respectively. The levels of Bcl-2 appeared to decrease in patients after received the treatment (0.36±0.06) compared with those didn't receive treatment (0.49±0.13) but the decreasing appears to be in significant. The same manner was repeated with Cytochrome C which decreased significantly in patients of with treatment group (6.29±0.74) versus those of without treatment group (9.62±1.24).

Table (4.3) Comparison of serum apoptotic biomarkers levels between control and patients groups.

Studied groups / Apoptotic Markers	Control group No. (30)	Patients group		P value
		Without treatment No. (20)	With treatment No. (40)	
	Means ± SE	Mean± SE	Mean± SE	
sFas (ng/ml)	4.86±0.31 a	6.77±0.61 b	7.74±0.51 b	0.017
sFas ligand (pg/ml)	56.52±3.32 a	63.97±8.11 a	80.58±4.94 b	0.001
Bcl-2 (ng/ml)	0.25±0.04 a	0.49±0.13 b	0.36±0.06 ab	0.037
Cytochrome C (ng/ml)	5.43±0.46 a	9.62±1.24 b	6.29±0.74 a	0.001

- Similar letters refer to non-significant difference among studied groups.
- Different letters refer to significant difference among studied groups.

4.4 Oxidative stress and antioxidant biomarkers

According to table (4.4), there was significant increasing in the oxidative stress representing by measuring the concentration of TBARS in patients with hypothyroidism whose didn't received treatment (9.21±0.33) in comparison to subjects in control group (8.23±0.24) whereas after the patients treated with levothyroxine the level of TBARS decreased (8.89±0.23) nearly to that of persons in control group.

Table (4.4) also clarify that there was significant decrease in the level of SepP1 as a biomarker for anti-oxidation in patients of without treatment group (0.51±0.05) versus its level in subjects of control group (1.05±0.19).

Table (4.4) Comparison of serum Thiobarbituric acid reactive substance (TBARS) and selenoprotein P1 (SepP1) levels between control and patients groups.

Studied groups / Oxidative &Antioxidant Markers	Control group No. (30)	Patients group		P value
		Without treatment No. (20)	With treatment No. (40)	
	Means ± SE	Mean± SE	Mean± SE	
TBARS (nmol/ml)	8.23±0.24 a	9.21±0.33 b	8.89±0.23 ab	0.023
SepP1 (mg/L)	1.05±0.19 a	0.51±0.05 b	0.74±0.08 ab	0.025

- Similar letters refer to non-significant difference among studied groups.
- Different letters refer to significant difference among studied groups.

4.5 Red blood cells indices

Red blood cells indices are blood test provide information about (RBCs count, hemoglobin (Hb), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). The results of table (4.5) were explained the most important changes in the above hematological parameters in

all studied groups. There was a significant decreasing in the levels of (Hb=11.60±0.29, HCT=37.98±1.18, MCH=25.83±0.84, MCHC=30.49±0.62) in the patients of without treatment groups compared to those in control (12.51±0.18, 40.79±0.65, 27.95±0.47 and 33.20±0.15) respectively. While the MCV recorded significant increasing in the patients of with treatment group (86.56±1.31) versus to persons in control group (81.19±1.17).

Table (4.5) Comparison of red blood cells indices between control and patients groups.

Studied groups / RBCs Indices	Control group No. (30)	Patients group		P value
		Without treatment No. (20)	With treatment No. (40)	
	Means ± SE	Mean± SE	Mean± SE	
RBCs Count /(10^6)	4.52±0.06 a	4.46±0.17 a	4.47±0.10 a	0.755
Hb (g/dl)	12.51±0.18 a	11.60±0.29 b	12.11±0.21 ab	0.014
HCT (%)	40.79±0.65 a	37.98±1.18 b	38.80±0.65 b	0.004
MCV (fl)	81.19±1.17 a	84.65±2.01 ab	86.56±1.31 b	0.006
MCH (pg/cell)	27.95±0.47 a	25.83±0.84 b	25.77±0.55 b	0.028
MCHC (g/dl)	33.20±0.15 a	30.49±0.62 b	29.69±0.28 b	0.001
RDW (fl)	14.31±0.28 a	15.72±1.63 a	15.73±1.14 a	0.418

- Similar letters refer to non-significant difference among studied groups.
- Different letters refer to significant difference among studied groups.

4.6 Changes in the number of different types of leucocytes

The different white blood cells (WBCs) including (granulocytes, lymphocytes and monocytes) were decreased in patients groups (without & with treatment) when compared to control group as shown in table (4.6).

The decreasing was significant in the number of lymphocytes in patients of without and with treatment groups (1.77±0.11, 1.89±0.12) respectively versus persons in control group (2.29±0.13). While monocytes numbers was decreased significantly only in patients of with treatment group (0.42±0.03) when compared to control group (0.56±0.03).

Table (4.6) Comparison of different types of leukocytes between control and patients groups.

Studied groups / Leukocytes	Control group No. (30)	Patients group		P value
		Without treatment No. (20)	With treatment No. (40)	
	Means ± SE	Mean± SE	Mean± SE	
WBCs (10^9/L)	7.00±0.37 a	6.63±0.36 a	7.02±0.39 a	0.562
Lymphocytes (10^9/L)	2.29±0.13 a	1.77±0.11 b	1.89±0.12 b	0.014
Monocytes (10^9/L)	0.56±0.03 a	0.50±0.05 ab	0.42±0.03 b	0.010
Granulocytes (10^9/L)	4.11±0.29 a	4.05±0.25 a	4.08±0.26 a	0.985

- Similar letters refer to non-significant difference among studied groups.
- Different letters refer to significant difference among studied groups.

4.7 Platelets Indices

Platelets Indices (PI) or platelets parameters refer to (platelets count, mean platelets volume (MPV), plateletcrit (PCT) and platelets distribution

width (PDW). Developing evidence submits that these (PI) may have diagnostic and prognostic value in certain diseases.

Table (4.7) was demonstrated that platelets count was recorded significant decreasing in patients of with treatment group (226.40±8.99) versus control group (250.1±7.82). The plateletcrit (PCT) and platelets distribution width (PDW) were decreased significantly in both patients groups (without treatment had 0.29±0.08, 14.57±0.30 of PCT and PDW) respectively, with treatment had (0.43±0.10, 14.42±0.21 of PCT and PDW) when compared to control group (PCT= 1.01±0.21, PDW= 15.63±0.05).

Table (4.7) Comparison of platelets indices between patients and control groups.

Studied groups / Platelets Indices	Control group No. (30)	Patients group		P value
		Without treatment No. (20)	With treatment No. (40)	
	Means ± SE	Mean± SE	Mean± SE	
Platelets (10^9/L)	250.1±7.82 a	245.6±8.80 ab	226.40±8.99 b	0.048
MPV (fl)	9.38±0.18 a	8.91±0.21 a	9.00±0.15 a	0.117
PCT (%)	1.01±0.21 a	0.29±0.08 b	0.43±0.10 b	0.003
PDW (fl)	15.63±0.05 a	14.57±0.30 b	14.42±0.21 b	0.002

- Similar letters refer to non-significant difference among studied groups.
- Different letters refer to significant difference among studied groups.

4.8 Differences in thyroid profile with respect to gender

Table (4.8) was demonstrated differences between males and females among control, without and with treatment groups with respect to (TSH, T3 and T4). There was a non- significant increasing in the levels of TSH in the females of studied groups: control, without and with treatment (1.20±0.20, 12.58±1.71, 9.69±1.08) respectively when compared to males in the same groups (0.75±0.24, 9.73±2.71, 6.96±2.63) respectively. The levels of T3 decreased significantly in the females of without treatment group (0.51±0.09) versus males of the same group (1.16±0.27).

On the other hand, the comparison among females of studied groups was revealed a significant increasing in the levels of TSH in the females of both patients group (12.58±1.71, 9.69±1.08) compared to control (1.20±0.20). The same manner was repeated with males in the studied groups. The levels of thyroid hormones were decreased significantly in females of without treatment group (T3=0.51±0.09, T4=3.31±0.20) in comparison to healthy females (1.03±0.18, 4.00±0.11) respectively.

Table (4.8) Mean differences of thyroid profile according to gender among studied groups.

Thyroid hormones variable	Studied groups	Gender				P. value
		Male	No.	Female	No.	
		Mean± SE		Mean± SE		
TSH (µIU/ml)	Control	0.75±0.24 [a]	5	1.20±0.20 [a]	25	0.363
	Without treatment	9.73±2.71 [b]	2	12.58±1.71 [b]	18	0.599
	With treatment	6.96±2.63 [b]	6	9.69±1.08 [b]	34	0.926
T3 (pg/ml)	Control	2.01±0.28 [a]	5	1.03±0.18 [a]	25	0.461
	Without treatment	1.16±0.27 [a]	2	0.51±0.09 [b]	18	0.046*
	With treatment	0.88±0.35 [a]	6	0.85±0.10 [a]	34	0.984
T4 (mg/dl)	Control	4.60±0.09 [a]	5	4.00±0.11 [a]	25	0.070
	Without treatment	3.06±0.95 [b]	2	3.31±0.20 [b]	18	0.713
	With treatment	3.42±0.33 [b]	6	4.12±0.15 [a]	34	0.080

*** Significant difference P≤ 0.05 between males and females each studied groups.**

- Similar letters refer to non-significant difference among the same gender in the studied groups.

- Different letters refer to significant difference among the same gender in the studied groups.

4.9 Differences in apoptotic markers with respect to gender.

The levels of studied apoptotic biomarkers among males and females of the current study were explained in table (4.9). The comparison between males and females with regard to levels of Fas and Cytochrome C in all studied groups wasn't significant.

Females of without treatment group had significant increasing in the levels of FasL (68.30±8.39) when compared to males of the same group (25.00±5.93). The levels of Bcl-2 decreased significantly in females of with treatment group (0.32±0.05) in comparing to males of the same group (0.61±0.24). Comparison among females of the studied groups revealed significant increasing of sFas in both patients group (without treatment 7.08±0.63, with treatment 7.72±0.58) when compared to control (4.94±0.35).

The levels of sFasL in females of with treatment group increased significantly (83.19±5.43) versus to that in control (57.23±3.85). the concentration of both Bcl-2 and Cytochrome C increased significantly in females of without treatment (0.50±0.14, 9.79±1.36) respectively when compared to control (0.24±0.05, 5.08±0.49) respectively.

Table (4.9) Mean differences of apoptotic biomarkers according to gender among studied groups.

Apoptotic variable	Studied groups	Gender				P. value
		Male	No.	Female	No.	
		Mean ± SE		Mean ± SE		
Fas (ng/ml)	Control	4.93±0.79 a	5	4.94±0.35 a	25	0.611
	Without treatment	4.00±1.43 a b	2	7.08±0.63 b	18	0.138
	With treatment	7.82±0.83 b	6	7.72±0.58 b	34	0.948
FasL (pg/ml)	Control	52.96±5.70 a b	5	57.23±3.85 a	25	0.641
	Without treatment	25.00±5.93 a	2	68.30±8.39 a b	18	0.004*
	With treatment	65.78±10.80 b	6	83.19±5.43 b	34	0.213
Bcl-2 (ng/ml)	Control	0.25±0.08 a	5	0.24±0.05 a	25	0.649
	Without treatment	0.46±0.23 a	2	0.50±0.14 b	18	0.425
	With treatment	0.61±0.24 a	6	0.32±0.05 a	34	0.005*
Cytochrome C (ng/ml)	Control	7.18±0.90 a	5	5.08±0.49 a	25	0.505
	Without treatment	8.11±3.25 a	2	9.79±1.36 b	18	0.322
	With treatment	4.82±1.25 a	6	6.55±0.84 a	34	0.391

*** Significant difference P≤ 0.05 between males and females each studied groups.**

- Similar letters refer to non-significant difference among the same gender in the studied groups.

- Different letters refer to significant difference among the same gender in the studied groups

4.10 Differences in oxidative stress and anti-oxidant markers with respect to gender.

Oxidative stress represented by measuring the levels of TBARS recorded significant increasing in females of with treatment group (9.05±0.26) in comparison to its level in the males of the same group (6.98±0.32). There was also a significant increasing in the concentration of TBARS in the females of without treatment group (9.34±0.35) compared to females of control group (8.29±0.27). The results of SepP1 as an anti-oxidant marker recorded significant increasing in females of without treatment groups (0.54±0.05) versus to males of the former group (0.21±0.01). The levels SepP1 in females of without and with treatment groups appeared to be increased significantly (0.54±0.05, 0.72±0.09) compared to females of control group (1.13±0.22). As explained in table (4.10).

Table (4.10) Mean differences of TBARS and SepP1 according to gender among studied groups.

Oxidative stress and antioxidant variable	Studied groups	Gender				P. value
		Male Mean ± SE	No.	Female Mean ± SE	No.	
TBARS (nmol/ml)	Control	7.97±0.55 [a]	5	8.29±0.27 [a]	25	0.631
	Without treatment	7.98±0.55 [a]	2	9.34±0.35 [b]	18	0.229
	With treatment	6.98±0.32 [a]	6	9.05±0.26 [a]	34	0.025*
SepP1 (mg/L)	Control	0.37±0.04 [a]	5	1.13±0.22 [a]	25	0.003*
	Without treatment	0.21±0.01 [a]	2	0.54±0.05 [b]	18	0.000*
	With treatment	0.82±0.31 [a]	6	0.72±0.09 [b]	34	0.692

*** Significant difference P≤ 0.05 between males and females each studied groups.**

- Similar letters refer to non-significant difference among the same gender in the studied groups.

- Different letters refer to significant difference among the same gender in the studied groups.

4.11 Results of correlation among the study biomarkers.

4.11.1 Correlation among biomarkers of without treatment patients group

The correlations among all studied biomarkers in this patients group were showed in the appendix B. The correlation between apoptosis and oxidative stress was positive significant correlation (P value 0.002, r= 0.659) as shown in figure below.

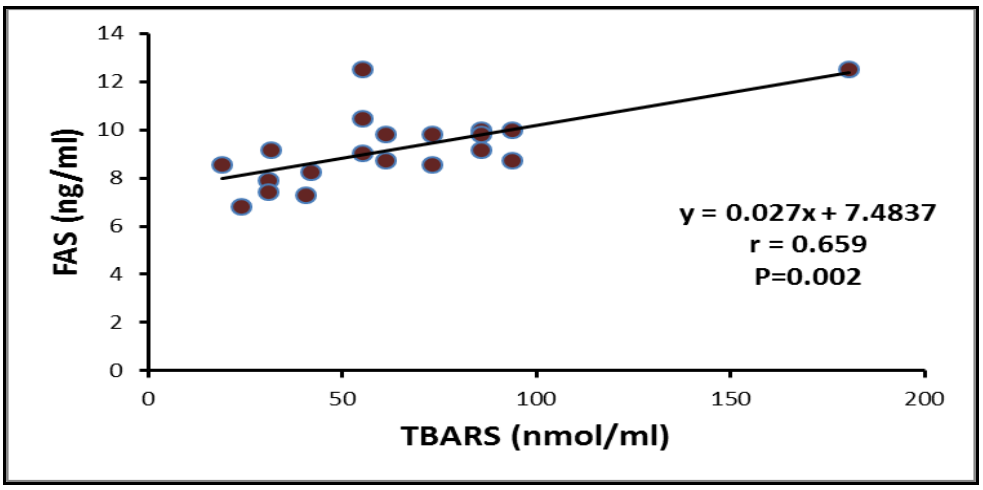

Figure (4-1): The correlation between Fas (ng/ml) and TBARS (nmol/ml) in hypothyroidism patients (without treatment group).

There was a significant negative correlation (P value 0.047, r= -0.449) between apoptosis representing by levels of Cytochrome C and the number of lymphocytes in hypothyroid patients without treatment as explained in figure (4.2).

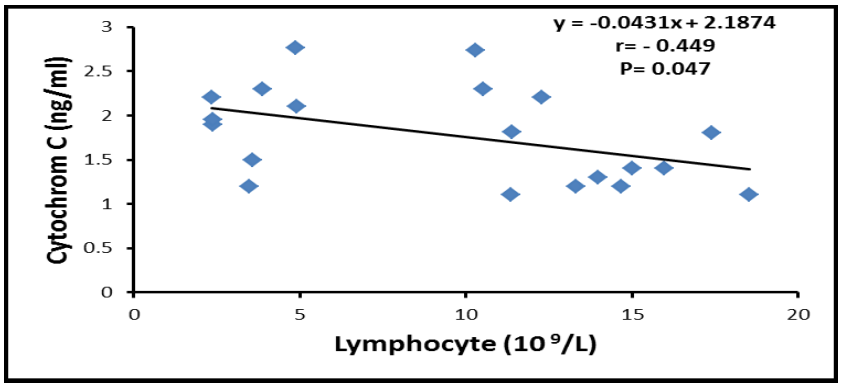

Figure (4-2): The correlation between cytochrome C (ng/ml) and lymphocytes number (10^9/L) in hypothyroidism patients (without treatment group).

4.11.2 Correlation among study biomarkers of patients with treatment group.

The correlation among all studied biomarkers in this patients group was showed in the appendix C. There was a negative significant correlation (P value 0.028, r= -0.347) between apoptosis represented by the levels of FasL and hemoglobin which consider a parameters of red cells indices in patients who continue to receive a levothyroxine as a treatment to hypothyroidism as shown in figure (4-3).

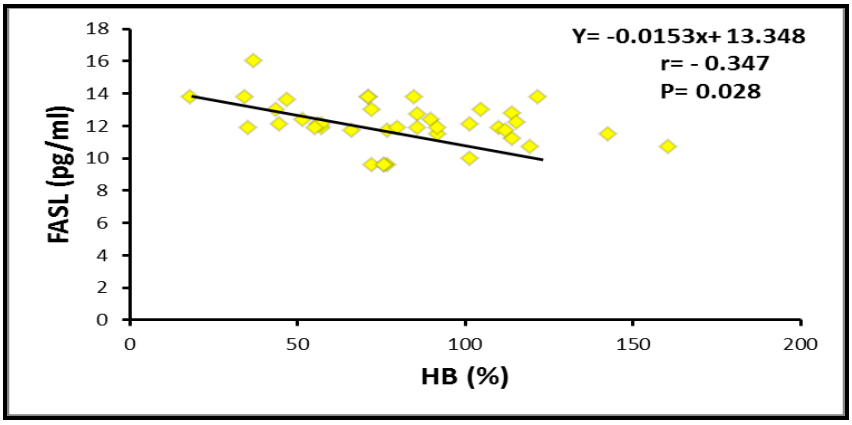

Figure (4-3): The correlation between FasL (pg/ml) and hemoglobin (Hb) in hypothyroidism patients (with treatment group).

The effect of oxidative stress on the numbers of RBCs in the patients of with treatment group was explained in figure (4-4) that reflect a negative significant correlation between TBARS and RBCs. (P value 0.048, r= -0.314).

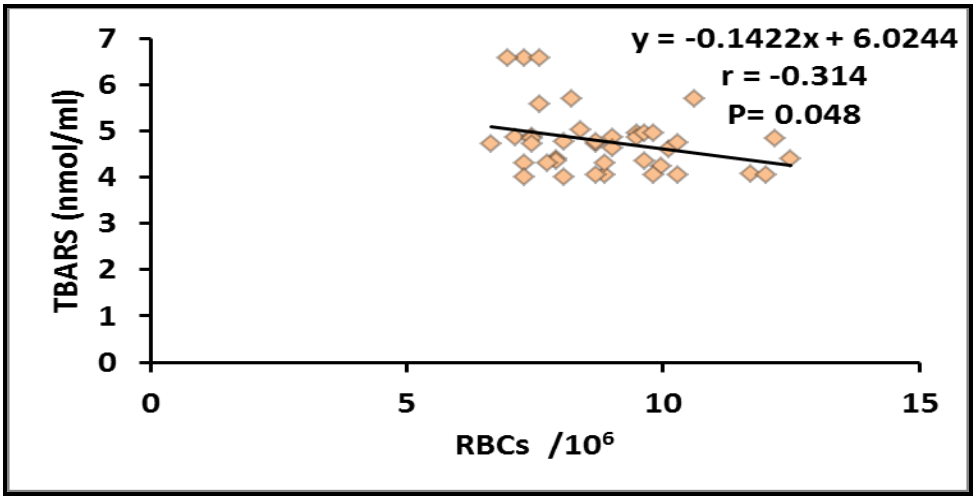

Figure (4-4): The correlation between TBARS (nmol/ml) and the number of Erythrocytes in hypothyroidism patients (with treatment group).

The correlation between triiodothyronin (T3) and mean corpuscular volume (MCV) which was one of red cells indices was negative significant correlation (P value 0.025, r= -0.353) as demonstrated below in figure (4-5).

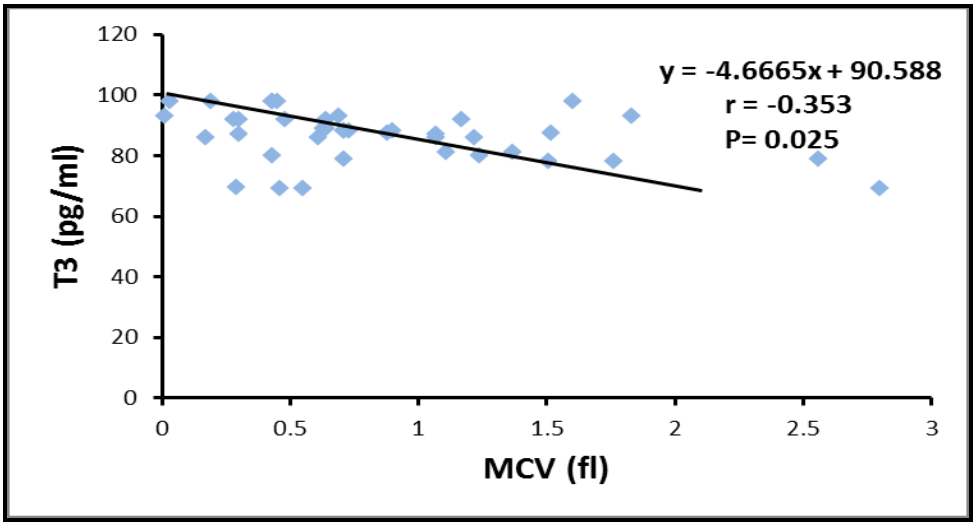

Figure (4-5): The correlation between Triiodothyronin (T3) (pg/ml) and the mean corpuscular volume (MCV) in hypothyroidism patients (with treatment group).

4.12 The sensitivity and specificity.

The results of table (4.11) was clarified that the studied biomarkers including (Fas, FasL, Bcl-2 and Cytochrome C) as apoptosis markers and (TBARS & SepP1) as oxidative and anti-oxidant markers had increasing in the sensitivity and decreasing in the specificity.

Table (4.11) The sensitivity and Specificity of studied biomarkers

Studied biomarkers	Sensitivity	Specificity	Cut off point
Fas	80.00	26.70	5.56
FasL	76.70	60.00	48.30
Bcl-2	58.00	53.20	0.17
Cytochrome C	50.17	42.10	5.23
TBARS	71.00	53.00	7.60
SepP1	65.00	46.70	0.44

4.13 Receiver Operating Characteristic (ROC) curve of studied biomarkers

Table (4.12) was demonstrated that area under curve (AUC) of Fas biomarker was 0.846 this refers to the possibility of well using this biomarker in the predicting of the prognosis of hypothyroidism. While the other studied markers can't use for the above purpose as their AUC located in poor area.

Table (4.12) Results of ROC curve of studied biomarkers

Factors	AUC	Asymptotic significant	Asymptotic 95% Confidence Interval	
			Lower bound	Upper bound
Fas	0.846	0.001	0.763	0.928
FasL	0.669	0.009	0.557	0.780
Bcl-2	0.558	0.373	0.435	0.681
Cytochrome C	0.561	0.351	0.442	0.679
TBARS	0.663	0.012	0.547	0.779
SepP1	0.503	0.969	0.361	0.644

Figure (4-6) explained that there was an increasing in the AUC of Fas bio marker with sensitivity (80%) and specificity (26%) at cut off (5.56) while FasL have decreased in AUC with sensitivity (76%) and specificity (60%) at cut off (48.3).

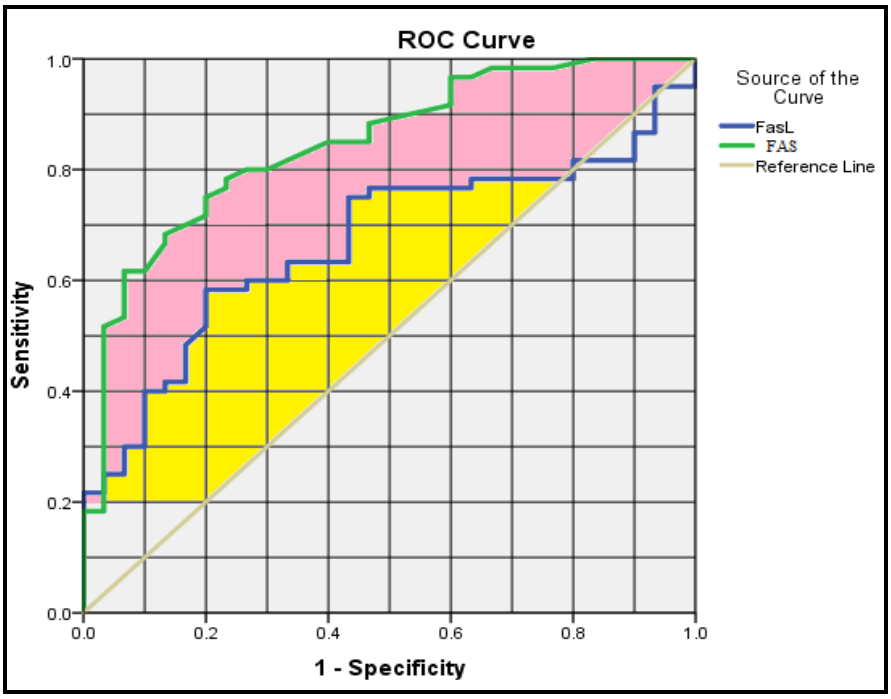

Figure (4-6): Receiver operator characteristic (ROC) curve of Fas & FasL in hypothyroidism patients.

Receiver operator characteristic (ROC) curve of Bcl-2 and Cytochrome C was shown in figure (4-7). This figure clarified that both biomarkers had low sensitivity and specificity (58.00, 53.20) respectively for Bcl-2 and (50.17, 42.10) respectively for Cytochrome C. Area under curve (AUC) of the formers biomarkers located in poor area with cutoff point 0.17 of Bcl-2 and 5.23 of Cytochrome C.

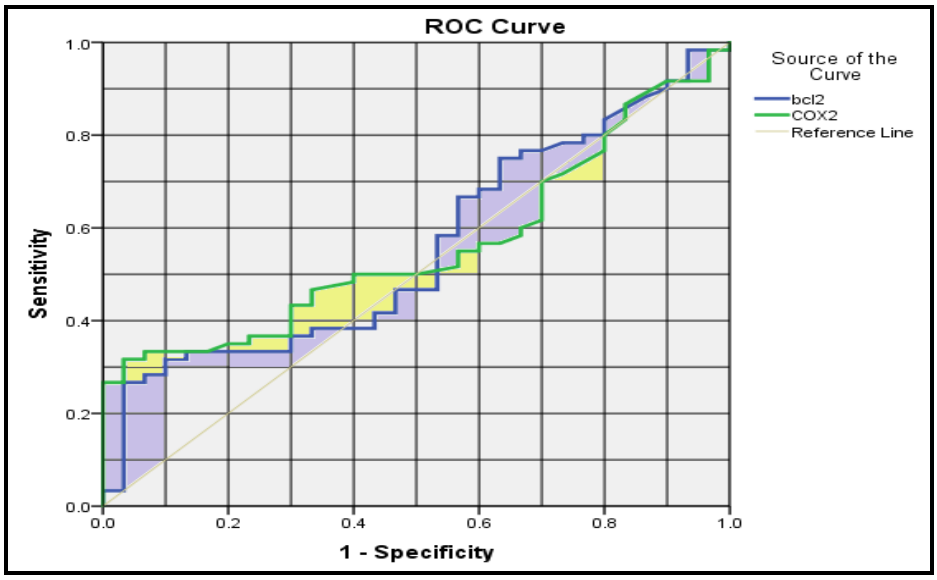

Figure (4-7): Receiver operator characteristic (ROC) curve of Bcl-2 & Cytochrome C in hypothyroidism patients.

The results of figure (4-8) were clarified that both TBARS and SepP1 had decreased in their AUC with sensitivity (71, 65%) respectively and specificity (53, 46%) respectively with TBARS cut off (7.6) and SepP1 cut off (0.44).

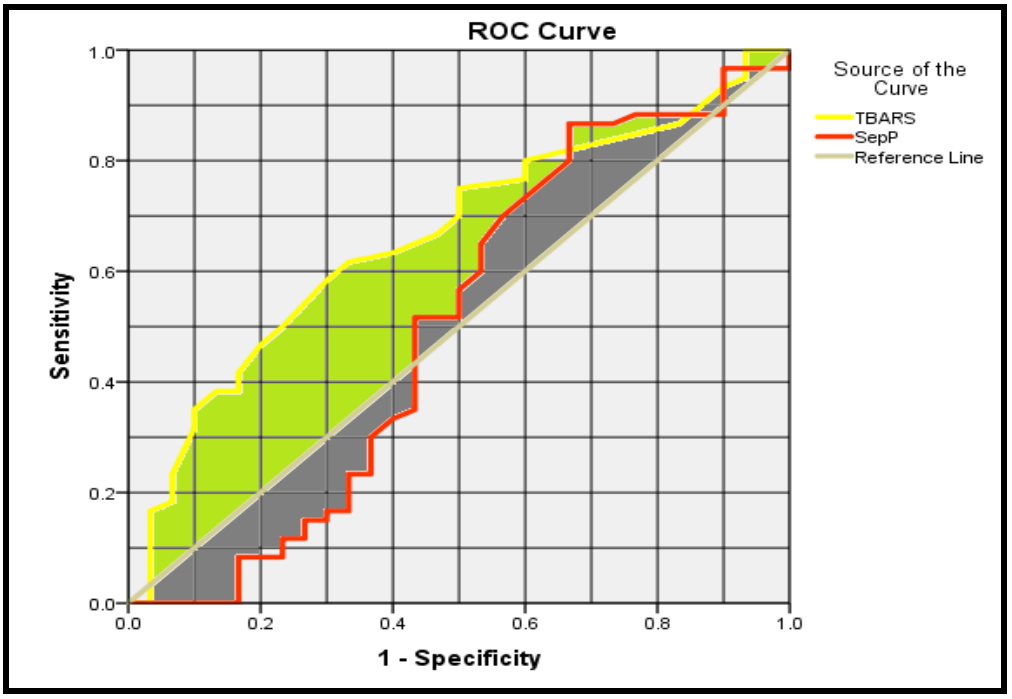

Figure (4-8): Receiver operator characteristic (ROC) curve of TBARS & SepP1 in hypothyroidism patients

4.14 Relative risk (RR) of studied biomarkers

The table (4.13) above was clarified that Fas had a relative risk (RR) = 2.9 with P value of 0.001 compared to the rest biomarkers which have RR larger than 1 but weren't significant. This increasing in the level of Fas might have a bad out come in patients with hypothyroidism.

Table (4.13) Relative risk (RR) of studied biomarkers

Studied markers	Relative risk (RR)	Confidence interval (CI 95%)	P value
Fas	2.9	(1.49-5.72)	0.001
FasL	1.2	(0.92-1.76)	0.137
Bcl-2	1.09	(3.74-5.99)	0.658
Cytochrome C	1.15	(3.50-6.58)	0.559
TBARS	1.3	(0.90-1.90)	0.153
SepP1	1.2	(0.83-1.78)	0.311

CHAPTER FIVE

DISCUSSION

Thyroid dysfunction is one of the most common endocrine disorders seen in clinical practice. The prevalence of thyroid dysfunction varies by age, sex, race/ethnicity, and geographically through variations in dietary iodine intake (Ittermann *et al.,* 2015). One of the most important type of thyroid dysfunction is hypothyroidism which is a chronic disease associated with deficiency in the thyroid hormones affects up to 5% of the general population, with a further estimated 5% being undiagnosed (Guglielmi *et al.,* 2018). Hypothyroidism is associated with decreased quality of life, increased number of sick leave days, and even increased mortality (Thvilum *et al.,* 2013).

5.1 Demographic and characteristics of study population

Through the results of table (4.1) which reflect that hypothyroidism increased in the fourth and fifth decades which are consistent with finding of (Shao *et al.,* 2017) that reported increased overt and sub clinical hypothyroidism at (46-55) and (≥50) years. Most previous studies deal with hypothyroidism from different aspects reported that the prevalence of this disease is increased with age especially at forties such as (Masullo *et al.*, 2018; Marchiori *et al.,* 2015). Bremner *et al.* (2012) suggested that with aging, the set point for TSH secretion is altered, resulting in higher serum TSH concentrations due to diminished sensitivity of thyrotropes to negative feedback of thyroid hormones.

Other studies demonstrated that if age-adjusted normal ranges are used in older adults, the prevalence of thyroid dysfunction may not increase with old age and some individuals might be reclassified from “abnormal” to “normal”, avoiding unnecessary treatment (Vadiveloo *et al.,* 2013). The debate regarding age-specific cut-points for thyroid dysfunction has clinical and economic implications, given the high prevalence in older adults and decreasing TSH threshold levels for treatment over the years (Taylor *et al.,* 2014).

In this study there is a predominance of hypothyroidism in females with 90% and 85% in both patients groups while the percentage of males was 10%

and 15% of patients. This is nearly as a result of an investigation for an unselected population in Mid-Norway stated that the prevalence of hypothyroidism was 4.8% and 0.9% for female and male, respectively (Bjùro *et al.,* 2000). Diab and his colleagues in 2019 reported women had a higher prevalence of treated hypothyroidism compared to males ($P < 0.001$). Even though higher prevalence of sub clinical hypothyroidism in women is unclear, it could be due to estrogen effect, higher prevalence of autoimmune thyroid diseases, and higher concentration of thyroperoxidase antibodies and thyroglobulin antibodies in females (Tekle *et al.,* 2018).

This study indicated that urban residency increased risk of developing hypothyroidism. There was about 65% of all patients living in urban area while 35% of them living in rural area. This result is quite similar to (Ha *et al.*,2018) who explain that 74.3% of their Korean patients with subclinical hypothyroidism living in urban area and 25.7% living in rural area. Ha and his research team reported increased risk of hypothyroidism associated with the area of residence. In women, the risk of SCH was significantly higher in urban area residents than in rural area residents regardless of the TSH cut-off level used. The risk of SCH in urban residents was 1.78 times higher using SCH-M and 2.18 times higher using SCH-P. Although the underlying mechanism of this difference is unclear, it is probably due to that urban women have more opportunities to consume high-iodine meals or supplementation.

5.2 Thyroid profile

The present study assessed the levels of TSH, T3 and T4 hormones in patients of both groups and control. The result revealed significant increase in TSH levels in patients compared to control on other hand the levels of thyroid hormones have significant decreased in patients versus to control. This results is identical to (Dahiya *et al.*,2016) who recorded high levels of TSH and low levels of T3 and T4. The levels of TSH is decreased after treatment by levothyroxine whereas thyroid hormone tend to increase in their levels this

result is in consistent with (Bolk *et al.*,2010) results which revealed a decrease in thyrotropin level of 1.25 μIU/L and an increase in FT4 and TT3 levels when levothyroxine is taken at bedtime. The management of primary hypothyroidism with levothyroxine (L-T4) is simple, effective and safe, and most patients report improved well-being on initiation of treatment. However, a proportion of individuals continue to suffer with symptoms despite achieving adequate biochemical correction (Okosieme *et al.*,2015).

5.3 Apoptotic biomarkers

Most of the previous studies that investigate the role of apoptosis in thyroid disorders were concerning the percentage of in situ apoptotic thyrocytes which increase in Hashimoto's thyroiditis but decreases in Graves' disease, suggesting that apoptosis has a role in function, regulation and cell proliferation of thyroid gland. Most of the apoptotic cells are detected in disrupted follicles on the periphery of infiltrating lymphoid cells (Hammond *et al.*,1997). But the present study was focused on studying the soluble apoptotic biomarkers that reflect the state of apoptosis in patients with hypothyroidism as a type of thyroid disorders.

According to the results of table (4.3) there was significant increasing in the level of sFAS and FASL in patients of both groups compared to control. The results also demonstrated that the increase continues after the patients had received their treatment. This is going with what Myśliwiec *et al.(*2006) concluded in his study in Poland which reported increased sFas levels in all the AITD patients, however, the highest concentrations were found in Hashimoto's thyroiditis. This is may be a consequence of the enhanced releasing of circulating forms of Fas that seem to reflect the intensity of Fas/FasL mediated apoptosis (Hiromatsu *et al.*,2004). As shown by Salmaso *et al.*(2002) in HT apoptosis in a higher degree involves thyrocytes than infiltrating lymphocytes that is connected with a more intense Fas expression on follicular cells.

Another study by (Fountoulakis *et al.*,2008) shown the proportion of CD4 lymphocytes expressing Fas was higher in patients with subclinical HT and those with GD when compared with controls. Cell apoptosis occurs in the normal thyroid at a low level. As new thyrocytes are produced, old cells are destroyed in order to maintain normal thyroid volume and function (Chistiakov, 2005).

Initiation of an out-of-control apoptotic mechanism in thyroid cells may be caused by various non-genetic injuries that affect expression of apoptosis inhibitor molecule Bcl-2 or membrane ligand FasL (Giordano *et al.*,2001). Thyrocytes from HT thyroid glands are able to hyperproduce Fas and FasL on their surfaces thus inducing fratricide apoptosis (Limachi & Basso, 2002). IL-1β, abundantly produced in HT glands, induces Fas expression in normal thyrocytes, the cross-linking of Fas resulting in massive thyrocyte apoptosis this can play a role in the progression of Hashimoto's thyroiditis (Giordano *et al.*,1997).

Serum concentration of Bcl-2 was increased significantly in hypothyroid patients of both groups (without and with treatment) comparable to control as table (4.3) showed. This is concordant with a study in 2009 by a Czech research team who explained that Bcl-2 increased in hypothyroid patients in comparison to control and patients with Graves' disease (Jiskra *et al.*,2009). Another study by (Myśliwiec *et al.*,2006) was reported that the levels of sBcl-2 values were increased in all the autoimmune thyroid diseases (AITD) groups however significantly higher sBcl-2 values were found only in euHashimoto thyroiditis (euHT) as compared to the controls. This is nearly similar to the result of current study which recorded continued increasing in the levels of Bcl-2 after hypothyroid patients received levothyroxine as a treatment compared to control. Bcl-2 may have a role in the pathogenesis of thyroiditis by inhibiting other pathways of apoptosis in thyrocytes.

Elevated Bcl-2 expression might be particularly beneficial at the early stages of thyroiditis (before Fas mediated, CD8 cytotoxicity is induced). At this early time point, apoptosis may be induced by oxidative stress or cytokines, and this might then result in a specific immune response. Therefore, increasing Bcl-2 concentrations may have a role in preventing thyroiditis- induced hypothyroidism by impeding the development of specific autoimmunity or blocking immune mediated cytotoxicity (Wang *et al.*,1999)

Release of cytochrome c from mitochondria is a major event during apoptosis. Released cytochrome c has been shown to activate caspase-dependent apoptotic signals (Kamal *et al.*,2004). The current study was assessed the levels of extracellular cytochrome C in hypothyroid patients and revealed that its levels were increased significantly in patients of newly diagnosed with hypothyroidism as well in treated hypothyroid patients compared to control. This result approximates to what Hara and his colleagues was found in 2001 when measured the levels of cytochrome c in the patients with Hashimoto's thyroiditis and confirm that in this disorder, cytochrome c levels of the supernatants were significantly higher than those in control subjects ($p<0.02$) (Hara *et al.*,2001).

Another study conducted by (Singh *et al.*,2003) found in the hypothyroid condition, a significantly higher level ($P<0.001$) of cytochrome c was detected in the cytosol at the early developmental stage from P_0 to P_8 and the decreased intensity was observed in the mitochondrial pellet ($P<0.001$). However, at late stages of development enhanced intensity of the cytochrome c band was observed in the mitochondrial pellet, with a concomitant decrease in the cytosol. It is suggested that hypothyroidism induced cytochrome c release to cytosol during early development might contribute to initiation of apoptosis through formation of apoptosomes and activation of caspase cascade (Huang *et al.*,2005). Bobba *et al.* (1999) reported that cytochrome c release is an event that occurs early in the commitment phase of the apoptotic process, and that

after accumulation, this protein might be progressively degraded by induced caspases.

5.4 Oxidative stress and antioxidant biomarkers

This study revealed an increasing in the oxidative stress in patients with hypothyroidism compared to control reflect by the levels of TBARS which elevated significantly in both patients groups versus to control. On other hand there was a slight decreasing after patients received the treatment. This results is confirm by (Pasupathi & Latha, 2008) and (Santi *et al.*,2012) who came to a conclusion that TBARS were significantly higher in patients with subclinical hypothyroidism than in healthy control. Marchiori *et al.*(2015) in their study reported non-significant reduction in the TBARS by 12 months of levothyroxine replacement therapy (LRT). Baskol *et al.*(2007) confirm the result of current study by recording increased in ROS before treatment and reduced after treatment but they were measure MDA instead of TBARS and both of them consider a products of lipid peroxidation.

These results indicate that thyroid dysfunction, in particular hypothyroidism, may enhance oxidative stress (Erdamar *et al.*,2008). Thyroid dysfunctions increase lipid peroxidation (LPO) reactions and ROS as documented by (Messarah *et al.*,2007). LPO is an autocatalytic mechanism leading to oxidative destruction of cellular membranes. Such destruction can lead to cell death and to the production of toxic and reactive aldehyde metabolites called free radicals, where malondialdehyde (MDA) is the most important. It is known that ROS would lead to oxidative damage of biological macro molecules, including lipids, proteins, and DNA (Messarah *et al.*,2010).

Free fatty acids are probably the main source of energy in thyroid cells, and free radicals leaking from thyroid cells might react with free fatty acids and hence initiate lipid peroxidation, which modifies the functional characteristics of the cellular membranes, changing their permeability and causing inactivation of membrane-bound receptors and enzymes (Resch *et al.*,2002).

The results of table (4.4) demonstrated a significant decrease in the levels of selenoprotein P1 (SepP1)in patients with hypothyroidism of both groups (with and without treatment) when compared to control group. This finding is going with the observation of (Nourbakhsha *et al.*,2016) & (Federige *et al.*,2017) who recorded a decreasing in SepP1 in patients with hypothyroidism and Gravers diseases compared to healthy persons. Selenoprotein P1 consider one of selenoproteins which are belonging to hepatokines, which are proteins that include an amino acid selenocysteine in their polypeptide chain and can be found in all lineages of life (Zoidis *et al.*,2018). They are produced in the liver and involved in defense against oxidative stress by taking part in oxidation-reduction reactions neutralizing reactive oxygen species (ROS) (Pitts *et al.*,2014).

Increased ROS levels in hypothyroidism may result in a pro-oxidation environment, which in turn could result in decreased antioxidant SepP1 activity (Baskol *et al.*,2007). (Gerenova & Gadjeva) in 2007 reported that there is a deficiency of cellular anti-oxidative defense in Hashimoto's thyroiditis patients in all stages of disease. Accordingly, they supposed that the supplementation with antioxidants from an early stage of the disease, in addition to thyroid hormone replacement, may have a positive benefit in the treatment.

Furthermore, SeP is considered a multifunctional protein, which appears to have Glutathione peroxidase-lik enzyme activity (GPx) (Mita *et al.*,2017). Glutathione peroxidase (GPx) consider one of the major defenses against harmful side effects of ROS in cells, and in cultured thyrocytes, it has a high capacity to degrade exogenous H_2O_2 (Björkman and Ekhom, 1995). It is possible that the impaired GPx activity may lead to H_2O_2 -induced apoptosis to thyroid cells in Hashimoto's thyroiditis patients (Gerenova and Gadjeva, 2007).

5.5 Red blood cell indices

Among the patients groups there was a decreasing in the number of erythrocytes, the level of hemoglobin, hematocrit, content of hemoglobin per

red blood cell (MCH) and the amount of hemoglobin per unit volume (MCHC) in comparison to control group. This is quite similar to Iranian study conducted by (Dorgalaleh *et al.*,2013) who reported a significant decreasing in the above red blood cell indices in hypothyroidism patients versus control and hyperthyroidism patients. Another study in 2010 by Kawa *et al.* revealed that thyroid hormone deficiency led to a decrease in total blood counts which including (RBCs count, Hb, MCH , MCHC) and clonogenic potential of BFU-E.

The results of the current study recorded continued decreasing in the previous red cell indices in patients who received thyroid replacement therapy. This result is confirming by (Bashir *et al.*,2012) who suggest that thyroid dysfunction is frequently associated with anemia in subclinical hypothyroidism and primary hypothyroidism. The results of their study reported continued decreasing in red cell indices in treated hypothyroidism patients.

Mean cell volume (MCV) in this study appear to be increased in patients groups compared to control . This result goes with (Kawa *et al.*,2010) who were found the average RBC volume (MCV) was higher in patients with thyroid disorders (hypo and hyperthyroidism) than the reference values in healthy subjects, which indicates macrocytosis.

Thyroid hormones often have important effect on erythropoiesis. They enhance erythropoiesis through hyper proliferation of immature erythroid progenitors and increase secretion of erythropoietin (EPO) by inducing erythropoietin gene expression (Drews, 2003). Thyroid hormone is essential for terminal human erythropoiesis, and functions through the TRβ receptor (Gaoa *et al.*,2017). As far as the reduction in red blood cells count in patients with hypothyroidism shows that the bone marrow is depressed and that thyroid hormones play an important role in the regulation of the human hematopoiesis in the bone marrow (Iddah *et al.*,2013).

Detection significantly decreased levels of RBCs in hypothyroid patients and low Hb concentrations which clinically indicates anemia. In the same notion, determination that these patients presented a hypochromic state of RBCs, as indicated by the significantly diminished values of MCH and MCHC. These data corroborate the findings that CD34C-expanded BFU-E from hypothyroid patients reveals a significant growth reduction in vitro which might be related to the in vivo suppression of thyroid hormones production in these patients (Kawa *et al.*, 2010).

5.6 Changing in the number of different types of white blood cells

This study indicated a decreasing in all types of white blood cells (lymphocytes, monocytes and granulocytes) in patients with hypothyroidism in comparison to control. This result is consistent with a Turkish study conducted by (Yazar *et al.*,2018) who reported a decreasing in white blood cells (WBCs) and erythrocytes in patients with hypothyroidism and down syndrome . There were a research team in Kenya reached in 2013 that WBCs and RBCs levels in patients suffering from autoimmune thyroid diseases were low compared to healthy persons (Iddah *et al.*,2013), so this study is in agreement with the results of present study. Generally it seems that hypothyroidism causes hypoplasia in all myeloid cell lineages. With regard to lymphocytes, T3 is as a precursor substance for normal B cell formation in bone marrow through its mediation of pro-B cell proliferation. Therefore, thyroid disorders can induce different effects on various blood cell lineages (Drews, 2003). According to obtained data, (Dorgalaleh *et al.*,2013) suggested that all patients with hypothyroidism and hyperthyroidism should be periodically evaluated for probably hematological changes.

5.7 Platelet Indices

Thyroid hormone receptors are present on hematopoietic stem cells and serum levels of thyroid hormone may modulate the production of blood cells including platelets (Chute *et al.*,2010). However, clinical studies have shown

inconsistent associations between thyroid hormone levels and platelet count and mean platelet volume (MPV) (Erikci *et al.*,2009). Most of these studies were small and enrolled patients with clinical thyroid disease. Therefore, this study designed to examine the effects of decreased thyroid hormone and increased TSH in hypothyroidism patients on platelet count and MPV.

By examining the results of the present study, one can point to the statistical significance decreasing in the number of thrombocytes, plateletcrit (PCT) and platelets distribution width (PDW) in hypothyroidism patients versus to control subjects. This is similar to finding of (Ijaz *et al.*,2018) who refereed to an increase in serum TSH level was associated with a significant decrease in the platelet count and MPV.

This is possibly related to the effects of thyroid hormones on both platelet formation as well as prolong the survival of platelets. One possible mechanism through which thyroid hormones may increase the number of megakaryocytes may include modulation of bone marrow matrix proteins, such as fibronectin. Indeed, in several cell lines, thyroid hormones increase the expression of fibronectin gene. Moreover, individuals with hyperthyroidism have elevated blood levels of fibronectin (Huang et al.,2008). Fibronectin appears to affect megakaryocyte maturation and thrombopoiesis through interaction with integrin α4β1(Malara *et al.*,2011).

5.8 Differences in thyroid profile with respects to gender

There was a significant difference between male and female in this study with respects to thyroid profile (TSH, T3 and T4). This is clarified by the results of table (4.8) which showed an increasing in the levels of TSH in female than male of patients with hypothyroidism. This result is nearly similar to a study had done in 2018 by Tekle *et al.* who demonstrated that the mean of thyroid hormones in females with hypothyroidism was slightly higher than that of male but the difference between the two groups was statistically insignificant.

Higher prevalence of sub clinical hypothyroidism among females in the current study is in line with the findings of a study conducted in Northern Europe (Laurberg *et al.*,1998) and Indian study by (Dhadhal *et al.*,2015). Canaris *et al.*(2000) reported that the highest age and sex-specific rates of hypothyroidism incidence were in women older than 60 years of age . Its prevalence among men over the age of 74 years (16%) was almost as high as it was in women of the same age (21%). Some studies have demonstrated that higher estrogen levels contribute to elevated levels of TSH or lower levels of FT4, resulting in hypothyroid symptoms (Mazer, 2004).

5.9 Differences in apoptotic markers with respect to gender.

The results of table (4.9) presented the relation between apoptosis and gender in hypothyroid patients and revealed that all studied apoptotic biomarkers didn't confirm a significant difference between male and female except FasL and Bcl-2 which were increased significantly in female than in male in patients without treatment group. This may be due to that estrogen and its related steroid hormones that at higher than typical serum concentrations induce apoptosis in a pathway consists of phosphodiesterase 3A and Schlafen 12 (SLFN12), which triggers apoptosis by down regulating the level of Bcl-2 and Mcl-1 (Li *et al.*, 2019).

There was a study conducted in 2009 by (Lewis-Wambi & Jordan) investigate the induction of apoptosis by estrogen in breast cell *in vitro* and suggest that the apoptotic mechanisms of estradiol in breast cancer cells LTED (long term estrogen deprived) are thought to involve the death receptors as well as the mitochondrial pathways. Specific molecular events include the activation of the Fas death receptor/Fas ligand (FasL) complex (Song *et al.*, 2001), the release of cytochrome c from the mitochondria and alterations in Bcl-2(Lewis *et al.*, 2005).

5.10 Differences in oxidative stress and anti-oxidant markers with respects to gender.

The current study was revealed an imbalance in the cellular oxidant-antioxidant system in the patients with hypothyroidism compared to subjects in control group by having an increasing in oxidative stress and decreasing in anti-oxidant biomarkers. Also this study demonstrated that females with hypothyroidism have an increased in TBARS as a biomarker for oxidative stress versus to males who have the same disease. For decades, the thyroid gland has been known to be a target of estrogen. Estrogen up-regulates its own receptor in the thyroid of female and male rats, whereas gonadectomy reduces ER levels in the thyroid of both male and female rats (Banu *et al.*, 2002) . ERα and ERβ are expressed in the thyroid of both female and male rats, though female thyroid expresses higher ER levels than male thyroid (Stanley *et al.*, 2010). Lima *et al.* (2006) reported that administration of estradiol to both intact and ovariectomized rats increases TPO activity , indicating that estrogen stimulates not only thyroid iodide uptake but also iodide organification.

Higher prevalence of thyroid diseases in women could be, at least in part, due to sex-related differences in the thyroid redox environment. Utilizing rats as a model, have shown higher H_2O_2 production and NOX_4 (an enzyme play central role in production of Hydrogen peroxidase H_2O_2 in thyrocytes) expression in the thyroid of adult female rats in comparison with their male counterparts (Fortunato *et al.*, 2013). Furthermore, the previous author and his colleagues have found higher NOX_4 expression in the thyroid of female rats in the proestrus phase of the estrous cycle (characterized by an estrogen peak) and higher NOX_4 expression and H_2O_2 production in PCCL3 cells treated with 17β-estradiol, indicating a role for estrogen in this process. (Fortunato *et al.*,2014) suggest that a redox imbalance elicited by estrogen could be involved in the sex differences found in the prevalence of thyroid dysfunctions.

5.11 Correlation of the study biomarkers.

The results of the current study reported there was a significant positive correlation between oxidative stress and apoptosis in patients with hypothyroidism figure (4-1). The explanation of this may be as hypothyroidism disorder is characterized by increasing in an oxidative stress. This excess in oxidative stress kills cells either by necrosis or by apoptosis (Kannan & Jain, 2000). In many models of apoptosis, alterations in the redox status of the cell to a more oxidizing environment occurs prior to activation of the final phase of caspase activation (Morel & Barouki, 1999). Several studies have shown that the global shutdown of mitochondrial function under conditions of oxidative stress could contribute to apoptosis. Under severe oxidative stress, the mitochondrial permeability transition (PT) occurs. PT involves a sudden increase of the inner mitochondrial membrane permeability to solutes greater than 1500 Da (Susin *et al.*,1998). This study was reported a negative correlation between apoptosis and lymphocytes numbers as presented in figure (4-2). Normally T3 was associated with lower incidence of early lymphocyte apoptosis indicating that this thyroid hormone may facilitate maintenance of the lymphocyte population. Although evidence for thyroid hormone regulation of lymphocyte responses has been demonstrated previously in animal models (Klecha *et al.*,2006), the underlying cellular mechanisms are not clearly understood. The explanation of this correlation may be due to the patients included in this study appeared to had an increasing in apoptosis reflected by elevated the levels of cytochrome C which have adverse effects on the numbers of lymphocytes in the absence of thyroid hormones.

There is a negative correlation between apoptosis and hemoglobin as explained in figure (4-3), this mean that an increased in apoptosis associated with decreased in red cell indices in patients with hypothyroidism. This is may be elucidate by the fact that in normal state premature removal of red blood cells occurs by eryptosis, a form of stress-induced, programmed cell death

which leads to the removal of defective cells without the release of the cell content (Ghashghaeinia *et al,*.2012). Eryptosis can also be stimulated by oxidative stress, which activates Ca^{2+}-permeable cation channels, Cl^- channels and caspases (Qadri *et al.*,2017). Since the hypothyroidism has an increased in oxidative stress, therefore the patients will have an increasing in the eryptosis which ultimately result in decreasing in the number of erythrocytes and hemoglobin levels and other red blood cell indices as explained in previous results of current study.

This study demonstrated an increasing in oxidative stress in patients with hypothyroidism of both studied groups (with and without treatment) and this increasing appear to have an effects on erythrocytes by negative significant correlation between TBARS and red blood cell counts as clarify in figure (4-4). The causes of this result can be referred to erythrocytes may be exposed to high oxidative stress in the circulation of patients with thyroid disorders which could cause injury and trigger their suicidal death or eryptosis. Oxidative stress activates Ca2+-permeable nonselective cation channels in the cell membrane, thus, stimulating Ca2+ entry and subsequent cell membrane scrambling resulting in phosphatidylserine exposure and activation of Ca2+-sensitive K+ channels leading to K+ exit, hyperpolarization, Cl exit, and ultimately cell shrinkage due to loss of KCl and osmotically driven water (Bissinger et al.,2019). Oxidative stress results from an imbalance between free radicals and the body's antioxidant defense systems; resulting in red blood cell dysfunction, platelet destruction and tissue injury (Comazzi *et al.*,2004).

The association between thyroid hormones and red blood cell indices is demonstrated in figure (4-5) which explain that the decrease in T3 was accompanied by increased in MCV as one of RBCs indices. The finding of earlier studies were showed thyroid dysfunction was associated with abnormal red blood cell indices (den Elzen *et al.*,2015). Researchers investigating potential altered erythropoiesis as a result of thyroid dysfunction found red cell

abnormalities and a reduced proliferative potential of hematopoietic progenitor cells in patients with hypothyroidism (Kawa *et al.*,2010).

5.12 The sensitivity and specificity.

Sensitivity is defined as the probability that an individual with disease will have a positive test and represents the "true positivity rate" or TPR of the test. Simply put, it is the ability of a test to correctly classify an individual as ′diseased. While specificity is defined as the probability that a disease-free individual will have a negative test and represents the "true negativity rate" or TNR of the test. Simply put, this would be the ability of a test to correctly classify an individual as being disease- free (Šimundić, 2009). The result of table (4.11) reflects that the apoptotic biomarkers FAS has increased sensitivity and decreased in specificity and this results in many patients who are disease free being told of the possibility that they have the disease and are then subject to further investigation (Lalkhen & McCluskey, 2008).

5.13 Receiver Operating Characteristic (ROC) curve of studied biomarkers

Diagnostic tests play a vital role in modern medicine not only for confirming the presence of disease but also to rule out the disease in individual patient. The receiver operating characteristic (ROC) curve is the plot that displays the full picture of trade-off between the sensitivity and (1- specificity) across a series of cutoff points. Area under the ROC curve is considered as an effective measure of inherent validity of a diagnostic test. This curve is useful in:

1) Finding optimal cut-off point to least misclassify diseased or non-diseased subjects.

2) Evaluating the discriminatory ability of a test to correctly pick diseased and non-diseased subjects.

3) Comparing the efficacy of two or more tests for assessing the same disease.

4) Comparing two or more observers measuring the same test (inter-observer variability) (Kummar & Indrayan, 2011).

The area under the curve (AUC) is an effective and combined measure of sensitivity and specificity for assessing inherent validity of a diagnostic test. Maximum AUC = 1 and it means diagnostic test is perfect in differentiating diseased with non-diseased subjects (Indrayan, 2008). The larger the AUC, the better is overall performance of diagnostic test to correctly pick up diseased and non-diseased subjects (Zhou *et al.*,2002). AUC can be interpreted as follows: 90 -100 = excellent; 80 - 90 = good; 70 - 80 = fair; 60 - 70 = poor; 50 - 60 = fail.

5.14 Relative risk of studied biomarkers.

The relative risk (RR) of an event is the likelihood of its occurrence after exposure to a risk variable as compared with likelihood of its occurrence in a control or reference group. Relative risk of 1.00 means the risk of the event is identical in the exposed and control samples, while RR is the less than 1.00 means that the risk is lower in the exposed sample. Relative risk greater than 1.00 means that the risk is increased in the exposed sample (Citrome, 2010). Relative risk is used in the statistical analysis of the data of ecological, cohort, and intervention studies, to estimate the strength of the association between exposures (treatments or risk factors) and outcomes (Ilona, 2011).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

- Hypothyroidism associated with increased in the levels of Fas, FasL, Bcl-2 and Cytochrome C as indicators for apoptosis.
- An imbalance in redox state recorded in hypothyroid patients by increased TBARS and decreased SepP1.
- There was increased in MCV indicating a type of macrocytic anemia in hypothyroidism.
- Sex specific differences with regard to females were appeared in apoptotic, oxidative and antioxidant biomarkers.
- Increased AUC of Fas biomarker indicating the possibility of using it in the prognosis of hypothyroidism.
- Apoptotic biomarkers (Fas) has significant relative risk factor of hypothyroidism .

Recommendations:

- Studying the genetic association between hypothyroidism and apoptosis genes.
- Evaluation of apoptotic biomarkers according to subtype of hypothyroidism.
- Using transmission and scanning electron microscope for studying the effects of hypothyroidism on the ultrastructure of blood cells.
- Possibility of using the measurement of Fas and FasL in the prognosis of hypothyroidism .
- It is better to take precautions against the potentially harmful effects of hypothyroidism-related anemia.

REFERENCES

References

Adams J. M. and Cory S.(2018). The BCL-2 arbiters of apoptosis and their growing role as cancer targets. *Cell Death Differ*; 25: 27–36.

Alfadda A. A. and Sallam R. M.(2012). Reactive Oxygen Species in Health and Disease. *Biomed. Biotechnol;* 2012, 1-14.

Alipourzamani S. H.; Movassagh, M. S.; Nouri, M.(2011). Goiter in the sheep slaughtered in Tabriz slaughterhouse, Iran. Scholars Research. *Library Annals of Biological Research*; 2(3) 242–246.

Allen L.A., Felker G.M., Mehra M.R., Chiong J.R., Dunlap S.H., Ghali J.K., et al.(2010). Validation and potential mechanisms of red cell distribution width as a prognostic marker in heart failure. *J Card Fail*. ;16(3):230-8.

Alshahrani A. S. et al. (2016). The epidemiology of thyroid diseases in the Arab world: a systematic review. *J. Public Health Epidemiol.;* 8, 17–26.

Andrikoula M. and Tsatsoulis A.(2001). The role of Fas-mediated apoptosis in thyroid disease. *European Journal of Endocrinology ;*144 :561-568.

Arpin C., Pihlgren M., Fraichard A., Aubert D., Samarut J., Chassande O., Marvel J. (2000). Effects of T3R alpha 1 and T3R alpha 2 General deletion on T and B lymphocyte development. *J. Immunol*. ; 1: 152–160.

Asvold B., Bjøro T., Nilsen T. and Vatten L.(2007).Tobacco Smoking and Thyroid Function. *Arch Intern Med.* ;167(13):1428-1432.

Banu S.K., Govindarajulu P. & AruldhasM.M.(2002). Testosterone and estradiol up-regulate androgen and estrogen receptors in immature and adult rat thyroid glands in vivo. *Steroids* ;67: 1007–1014

Bartalena L., Wiersinga W.M., Tanda M.L., Bogazzi F., Piantanida E., Lai A., Martino E.(2004). Diagnosis and management of amiodarone-induced thyrotoxicosis in Europe: results of an international survey among members of the European Thyroid Association. *Clin. Endocrinol.*; 61: 494–502.

References

Bashir H.,Bhat M., Farooq R., Majid S., Shoib S., Hamid R., Mattoo A.,Rashid T., Bhat A.,Wani H. and Masood A. (2012). Comparison of hematological parameters in untreated and treated subclinical hypothyroidism and primary hypothyroidism patients. *MJIRI*; 26(4):172-178.

Baskol G., Atmaca H. ,Tanrverdi F. , Baskol M.,Kocer D. and Bayram F. (2007). Oxidative Stress and Enzymatic Antioxidant Status in Patients with Hypothyroidism before and after Treatment. *Exp Clin Endocrinol Diabetes* ; 115: 522 – 526.

Belin R.M., Astor B.C., Powe N.R., Ladenson P.W.(2004). Smoke exposure is associated with a lower prevalence of serum thyroid autoantibodies and thyrotropin concentration elevation and a higher prevalence of mild thyrotropin concentration suppression in the third National Health and Nutrition Examination Survey (NHANES III). *J Clin Endocrinol Metab.*;89(12):6077-6086.

Bijay V., Simon H . (2008). Management of hypothyroidism in adults. *BMJ ;337:a801*

Biondi B. and Cooper D.S.(2008).The Clinical Significance of Subclinical Thyroid Dysfunction. *Endocrine Reviews*; 29:76-131.

Biondi, B.& Wartofsky, L.(2014) Treatment with thyroid hormone. Endocr Rev.;35(3):433-512.

Bissinger R., Bhuyan A., Qadri S. and Lang F. (2019).Oxidative stress, eryptosis and anemia: a pivotal mechanistic nexus in systemic diseases. *The Federation of European Biochemical Societies Journal* ;286: 826–854 .

Björkman U. and Ekholm R. (1995). Hydrogen peroxide degradation and glutathione peroxidase activity in cultures of thyroid cells. *Mol Cell Endocrinol* ;111:99–107.

Bjùro T., Holmen J., Krüger O., Midthjell K., Hunstad K., Schreiner T., Sandnes L. and Brochmann H.(2000). Prevalence of thyroid disease, thyroid dysfunction and thyroid peroxidase antibodies in a large, unselected population. The health study of Nord-Trondelag (HUNT). *Eur J Endocrinol* ; 143: 639-647.

References

Bobba A., Atlante A., Giannattasio S., Sgaramella G., Calissano P., and Marra E. (1999). Early release and subsequent caspase-mediated degradation of cytochrome c in apoptotic cerebellar granule cells. *FEBS Lett.*; 457: 126–130.

Bober J., Kwiatkowska E., Kędzierska K., Olszewska M., Stachowska E., Ciechanowski K. and Chlubekc D. (2006). fluoride aggravation of oxidative stress in patients with chronic renal failure. *Fluoride;* 39(4):302–309 .

Boelaert K., Newby P.R., Simmonds M.J., Holder R.L., Carr-Smith J.D., Heward J.M., Manji N., Allahabadia A., Armitage M., Chatterjee K.V., Lazarus J.H., Pearce S.H., Vaidya B., Gough S.C., Franklyn J.A.(2010). Prevalence and relative risk of other autoimmune diseases in subjects with autoimmune thyroid disease. *Am. J. Med.;* 123, 183.e1–183.

Bolk N.,Visser T., Nijman J., Jongste I.,Tijssen J.and BerghoutA. (2010). Effects of Evening vs Morning Levothyroxine Intake. *Arch Intern Med.*;170(22):1996-2003

Braun, E.M.; Windisch, G.; Wolf,G.; Hausleitner, L.; and Anderhuber, F. (2007). The pyramidal lobe: Clinical anatomy and its importance in thyroid surgery. *Surgical and Radiologic Anatomy;* 29: 21–27.

Bremner A.P., Feddema P., Leedman P.J., Brown S.J., Beilby J.P., Lim E.M.,Wilson S.G., O'Leary P.C.,Walsh P.J. (2012). Age-related changes in thyroid function: a longitudinal study of a community-based cohort. *The Journal of clinical endocrinology and metabolism*; 97: 1554–1562

Budenhofer B.K., Ditsch N.U., Jeschke R. and Gartner B.(2013). Thyroid dysfunction in normal and disturbed pregnancy. *Archives of Gynecology and Obstetrics*; 287: 1–7

Bulow P. I., Knudsen N., Carlé A., Vejbjerg P., Jørgensen T., Perrild H., Ovesen L., Rasmussen L.B., Laurberg P.A.(2011). A cautious iodization program bringing iodine intake to a low recommended level is associated with an increase in the prevalence of thyroid autoantibodies in the population. *Clin. Endocrinol.*; 75, 120–126 .

References

Bulow P.I., Knudsen N., Carlé A., Schomburg L., Köhrle J., Jørgensen T., Rasmussen L.B., Ovesen L., Laurberg P.(2013). Serum selenium is low in newly diagnosed Graves' disease: a population-based study. *Clin. Endocrinol*.; 79: 584–590.

Burk R.F., Hill K.E., Motley A.K.(2003). Selenoprotein metabolism and function: evidence for more than one function for selenoprotein P. *J. Nutr*.;133 :1517S–1520S.

Campbell K.J. and Tait S.G. (2018). Targeting BCL-2 regulated apoptosis in cancer. *Open Biol.* 8: 180002.

Canaris G.J., Manowitz N.R., Mayor G., Ridgway E.C.(2000). The Colorado thyroid disease prevalence study. *Arch Intern Med.* ;160(4):526–534.

Cano-Europa B.V., Margarita F.C., Rocio O.B.(2012). The relationship between thyroid states, oxidative stress and cellular damage. In: Oxidative stress and diseases; Lushchak V (ed.):413-436. InTech, Croatia.

Carmeli E., Bachar A., Barchad S., Morad M., and Merrick J.(2008). Antioxidant status in the serum of persons with intellectual disability and hypothyroidism: A pilot study. *Res. Development. Disab*.; 29:431-438.

Caturegli P., De Remigis A., Rose N.R.(2014). Hashimoto thyroiditis: clinical and diagnostic criteria. *Autoimmun Rev*.;13:391–7.

Chaker L., Bianco A. C., Jonklaas,J. & Peeters R. P.(2017). Hypothyroidism. *Lancet* ;390, 1550–1562 .

Chandel R.S., Gangadhar C., Leela A. (2015). Impact of subclinical hypothyroidism on iron status and hematological parameters. *APALM*; 2: 21-25.

Chang L., Kamata H., Solinas G., Luo J.L., Maeda S., Venuprasad K., Liu Y.C., Karin M.(2006). The E3 ubiquitin ligase itch couples JNK activation to TNFalpha-induced cell death by inducing c-FLIP(L) turnover. *Cell*;124:601- 613.

References

Chistiakov D.A.(2005).Immunogenetics of Hashimoto's thyroiditis *Journal of Autoimmune Diseases*; 2:1

Choksi N.Y., Jahnke G.D., Hilaire C., Shelby M.(2003). Role of thyroid hormones in human and laboratory animal reproductive health. Birth Defects *Res Dev Reprod Toxicol*.; 68:479- 491.

Chute J.P., Ross J.R., McDonnell D.P.(2010). Mini review: nuclear receptors, hematopoiesis, and stem cells. *Mol Endocrinol*.; 24:1-10

Citrome L.(2010). relative vs absolute measures of benefit and risk : what is the difference? *Acta Psychiatr Scand*.;121(2):94-102.

Comazzi S., Spagnolo V., Bonfanti U.(2004). Erythrocyte changes in canine diabetes mellitus: in vitro effects of hyperglycaemia and ketoacidosis. *J Comp Clin Path*.;12:199–205.

Combs G.F. , Midthune D.N., Patterson K.Y., Canfield W.K., Hill A.D., Levander O.A., et al. (2009). Effects of selenomethionine supplementation on seleniumstatus and thyroid hormone concentrations in healthy adults, *Am. J. Clin.Nutr*. ;89 :1808–1814.

Cominetti C., Bortoli M.C., Abdalla D.P., Cozzolino S.F.(2011). Considerations about oxidative stress, selenium and nutrigenetics. *Nutrire*.;36:131-53.

Cooper D and Biondi B.(2012). Subclinical thyroid disease. *Lancet* ; 379: 1142–54.

Dahiya K.,Verma M. , Dhankhar R. , Ghalaut V.,Ghalaut P. ,Sachdeva A., Malik I. and Kumar R.(2016).Thyroid profile and iron metabolism: mutual relationship in hypothyroidism. *Biomedical Research* ; 27 (4): 1212-1215.

Dardano A., Ghiadoni L., Plantinga Y. et al. (2006). Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. *Journal of Clinical Endocrinology & Metabolism*; 91(10): 4175–4178.

den Elzen W.P., de Craen A.J., Mooijaart S.P., Gussekloo J.(2015). Low thyroid function and anemia in old age: the Leiden 85-plus study. *J Am Geriatr Soc.*;63(2):407–409.

References

Dhadhal R., Mulchandani V., Parakh R., Ashish J., Ketan M., Nihari B.(2015). A cross sectional study of prevalence of hypothyroidism in adult population of udaipur district. *GJBB.*;4(1):103–6.

Diab N., Daya N., Juraschek S., Martin S., McEvoy J., Schulthei U.,Köttgen A. & Selvin E.(2019). Prevalence and Risk Factors of Thyroid Dysfunction in Older Adults in the Community. *Nature research* ; 9:13156

Dickens L.S., Powley I.R., Hughes M.A., MacFarlane M. (2012). The 'complexities' of life and death: death receptor signalling platforms. *Exp Cell Res* ;318(11):1269–1277

Dorgalaleh A., Mahmoodi M.,Varmaghani B., Kiani node F., Saeeidi O., Alizadeh Sh., Tabibian Sh., Bamedi T., Momeni M., Abbasian S., Kashani Z. (2013). Effect of Thyroid Dysfunctions on Blood Cell Count and Red Blood Cell Indice. *Iranian Journal of Pediatric Hematology Oncology* ;l3(2).

Dorgan,F.J.;Stanczyk,Z.F.;Kahle,L.L.;and Brinton,A.L.(2010). Prospective case-control study of premenopausal serum estradiol and testosterone levels and breast cancer risk. *J.Breast cancer research*;12:98.

Drews R.E.(2003). Critical issues in hematology: anemia, thrombocytopenia, coagulopathy, and blood product transfusions in critically ill patients. *Clin. Chest. Med* .; 24(4):607-622.

Duan D., Zhang B., Yao J., Liu Y. & Fang J. (2014). Shikonin targets cytosolic thioredoxin reductase to induce ROS-mediated apoptosis in human promyelocytic leukemia HL-60 cells. *Free Radic Biol Med*; 70, 182–193.

Duntas L.H. and Benvenga S.(2015). Selenium an element for life, *Endocrine*; 48 :756–775.

Durackova Z.(2010). Some current insights into oxidative stress. *Physiol Re.*;59(4):459-69.

Effraimidis G. and Wiersinga W.M. (2014). Mechanisms in endocrinology: autoimmunethyroid disease: old and new players, *Eur. J. Endocrinol*.;170 : R241–R252.

References

Eleftheriadis T., Pissas G., Liakopoulos V. and Stefanidis I.(2016). Cytochrome c as a Potentially Clinical Useful Marker of Mitochondrial and Cellular Damage. *Front. Immunol*. 7:279.

Elizabeth D.A. and Agabegi, SS. (2008). Step-Up to Medicine (Step-Up Series). Hagerstwon, MD. Lippincott Williams & Wilkins. 3rd Edition

Erdamar H.,Demirci H., Yaman H., Erbil M.K., Yakar T., Sancak B., et al.(2008). The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. *Clin Chem Lab Med*.; 46(7):1004– 1010.

Erikci A.A., Karagoz B., Ozturk A., Caglayan S., Ozisik G., Kaygusuz I., Ozata M.(2009). The effect of subclinical hypothyroidism on platelet parameters. *Hematology*; 14:115-117.

Fandrey J., Pagel H., Frede S., Wolff M., Jelkmann W.(1994). Thyroid hormones enhance hypoxia-induced erythropoietin production in vitro. *Exp Hematol*.;22(3):272–277.

Federige M.,Romaldini J., Miklos A., Koike M.,Takei K. and Portes E. (2017). Serum selenium and selenoprotein-P levels in autoimmune thyroid diseases patients in a select center: a transversal study *Arch Endocrinol Metab.* ;61/6

Ferrucci L., Guralnik J.M., Bandinelli S., Semba R.D., Lauretani F., Corsi A., et al.(2007). Unexplained an aemia in older persons is characterized by low erythropoietin and low levels of pro-inflammatory markers. *Br J Haematol*.;136(6):849-55.

Fogarty M. C., Hughes C. M., Burke G. et al. (2011). Exercise induced lipid peroxidation: implications for deoxyribonucleic acid damage and systemic free radical generation. *Environmental and Molecular Mutagenesis*; 52(1): 35–42.

Fortunato R., Braga W.M., Ortenzi V.H., Rodrigues D.C., Andrade B.M., Miranda-Alves L., Rondinelli E., Dupuy C., Ferreira A.C. & Carvalho D.P.(2013). Sexual dimorphism of thyroid reactive oxygen species production due to higher NADPH oxidase 4 expression in female thyroid glands. *Thyroid* ;23: 111–119.

References

Fortunato R., Ferreira A.,Hecht F., Dupuy C.and Carvalho D. (2014).Sexual dimorphism and thyroid dysfunction: a matter of oxidative stress? *Journal of Endocrinology* ; 221: R31–R40.

Fountoulakis S.,Vartholomatos G., Kolaitis N., Frillingos S.,Philippou G. and Tsatsoulis A.(2008). Differential expression of Fas system apoptotic molecules in peripheral lymphocytes from patients with Graves' disease and Hashimoto's thyroiditis. *European Journal of Endocrinology* ; 158: 853–859.

Franco M., Chavez E., and Perez-Mendez O. (2011). Pleiotropic effects of thyroid hormones: learning from hypothyroidism. *Journal of Thyroid Research;*2011:17.

Gabellini C., Trisciuoglio D., Del Bufalo D.(2017). Non-canonical roles of Bcl-2 and Bcl-xL proteins: Relevance of BH4 domain. *Carcinogenesis* ; 38, 579–587.

Gajate C. and Mollinedo F. (2015). Lipid rafts and raft-mediated supra molecular entities in the regulation of CD95 death receptor apoptotic signaling. *Apoptosis* ;20(5):584–606.

Gaoa X., Leea H.,Lib W., Plattd R., Barrasaa I.,Mac Q., Elmesa R., Rosenfeldb M. and Lodisha H.(2017). Thyroid hormone receptor beta and NCOA4 regulate terminal erythrocyte differentiation. *PNAS*; 114(38): 10107–10112.

Garmendia M. A., Santos P.S., Guillen-Grima, F. & Galofre, J. C. (2014). The incidence and prevalence of thyroid dysfunction in Europe: a meta-analysis. *J. Clin. Endocrinol. Metab.*; 99, 923–931.

Gavathiotis E.(2019).BCL-2 Family Proteins Methods and Protocols. First edition, Springer Science+Business Media, LLC, part of Springer Nature. pp (1-2).

Gerenova J & Gadjeva V.(2007). Oxidative stress and antioxidant enzyme activities in patients with Hashimoto's thyroiditis. *Comp Clin Pathol* .;16:259–264.

References

Ghashghaeinia M., Cluitmans J.C., Akel A., Dreischer P., Toulany M., Köberle M., Skabytska Y., Saki M., Biedermann T., Duszenko M., et al.(2012). The impact of erythrocyte age on eryptosis. *Br. J. Haematol*.; 157: 606–614.

Gietka-Czernel M.(2008). Progress in biochemical diagnosis of thyroid function. *Post Nauk Med*.; 2: 83–91.

Giordano C., Richiusa P., Bagnasco M., Pizzolanti G., Di Blasi F., Sbriglia M., Mattina A., Pesce G., Montagna P., Capone F., Misiano G., Scorsone A., Pugliese A., Galluzzo A.(2001). Differential regulation of Fas-mediated apoptosis in both thyrocyte and lymphocyte cellular compartments correlates with opposite phenotypic manifestations of autoimmune thyroid disease. *Thyroid* ; 11:233-244.

Giordano C., Stassi G., De Maria R., Todaro M., Richiusa P., Papoff G., Ruberti G., Bagnasco M., Testi R., Galluzzo A.(1997). Potential involvement of Fas and its ligand in the pathogenesis of Hashimoto's thyroiditis. *Science* ; 275:960-963.

Goldar S., Khaniani M.S., Derakhshan S.M., Baradarn B.(2015). Molecular mechanisms of apoptosis and roles in cancer development and treatment. *Asian Pac. J. Cancer Prev*. ; 16: 2129–2144.

Green D.R. and Llambi F. (2015). Cell death signaling. Cold Spring *Harb. Perspect. Biol*.; 7: a006080.

Grymuła K.; Paczkowska, E.; Dziedziejko, V.; Bas´kiewicz-Masiuk, M.; Kawa, M.; Baumert, B.; Celewicz, Z.; Gawrych, E. & Machalin´ ski, B.(2007). The influence of 3,30,5-triiodo-L-thyronine on human haematopoiesis. *Cell Proliferation* .; (40): 302–315.

Guglielmi R., Grimaldi F., Negro R., et al.(2018). Shift from levothyroxine tablets to liquid formulation at breakfast improves quality of life of hypothyroid patients. *Endocr Metab Immune Disord Drug Targets*.;18:235–40.

Gussekloo, J., van Exel E., de Craen A.J., Meinders A.E., Frölich M., Westendorp R.G.(2004). Thyroid status, disability and cognitive function, and survival in old age. *JAMA*; 292, 2591–2599.

References

Guyton A.and Sepehhri H. (2011). Textbook of medical physiology. 12th ed.Tehran. Andish rafi,pp1158-71.

Ha J., Lee J., Jo K., Lim D., Kang M., Cha B. and Kim M.(2018). Sex differences in risk factors for subclinical hypothyroidism *Endocrine Connection ;* 7, 511–522

Halliwell B. (2007).Biochemistry of oxidative stress . *Biochem. Soc. Trans.*; 35 (5): 1147 – 1150 .

Hamdi Y., Kaddour H., Vaudry D. et al. (2015) Octadecaneuropeptide (ODN) prevents hydrogen peroxide-induced oxidative damage of biomolecules in cultured rat astrocytes. *Peptides* ;71, 56–656.

Hammond L.J., Lowdell M.W., Cerrano P.G., Goode A.W., Bottazzo G.F.& Mirakian R. (1997). Analysis of apoptosis in relation to tissue destruction associated with Hashimoto's autoimmune thyroiditis. *Journal of Pathology*; 182: 138-144.

Hara H., Ryuji S. and Yosmo B.(2001). Production of Mononuclear 8-OHdG and Cytochrome c by Cultured Human Cells in Patients with Autoimmune Thyroid Diseases . *Endocrine Journal* ; 48 (6), 671-675.

Harris K.B., Pass K.A.(2007). Increase in congenital hypothyroidism in NewYork state and in the United States. *Mol Genet Metab* ;91:268-77.

Hassan M., Watari H., AbuAlmaaty A., Ohba Y., Sakuragi N.(2014). Apoptosis and molecular targeting therapy in cancer. *BioMed Res. Int.;*2014:23.

Helfand M. (2004).Screening for subclinical thyroid dysfunction in nonpregnant adults: a summary of the evidence for the U.S. Preventative Services Task Force. *Ann Intern Med.*;140:128-141.

Hillier L.W., Fulton R.S., Fulton L.A., Graves T.A., Pepin K.H., Wagner- McPherson C., et al.(2003). The DNA sequence of human chromosome 7. *Nature* ; 424(6945):157–64.

References

Hiromatsu Y., Kakau H., Mukai T., Miyake I., Fukutani T., Koga M.(2004). Immunohistochemical analysis of Bcl-2, Bax and Bak expression in thyroid glands from patients with Graves' disease. *Endocrinol J* ; 51: 399-405.

Hollowell J.G., Staehling N.W., Flanders W.D., et al.(2002). Serum TSH, T(4), and thyroid antibodies in the United States population (1988 to 1994). *J Clin Endocrinol Metab*.;87(2):489-499.

Huang X.W., Zhao Z.Y., and Ji C.(2005). Effects of hypothyroidism on apoptosis and the expression of Bcl-2 and Bax gene in the neonatal rat hippocampus neurons. *Chinese Journal of Pediatrics*;43(1): 48–52.

Huang Y.H., Tsai M.M., Lin K.H.(2008). Thyroid hormone dependent regulation of target genes and their physiological significance. *Chang Gung Med J*.; 31:325-334.

Huber G.,Staub J., Meier C., et al.(2002). Prospective study of the spontaneous course of subclinical hypothyroidism: prognostic value of thyrotropin, thyroid reserve, and thyroid antibodies. *J Clin Endocrinol Metab*.;87:3221- 3226.

Hussain S., Amstad P., He P., Robles A., Lupold S.,Kaneko I., Ichimiya M.,Sengupta S.,Mechanic L.,Okamura S., Hofseth L.,Moake M.,Nagashima M., Forrester K. and Harris C. (2004). p53-Induced Up-Regulation of MnSOD and GPx but not Catalase Increases . *Journal of Pathology*; 182: 138-144.

Ibrahim A., Nurcan B., Nihat S. and Halil .LK.(2012). Evaluation of biochemical, hematological and thyroid function parameters in nondipper and dipper hypertensive patients. *Cent Eur J Med*; 124(13-14): 439-443.

Iddah M., Macharia N., Ng'wena A., Keter A. and Ofulla A. (2013). Thryroid Hormones and Hematological Indices Levels in Thyroid Disorders Patients at Moi Teaching and Referral Hospital, Western Kenya. *ISRN Endocrinology;* 2013: 6 pages.

Ijaz S.H., Jamal S. M., Qayyum R. (2018). Relationship Between Thyroid Hormone Levels and Mean Platelet Count and Volume: Quantitative Assessment. *Cureus*; 10(10).

References

Ilona C. (2011). Introduction to epidemiology. (2nd ed.). Maidenhead, Berkshire: Open University Press. p. 27

Indrayan A.(2008). Medical Biostatistics (Second Edition). Boca Raton: Chapman & Hall/ CRC Press; p. 263-7.

Ittermann T.; Khattak, R. M.; Nauck, M.; Cordova, C. M. & Volzke, H.(2015). Shift of the TSH reference range with improved iodine supply in Northeast Germany. *Eur J Endocrinol* ;172: 261–267.

Jaleco S., Swainson L., Dardalhon V., Burjanadze M., Kinet S., Taylor N.(2003). Homeostasis of naive and memory CD4+ T cells: IL-2 and IL-7 differentially regulate the balance between proliferation and Fas mediated apoptosis. *J Immunol.*; 171:61–8.

Jang S., Krammer P.H., Salgame P.(2003). Lack of proapoptotic activity of soluble CD95 ligand is due to its failure to induce CD95 oligomers. *J Interferon Cytokine Res*.; 23(8):441–7.

Jaume J.C., Mendel C.M., Frost P.H., Greenspan F.S., Laughton C.W.(1996). Extremely low doses of heparin release lipase activity into the plasma and can thereby cause artifactual elevations in the serum-free thyroxine concentration as measured by equilibrium dialysis. *Thyroid* ; 6: 79–83.

Jiskra J., Antošová M., Límanová Z., Telicˇka Z. and Lacinová Z.(2009). The relationship between thyroid function, serum monokine induced by interferon gamma and soluble interleukin-2 receptor in thyroid autoimmune diseases. *Clinical and Experimental Immunology*;156: 211–216.

Jorde R. and Sundsfjord J.(2006). Serum TSH levels in smokers and non-smokers: the 5th Tromso study. *Exp Clin Endocrinol Diabetes*. ;114(7):343-347.

Kale J., Osterlund E.J., Andrews D.W.(2018). BCL-2 family proteins: Changing partners in the dance towards death. *Cell Death Di*.; 25, 65–80.

Kamal A., Stephane R. , Zui P., Zita B., Jianjie M., and Leroy F. (2004). Nuclear Translocation of Cytochrome c during apoptosis. *the journal of biological chemistry*; 279(24): 24911–24914.

References

Kannan K. and Jain S. (2000).Oxidative stress and apoptosis. *Pathophysiology* ;7 (27): 153–163.

Katyare S.S. and Rajan R.R.(2005). Influence of thyroid hormone treatment on the respiratory activity of cerebral mitochondria from hypothyroid rats. A critical reassessment. *Experimental Neurology*. ;195(2):416–422.

Kawa M.P.; Grymuła, K., Paczkowska, E.; Baśkiewicz-Masiuk, M.; Dąbkowska, E.; Koziołek ,M. et al.(2010). Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. *Eur J Endocrinol*. ; 162(2):295-305.

Kendrick T.S., Payne C.J., Epis M.R., et al. (2008). Erythroid defects in TRalpha-/- mice. *Blood*; 111: 3245-3248.

Kim T.H., Kim K.W., Ahn H.Y., et al.(2013). Effect of seasonal changes on the transition between subclinical hypothyroid and euthyroid status. *J Clin Endocrinol Metab*; 98: 3420–29.

Klecha A.J., Genaro A.M., Gorelik G., Barreiro A.M., Silberman D.M., Schuman M., Garcia S.I., Pirola C. & Cremaschi G.A.(2006). Integrative study of hypothalamus–pituitary–thyroid–immune system interaction: thyroid hormone-mediated modulation of lymphocyte activity through the protein kinase C signaling pathway. *Journal of Endocrinology*; 189: 45–55.

Kleiner J., Altshuler L., Hendrick V., Hershman J.M.(1999). Lithium induced subclinical hypothyroidism: review of the literature and guidelines for treatment. *J Clin Psychiatry*;60(4):249-55

Kohrle J. and Gartner R.(2009). Selenium and thyroid. *Best Pract. Res. Clin. Endocrinol.Metab*.;23 : 815–827.

Kosenli A., Erdogan M., Ganidagli S., Kulaksizoglu M., Solmaz S., Kosenli O.,Unsal C., Canataroglu A.(2009). Anemia frequency and etiology in primary hypothyroidism. *Endocr Abstr*; 20: 140.

Ku B., Liang C., Jung J. U. & Oh B.H.(2011). Evidence that inhibition of BAX activation by BCL-2 involves its tight and preferential interaction with the BH3 domain of BAX. *Cell Res*.;21, 627–641 .

References

Kuhlbrandt W.(2015). Structure and function of mitochondrial membrane protein complexes. *BMC Biol* ; 13:89.

Kumar A., Sinha R.A., Tiwari M., Singh R., Koji T., et al.(2007). Hyperthyroidism induces apoptosis in rat liver through activation of death receptor-mediated pathways. *Journal of Hepatology*. ;46(5):888-898.

Kummar R. and Indrayan A.(2011). Receiver operating characteristic (ROC) curve for medical researchers. *Indian Pediatr*; 48: 277-89.

Lalkhen A. and McCluskey A.(2008). Clinical tests: sensitivity and Specificity. *Trustees of the British Journal of Anaesthesia* ;8(6) .

Laurberg P., Cerqueira C., Ovesen L., et al.(2010). Iodine intake as a determinant of thyroid disorders in populations. *Best Pract Res Clin Endocrinol Metab* ; 24: 13–27

Laurberg P., Pedersen K.M., Hreidarsson A., Sigfusson N., Iversen E., Knudsen P.R.(1998). Iodine intake and the pattern of thyroid disorders: a comparative epidemiological study of thyroid abnormalities in the elderly in Iceland and in Jutland, Denmark. *J Clin Endocrinol Metab*.;83:765–9.

Leberbauer C., Boulme F., Unfried G., Huber J., Beug H., and Mullner E. W. (2005). Different steroids co-regulate long-term expansion versus terminal differentiation in primary human erythroid progenitors. *Blood;* 105, 85–94.

LeFevre M.L.(2015). Screening for thyroid dysfunction: U.S. Preventive Services Task Force recommendation statement. *Ann Intern Med* ; 162: 641–50.

Lewis J.S., Meeke K., Osipo C., Ross E.A., Kidawi N., Li T., Bell E., Chandel N.S., Jordan V.C. (2005). Intrinsic mechanism of estradiol induced apoptosis in breast cancer cells resistant to estrogen deprivation. *J Natl Cancer Inst*; 97:1746-1759.

Lewis-Wambi J.and Jordan C.(2009). Estrogen regulation of apoptosis: how can one hormone stimulate and inhibit? *Breast Cancer Research*;11(3).

References

Li D.,Chen J., Ai Y., Gu X., Li L.,Che D.,Jiang Z., Li L., Chen S., Huang H.,Wang J., Cai T., Cao Y.,Qi X., and Wang X. (2019). Estrogen-Related Hormones Induce Apoptosis by Stabilizing Schlafen-12 Protein Turnover. *Molecular Cell*; 75, 1103–1116.

Limachi F. and Basso S.(2002). Apoptosis: life trough planned cellular death regulating mechanisms, control systems, and relations with thyroid disease. *Thyroid* ; 12:27-34.

Lima C.S., Zantut W., Castro E. V. et al.(2006). Pancytopenia in untreated patients with Graves' disease. *Thyroid,* 16,(4),: 403–409.

Lima L.P., Barros I.A., Lisboˆa P.C., Arau´ jo R.L., Silva A.C., Rosenthal D., Ferreira A.C. & Carvalho D.P.(2006). Estrogen effects on thyroid iodide uptake and thyroperoxidase activity in normal and ovariectomized rats. *Steroids* ;71: 653–659.

Lippi G., Filippozzi L., Montagnana M., Salvagno G.L., Franchini M., Guidi G.C., et al.(2009). Clinical usefulness of measuring red blood cell distribution width on admission in patients with acute coronary syndromes. *Clin Chem Lab Med*.;47(3):353-7.

Lippi G., Montagnana M., Salvagno G.L. and Guidi G.C.(2008). Should women with abnormal serum thyroid stimulating hormone undergo screening for Anaemia? *Arch Pathol Lab Med* ; 132(3):321-2.

Liu Y., Zhang W., Zhao J.T., et al.(2018). Selenoprotein P as the major transporter for mercury in serum from methylmercury-poisoned rats. *J Trace Elem Med Bio* ; 50: 589–595.

Lomonosova E., Chinnadurai G.(2008). BH3-only proteins in apoptosis and beyond: An overview. *Oncogene* ; 27, S2–S19.

Lopez J. and Tait S.(2015). Mitochondrial apoptosis: Killing cancer using the enemy within. *Br. J. Cancer* ; 112: 957–962.

Ma S., Hill K.E., Caprioli R.M., Burk R.F.(2002). Mass spectrometric characterization of full-length rat selenoprotein P and three isoforms shortened at the C terminus. Evidence that three UGA codons in the mRNA open reading frame have alternative functions of specifying selenocysteine insertion or translation termination. *J Biol Chem*; 277: 12749–12754.

References

Maenhaut, C.; Christophe, D.; Vassart, G.; Dumont, J.; Roger, P.P and Opitz, R. (2015). Ontogeny, anatomy, metabolism and physiology of the thyroid. editors. Endotext. South Dartmouth (MA): MDText.com, Inc.

Malara A., Gruppi C., Rebuzzini P., et al.(2011). Megakaryocyte-matrix interaction within bone marrow: new roles for fibronectin and factor XIII-A. *Blood.*; 117:2476-2483.

Mao X., Yu C.R., Li W.H., (2008). Induction of apoptosis by shikonin through a ROS/JNK-mediated process in Bcr/Abl-positive chronic myelogenous leukemia (CML) cells. *Cell Res*;18:879-888.

Marchiori R.C., Pereira L., Naujorks A., Rovaris D., Meinerz D., Marinò M., Latrofa F., Menconi F., Chiovato L. & Vitti P.(2015). Role of genetic and non-genetic factors in the etiology of Graves' disease. *J. Endocrinol. Invest.*; 38, 283–294..

Marchiori R., Pereira L., Naujorks A., Rovaris D., Meinerz D., Duarte M. and Rocha J.(2015). Improvement of blood inflammatory marker levels in patients with hypothyroidism under levothyroxine treatment *BMC Endocrine Disorders*; 15:32

Marinò M., Latrofa F., Menconi F., Chiovato L. & Vitti P.(2015). Role of genetic and non-genetic factors in the etiology of Graves' disease. *J. Endocrinol. Invest.*; 38, 283–294..

Maruoka H.,Watanabe M., Matsuzuka F., Takimoto T., Miyauchi A. & Iwatami Y.(2004). Increased intensities of Fas expression on peripheral T cell subsets in severe autoimmune thyroid disease. *Thyroid*;14: 417–423.

Masullo L.F., Magalhães R.A., Lemes R.P., de Almeida F.T., de Castro M.F., Maia F.P., Cunha T.O., Quidute A.R., Fontenele E.G., Sun G. and Martins M.R. (2018). Levothyroxine Replacement Improves Oxidative Status in Primary Hypothyroidism. *Front. Endocrinol.*; 9:655.

Mazer, N. A.(2004). Interaction of estrogen therapy and thyroid hormone replacement in postmenopausal women. *Thyroid: official journal of the American Thyroid Association* ;14(1): S27–34.

References

Messarah M., Boulakoud M. S., Boumendjel A., Abdennour C., and El Feki A. (2007).The impact of thyroid activity variations on some oxidizing-stress parameters in rats. *Comptes Rendus—Biologies*;330(2): 107–112.

Messarah M., Boumendjel A., Chouabia A. et al.(2010). Influence of thyroid dysfunction on liver lipid peroxidation and antioxidant status in experimental rats. *Experimental and Toxicologic Pathology*; 62(3): 301–310.

Mihara S., Suzuki N., Wakisaka S., et al.(1999). Effects of thyroid hormones on apoptotic cell death of human lymphocytes. *J Clin Endocrinol Metab.;* 84(4):1378-85.

Miłosz P. K.; Katarzyna, G.; Edyta, P.; Magdalena, B.; Elz˙bieta, D.; Monika, K.; Maciej, T. et al .(2010). Clinical relevance of thyroid dysfunction in human haematopoiesis: biochemical and molecular studies. *European Journal of Endocrinology* . 162; 295–305.

Misu H., Ishikura K., Kurita S., Takeshita Y., Ota T., Saito Y., et al.(2012). Inverse correlation between serum levels of selenoprotein p and adiponectin in patients with type 2 diabetes. *PLoS One*. ;7:1-7.

Mita Y., Nakayama K., Inari S., Nishito Y., Yoshioka Y., Sakai N., Sotani K., Nagamura T., Kuzuhara Y., Inagaki K. et al. (2017). Selenoprotein P-neutralizing antibodies improve insulin secretion and glucose sensitivity in type 2 diabetes mouse models. *Nat. Commun.*; 8: 16-58.

Mitra N., Fatemeh A., Behnam C.,Zahra M., Mona N., Sey R.,Amirhossein D., Abolfazl G., Maryam R.(2016). Selenium and its relationship with selenoprotein P and glutathione peroxidase in children and adolescents with Hashimoto's thyroiditis and hypothyroidism. *Journal of Trace Elements in Medicine and Biology ;*34: 10–14.

Mitsiades N., Poulaki V., Kotoula V., Mastorakos G., Tseleni- Balafouta S.T., Koutras D.A. et al.(1998). Fas/Fas ligand up-regulation and Bcl-2 down-regulation may be significant in the pathogenesis of Hashimoto's thyroiditis. *Journal of Clisnical Endocrinology and Metabolism* ;83: 2199- 2203.

References

Mittag J., Behrends T., Hoefig C.S., Vennstrom B., Schomburg L.(2010). Thyroid hormones regulate selenoprotein expression and selenium status in mice, *PLoS One*; 5: e12931

Morel Y. and Barouki.(1999). Repression of gene expression by oxidative Stress. *Biochem J* ; 342: 481–496.

Muzembo B.A., Dumavibhat N., Ngatu N.R., Eitoku M., Hirota R., Kondo S., et al.(2013). Serum selenium and selenoprotein P in patients with silicosis. *J Trace Elem Med Biol*.;27:40-4.

Myśliwiec J., Okłota M., Nikołajuk A. and Górska M.(2006). Soluble Fas, Fas ligand and Bcl-2 in autoimmune thyroid diseases: relation to humoral immune response markers .*Advanced in medical science* ; 51:119-22.

Nanba T., Watanabe M., Inoue N., Iwatani Y.(2009). Increases of the Th1/Th2 cell ratio in severe Hashimoto's disease and in the proportion of Th17 cells in intractable graves' disease. *Thyroid*;19:495–501.

Nanda N., Bobby Z. & Hamide A.(2012). Oxidative stress in anti thyroperoxidase antibody positive hypothyroid patients. *Asian Journal of Biochemistry* ;7: 54–58.

Nanda N., Bobby Z., Hamide A., Koner B. , and Sridhar M. G.(2007). Association between oxidative stress and coronary lipid risk factors in hypothyroid women is independent of body mass index. *Metabolism*; 56(10) pp. 1350–1355.

Nejar A., Pochmann D., Klein R., et al. (2005). 5′-nucleotidase activity is altered by hypo- and hyperthyroidism in platelets from adult rats. *Platelets*;16: 25-30.

Navarro R., Busnadiego I., Ruiz-Larrea M. B. & Ruiz-Sanz J. I.(2006). Superoxide Anions Are Involved in Doxorubicin-Induced ERK Activation in Hepatocyte Cultures. *Ann N Y Acad Sci* ;1090: 419–428 .

References

Nourbakhsha M.,Ahmadpoura F.,Chahardolia B.,Dehkordic Z., Hosseini-Farda S., Doustimotlaghe A., Golestanie A. and Razzaghy-Azar M.(2016). Selenium and its relationship with selenoprotein P and glutathione peroxidase in children and adolescents with Hashimoto's thyroiditis and hypothyroidism. *Journal of Trace Elements in Medicine and Biology*; 34: 10–14.

Obulesu, M.& Lakshmi,M.J.(2014). Apoptosis in Alzheimer's disease: an understanding of the physiology, pathology and therapeutic avenues. *Neurochem Res*. ; 39(12): 2301–2312.

Okosieme O., Gilbert J., Abraham P., Boelaert K., Dayan C., Gurnell M., Leese G.,McCabe C., Perros P.,Smith V., Williams G. and Vanderpump M.(2015). Management of primary hypothyroidism: statement by the British Thyroid Association Executive Committee *Clinical Endocrinology*; 0, 1–10

Olson G.E., Winfrey V.P., Nagdas S.K., Hill K.E., Burk R.F.(2007). Apolipoprotein E receptor-2 (ApoER2) mediates selenium uptake from selenoprotein P by the mouse testis. *J Biol Chem* ;282:12290–12297

Öztürk Ü., Vural P., Özderya A., Karadag B., Dogru-Abbasoglu S., Uysal M.(2012). Oxidative stress parameters in serum and low density lipoproteins of Hashimoto's thyroiditis patients with subclinical and overt hypothyroidism. *Int Immunopharmacol*.; 14:349–52.

Parritz R. H., & Troy M. F. (2018). Disorders of childhood: Development and psychopathology. 3rd edition, publisher: Boston, MA : Cengage Learning.

Pasupathi P. and Latha R.(2008). Free Radical Activity and Antioxidant Defense Mechanisms in Patients with Hypothyroidism. *Thyroid Science* ;3(12):1-6.

Patel K.V., Ferrucci L., Ershler W.B., Longo D.L., Guralnik J.M.(2009). Red blood cell distribution width and the risk of death in middle-aged and older adults. *Arch Intern Med*. ;169(5):515-23.

References

Pearce E.N. and Braverman L.E. (2009). Environmental pollutants and the thyroid, Best Practice and Research. *Clinical Endocrinology and Metabolism;* 23 , 801-813.

Pearce E.N., Farwell A.P. Braverman L.E. (2003). Thyroiditis. *The New England Journal of Medicine*;348, 2646-55.

Pedersen I.B., Laurberg P., Knudsen N., Jørgensen T., Perrild H., Ovesen L., Rasmussen L.B.(2007). An increased incidence of overt hypothyroidism after iodine fortification of salt in Denmark: a prospective population study. *J. Clin. Endocrinol. Metab.* 92, 3122–3127.

Peter M.E., Hadji A., Murmann A.E., Brockway S., Putzbach W., Pattanayak A. and Ceppi P . (2015). The role of CD95 and CD95 ligand in cancer. *Cell Death and Differentiation;* 22, 549–559.

Pierce M. J., LaFranchi S. H. & Pinter J. D. (2017). Characterization of thyroid abnormalities in a large cohort of children with Down syndrome. Hormone Res. *Paediatr.* 87, 170–178.

Pitts M.W., Byrns C.N., Ogawa-Wong A.N., Kremer P., Berry M.J.(2014). Selenoproteins in nervous system development and function. *Biol. Trace Elem. Res.*; 161: 231–245.

Polzar B., Barbosa B.F., Sogani P., Vagaiwala M. (2014). A pilot study of Recent parameter in Hematology Laboratory . *J. Cell Biol*.; 12(6):124-31.

Poncin S., Gerard A.C., Boucquey M., Senou M., Calderon P.B., Knoops B., Lengele B., Many M.C. & Colin I.M.(2008). Oxidative stress in the thyroid gland: from harmlessness to hazard depending on the iodine content. *Endocrinology* ;149: 424–433.

Qadri S.M., Bissinger R., Solh Z., Oldenborg P.A.(2017). Eryptosis in health and disease: A paradigm shift towards understanding the (patho)physiological implications of programmed cell death of erythrocytes. *Blood Rev.* ; 31: 349–361.

Radosevich J.(2018). Apoptosis and Beyond the Many Ways Cells Die. John Wiley& Sons Inc , first edition , UK. pp: 2-17

References

Raymond F., Kristina E., Hill B.(2009). Selenoprotein P – Expression, Functions, and Roles in Mammals. *Biochim Biophys Acta*. ; 1790(11): 1441–1447.

Razvi S., Ingoe L., Keeka G., Oates C., McMillan C., Weaver J.U.(2007). The beneficial effect of L-thyroxine on cardiovascular risk factors, endothelial function, and quality of life in subclinical hypothyroidism: randomized, crossover trial. *J Clin Endocrinol Metab*. ;92(5):1715-23.

Reed J. C. and Green D. R.(2011). Apoptosis physiology and pathology. Cambridge University Press 2011, first edition , USA. pp: 1

Resch U., Helsel G., Tatzber F., Sinzinger H.(2002). Antioxidant status in thyroid dysfunction. *Clin Chem Lab Med*; 40:1132–4.

Rieux-Laucat F., Le Deist F., Fischer A.(2003). Autoimmune lymphoproliferative syndromes: genetic defects of apoptosis pathways. *Cell Death Differ*.; 10:124–33.

Roberts C. and Ladenson P.(2004). Hypothyroidism. *Lancet* ; 363 (9411): 793–831.

Rock C. and Moos P.J. (2010). Selenoprotein P protects cells from lipid hydroperoxides generated by 15-LOX-1. *Prostaglandins Leukot Essent Fatty Acids* ; 83: 203–210.

Rodríguez-Gutiérrez G., Rubio-Senent F., Gómez-Carretero A., Maya I., Fernández-Bolaños J., Duthie G.G., de Roos B.(2019). Selenium and sulphur derivatives of hydroxytyrosol: inhibition of lipid peroxidation in liver microsomes of vitamin E-deficient rats. Eur J Nutr. ;58(5):1847-1851.

Roelfsema F., Pereira A.M., Adriaanse R., et al.(2010). Thyrotropin secretion in mild and severe primary hypothyroidism is distinguished by amplified burst mass and Basal secretion with increased spikiness and approximate entropy. *J Clin Endocrinol Metab*; 95: 928–34.

Rojas-Villarraga A., Amaya-Amaya J., Rodriguez-Rodriguez A., Mantilla R.D., Anaya J.M.(2012). Introducing poly autoimmunity: secondary autoimmune diseases no longer exist. *Autoimmune Dis*.;2012:1–9.

References

Rose S.R.(2001). Cranial irradiation and central hypothyroidism. *Trends Endocr Metab*;12:97-104.

Saito Y., Takahashi K., Junqiu L., Guimin L., Yang M.(2012). Selenoproteins and Mimics, Berlin: Springer-Verlag GmBH, ;77–88.

Salmaso C., Bagnasco M., Pesce G., Montagna P., Brizzolara R., Altrinetti V.(2002). Regulation of apoptosis in endocrine autoimmunity: insight from Hashimoto's thyroiditis and Graves' disease. *Ann N Y Acad Sci*; 966: 496-501.

Santi A., Duarte M., de Menezes C. , and Loro V.(2012). Association of lipids with oxidative stress biomarkers in subclinical hypothyroidism. *International Journal of Endocrinology*; 2012: 7 pages.

Saranac L.; Zivanovic, S.; Bjelakovic, B.; Stamenkovic, H.; Novak, M.; Kamenov,B.(2011). Why is the thyroid so prone to autoimmune disease? *Horm Res Paediatr*. ; 75(3):157-165.

Sarandol E., Tas S., Dirican M., and Serdar Z.(2005). Oxidative stress and serum paraoxonase activity in experimental hypothyroidism: Effect of vitamin E supplementation. *Cell Biochem. Funct*.; 23(1):1-8.

Sawka A.M.; Brierley, J.D.; Tsang, R.W.; Thabane, L.; Rotstein, L.; Gafni, A.; Straus, S. and Goldstein, D.P. (2008). An updated systematic review and commentary examining the effectiveness of radioactive iodine remnant ablation in well-differentiated thyroid cancer. *Endocrinol. Metab. Clin. North Am*. 37(2):457-480.

Schmiegelow M., Feldt-Rasmussen U., Rasmussen A.K., Poulsen H.S., Müller J.(2003). A Population-based study of thyroid function after radiotherapy and chemotherapy for a childhood brain tumor. *J Clin Endocr Metab*;88:136-140.

Schneider P., Holler N., Bodmer J.L., Hahne M., Frei K., Fontana A., et al.(1998). Conversion of membrane-bound Fas(CD95) ligand to its soluble form is associated with down regulation of its proapoptotic activity and loss of liver toxicity. *J Exp Med* ; 187(8):1205–13.

References

Schomburg L. and Bauer K.(1997). Regulation of the adenohypophyseal thyrotropin-releasing hormone-degrading ectoenzyme by estradiol. *Endocrinology*; 138 , 3587- 3593.

Schultheiss U.T., Teumer A., Medici M., Li Y., Daya N., Chaker L., Homuth G., Uitterlinden A.G., Nauck M., Hofman A., Selvin E., Völzke H., Peeters R.P., Köttgen A.(2015). A genetic risk score for thyroid peroxidase antibodies associates with clinical thyroid disease in community-based populations. *J. Clin. Endocrinol. Metab.*;100:799–807.

Shao F., Gao C., Wei L.,Yang Y., Zhou Z., Liu J., Yang R., Ma W.,Wang Y., Tian L. (2017).Prevalence, related lifestyle and metabolic risk factors of hypothyroidism in adults: across-sectional study in Gansu province, Northwestern China. *Int J Clin Exp Med* ;10(7):10890-10897.

Shetty S.P., Shah R., Copeland P.R.(2014). Regulation of selenocysteine incorporation into the selenium transport protein, selenoprotein P. *J Biol Chem* ; 289: 25317–25326.

Shine B., McKnight R. F., Leaver L. & Geddes J. R.(2015). Long-term effects of lithium on renal, thyroid, and parathyroid function: a retrospective analysis of laboratory data. *Lancet*; 386, 461–468.

Šimundić A.M.(2009). Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC*; 19:203-211.

Singh R. and Czaja M.(2007). Regulation of hepatocyte apoptosis by oxidative stress. *Journal of Gastroenterology and Hepatology* ;2 (1): S45–S48.

Singh R., Upadhyay G.and Godbole M. (2003). Hypothyroidism alters mitochondrial morphology and induces release of apoptogenic proteins during rat cerebellar development. *Journal of Endocrinology* ; 176, 321–329.

Somwaru L.L., Rariy C.M., Arnold A.M., Cappola A.R.(2012). The natural history of subclinical hypothyroidism in the elderly: the cardiovascular health study. *J Clin Endocrinol Metab.* ;97(6):1962-1969.

References

Song P., Zhang R., Wang X., He P., Tan L., Ma X.(2011). Dietary grape-seed procyanidins decreased postweaning diarrhea by modulating intestinal permeability and suppressing oxidative stress in rats. *J Agric Food Chem*;59:6227-6232.

Song R.X., Mor G., Naftolin F., McPherson R.A., Song J., Zhang Z., Yue W., Wang J., Santen R.J.(2001). Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17beta-estradiol. *J Natl Cancer Inst*; 93:1714-1723.

Stagnaro-Green A. and Pearce, E.(2012). Thyroid disorders in pregnancy. *Nature Reviews in Endocrinology*; 8: (11), 650-8.

Standring S. (2016). Gray's anatomy: The anatomical basis of clinical practice. Philadelphia: Elsevier Limited pp. 470–471 Section 4, Chapter 29

Stanley J.A., Aruldhas M.M., Yuvaraju P.B., Banu S.K., Anbalagan J., Neelamohan R., Annapoorna K. & Jayaraman G.(2010). Is gender difference in postnatal thyroid growth associated with specific expression patterns of androgen and estrogen receptors? *Steroids*; 75: 1058–1066.

Stojanović J., Stefanović D., Vulović D., Puškas L., Marković L.(2009). Role of apoptosis in pathogenesis of thyroiditis. *Med Pregl*.; 62(1-2): 49–52.

Stassi G. and De Maria R. (2002). Autoimmune thyroid disease: new models of cell death in autoimmunity. *Nat Rev Immunol*.; 2(3): 195–204.

Suda T., Hashimoto H., Tanaka M., Ochi T., Nagata S.(1997). Membrane Fas ligand kills human peripheral blood T lymphocytes, and soluble Fas ligand blocks the killing. *J Exp Med* ; 186(12):2045–50.

Sun Q., Faustman C., Senecal A., Wilkinson A. L., and Furr H.(2001). Aldehyde reactivity with 2-thiobarbituric acid and TBARS in freeze-dried beef during accelerated storage. *Meat Science*; 57(1), pp. 55–60.

References

Surks M.I., Ortiz E., Daniels G.H., Sawin C.T., Col N.F., Cobin R.H., Franklyn J.A., Hershman J.M., Burman K.D., Denke M.A., C., Gorman R.S. (2004). Subclinical thyroid disease: scientific review and guidelines for diagnosis and management. *JAMA*; 291, 228-238.

Susin S.A., Zamzami N., Kroemer G.(1998). Mitochondria as regulators of apoptosis; doubt no more. *Biochim. Biophys. Acta;* 1366: 151–165.

Tanaka M., Suda T., Takahashi T., Nagata S.(1995). Expression of the functional soluble form of human fas ligand in activated lymphocytes. *EMBO J*; 14(6):1129–35.

Taylor P.N., Albrecht D., Scholz A. et al.(2018). Global epidemiology of hyperthyroidism and hypothyroidism. *Nature Reviews Endocrinology* ;14: 301–316.

Taylor P. N., Iqbal A., Minassian C.,Sayers A.,Draman M.S.,Greenwood R.,Hamilton W., Okosieme O.,Panicker V.,Thomas S.L.,Dayan C.(2014). Falling threshold for treatment of borderline elevated thyrotropin levels-balancing benefits and risks: evidence from a large community-based study. *JAMA internal medicine* ;174: 32–39.

Tekle H., Bobe T, Tufa E and Solomon F.(2018). Age-sex disparities and sub-clinical hypothyroidism among patients in Tikur Anbesa Specialized Hospital, Addis Ababa, Ethiopia. *Journal of Health, Population and Nutrition* ;37:18

Thvilum M., Brandt F., Almind D., et al.(2013). Excess mortality in patients diagnosed with hypothyroidism: a nationwide cohort study of singletons and twins. *J Clin Endocrinol Metabol*.;98:1069–75.

Traulsen H., Steinbrenner H., Buchczyk D.P., Klotz L O., Sies H.(2004). Selenoprotein P protects low-density lipoprotein against oxidation. *Free Radic. Res*.; 38:123–28.

Tsikas D.(2017). Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Analytical Biochemistry*; 524, 13–30 .

References

Vadiveloo T., Donnan P. T., Murphy M. J. & Leese G. P.(2013). Age- and gender-specific TSH reference intervals in people with no obvious thyroid disease in Tayside, Scotland: the Thyroid Epidemiology, Audit, and Research Study (TEARS). *The Journal of clinical endocrinology and metabolism;* 98: 1147–1153.

Valko M., Leibfritz D., Moncol J., Cronin M.T., Mazur M., Telser J.(2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*.;39(1):44-84.

Van der Harst P. et. al. (2012). Seventy-five genetic loci influencing the human red blood cell. *Nature*; 492:369–375.

Vanderpump M. (2010). The epidemiology of thyroid disease. *Br. Med. Bull.* 99:39-51.

Vanderpump M. (2005). The Thyroid: A Fundamental and Clinical Textbook (JB Lippincott-Raven,)pp 398–496.

Vanderpump M.P.J. (2005). The epidemiology of thyroid diseases. In: Braverman LE, Utiger RD, editors. Werner and Ingbar's The Thyroid: A Fundamental and Clinical Text. 9th edn. Philadelphia: JB Lippincott-Raven pp. 398-496.

van Deventer H.E., Mendu D.R., Remaley A.T., Soldin S.J.(2011). Inverse log-linear relationship between thyroid-stimulating hormone and free thyroxine measured by direct analog immunoassay and tandem mass spectrometry. *Clin Chem*; 57: 122–27.

Vitale M., Di Matola T., D'Ascoli F., Salzano S., Bogazzi F., Fenzi G. et al.(2000). Iodide excess induces apoptosis in thyroid cells through a p53-independent mechanism involving oxidative stress. *Endocrinology*. ; 141(2): 598–605.

Volpe E., Sambucci M., Battistini L. and Borsellino G. (2016). Fas–Fas Ligand: Checkpoint of T Cell Functions in Multiple Sclerosis. *Front. Immunol.*;7:382.

Wajant H.(2002). The Fas signaling pathway: more than a paradigm. *Science* ;296:1635–6.

References

Wang C.Y., Chang T.C., Chen M.F.(2012). Associations between subclinical thyroid disease and metabolic syndrome. *Endocr J.* ;59(10):911-7.

Wang S.H., Bretz J.D., Phelps E., Mezosi E., Arscott P.T., Utsugi S.(2002). A unique combination of inflammatory cytokines enhances apoptosis of thyroid follicular cells and transforms nondestructive to destructive thyroiditis in experimental autoimmune thyroiditis. *J Immunol,*; 168: 2470-4.

Wang S., Koenig R., Giordano T., Thompson N. and Baker J.(1999). 1α,25-Dihydroxyvitamin D3 Up-Regulates Bcl-2 Expression and Protects Normal Human Thyrocytes from Programmed Cell Death. *Endocrinology*; 140, (4).

Weetman A.P.(2005). Spectrum of autoimmune diseases in thyroid disorders. *Thyroid International* .; (1): 1–12.

Weitzel J. M., Iwen K. A., and Seitz H. J.(2003). Regulation of mitochondrial biogenesis by thyroid hormone. *Experimental Physiology*; 88(1), pp. 121–128.

Xiaofei G., Hsiang Y., Wenbo L., Randall J., Inmaculada B., Qi Mac R., Michael G. and Harvey F. (2017).Thyroid hormone receptor beta and NCOA4 regulate terminal erythrocyte differentiation. *PNAS* ; 114(38).

Xiong Z., Sun D. W., Pu H., Xie A., Han Z., and Luo M.(2015). Nondestructive prediction of thiobarbituric acid reactive substances (TBARS) value for freshness evaluation of chicken meat using hyperspectral imaging. *Food Chemistry*; 179(1), pp. 175– 181.

Xu W., Jing L., Wang Q., Lin C., Chen X., Diao J., Liu Y., Sun X.(2015). Bas-PGAM5L-Drp1 complex is required for intrinsic apoptosis execution. *Oncotarget* ; 6: 30017–30034.

Yamada M and Mori M.(2008). Mechanisms related to the pathophysiology and management of central hypothyroidism. *Nat Clin Pract Endocr Metab*;4:683-694.

References

Yang J.C. and Cortopassi G.A.(1998). Induction of the mitochondrial permeability transition causes release of the apoptogenic factor cytochrome C. *Free Radic. Biol. Med*.; 24 : 624-631.

Yazar A.,Yorulmaz A.,Ture E.,Akin F. and Sert A. (2018). A relationship between subclinical hypothyroidism and hematologic parameters in patients with Down Syndrome .*Fam Pract Palliat Care.* 3(2):92-97

Yilmaz S., Ozan S., Benzer F., and Canatan H.(2003). Oxidative damage and antioxidant enzyme activities in experimental hypothyroidism. *Cell Biochem. Funct.*; 21(4):325-330.

Yu H., Park K. and Lee J.(2014).The value of red blood cell distribution width in subclinical hypothyroidism. *Arq Bras Endocrinol Metab.* ;58(1).

Yu X., Acehan D., Me´ ne´ tret J. F., Booth C. R., Ludtke S. J., Riedl S. J., Shi Y., Wang X., and Akey C. W. (2005). A structure of the human apoptosome at 12.8 Å resolution provides insights into this cell death platform. *Structure*; 13: 1725–1735.

Yukihito K. , Kengo O.,Takuya K., Miwako H., Junko Y., Mitsuru M. and Yoshimi H. (2007). Involvement of selenoprotein P in the regulation of redox balance and myofibroblast viability in idiopathic pulmonary fibrosis. *Genes to Cells;* 12: 1235–1244.

Zaman S., Wang R., Gandhi V.(2014). Targeting the apoptosis pathway in hematologic malignancies. *Leuk. Lymphoma* ; 55: 1980–1992.

Zhou X., Obuchowski N.A., McClish D.K.(2002). Statistical Methods in Diagnostic Medicine. New York: John Wiley and Sons, Inc.

Zimmermann M. B.(2009). Iodine deficiency. *Endocr. Rev*. ;30, 376–408.

Zimmermann M.B. and Köhrle J.(2002). The impact of iron and selenium deficiencies on iodine and thyroid metabolism: Biochemistry and relevance to public health. *Thyroid;* 12, 867–878

References

Zoidis E., Seremelis I., Kontopoulos N., Danezis G.P. (2018). Selenium-dependent antioxidant enzymes: Actions and properties of selenoproteins. *Antioxidants* ;8(7): 66.

APPENDEXES

Appendix A

Patient sheet

الرقم:

اسم المريض	
العمر	
الجنس	
العنوان	
رقم الموبايل	
مدة الاصابة	
التدخين	
نوع العمل	
وجود امراض مزمنة	
نوع العلاج	
الفترة المستمر بها في العلاج	
وجود امراض مناعية	
نوع الغذاء	
الحالة الزوجية وعدد الاطفال	
المستوى الدراسي	
نوع هبوط الدرقية	

Appendix B

Correlation among studied biomarkers of patients without treatment group

		Fas	FasL	TBARS	SepP	TSH	T3	T4	RBCcount	HB	HCT	MCV	MCH	MCHC	RDW	WBC	LYMPH	MONO	GRAN	PLT	MPV	PCT	PDW
Fas	Pearson Correlation	1	.597**	.338	.592**	-.098	.009	-.331	-.172	-.015	-.399	-.241	-.148	.033	.093	-.377	-.207	.079	-.234	-.305	.075	-.215	-.037
	Sig. (2-tailed)		.005	.144	.006	.681	.969	.154	.470	.949	.081	.307	.532	.891	.697	.101	.380	.742	.321	.190	.755	.364	.876
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
FasL	Pearson Correlation	.597**	1	.659**	.444	-.084	-.071	-.282	-.067	-.342	-.299	-.309	-.310	-.163	.253	.009	.150	.234	-.198	-.355	.306	-.260	.116
	Sig. (2-tailed)	.005		.002	.050	.723	.765	.228	.780	.140	.201	.185	.183	.491	.282	.971	.528	.322	.403	.125	.190	.268	.627
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
TBARS	Pearson Correlation	.338	.659**	1	.293	.119	-.159	.076	.345	-.137	.106	-.353	-.420	-.341	.148	-.109	-.143	.152	-.172	-.096	.097	-.375	-.128
	Sig. (2-tailed)	.144	.002		.210	.618	.503	.749	.136	.565	.656	.127	.065	.141	.533	.648	.547	.521	.470	.686	.683	.103	.590
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
SepP	Pearson Correlation	.592**	.444	.293	1	-.252	.027	-.092	-.024	-.017	-.196	-.220	-.040	.184	-.013	-.160	-.162	.095	-.356	-.276	.097	-.066	.134
	Sig. (2-tailed)	.006	.050	.210		.284	.909	.699	.921	.943	.407	.351	.867	.437	.957	.500	.494	.692	.123	.239	.683	.783	.573
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
TSH	Pearson Correlation	-.098	-.084	.119	-.252	1	.275	.043	-.004	.215	.020	-.008	.021	.063	.418	-.090	.057	.531*	-.104	.093	.332	.098	.087
	Sig. (2-tailed)	.681	.723	.618	.284		.241	.856	.986	.363	.933	.974	.930	.793	.067	.707	.813	.016	.662	.695	.153	.680	.716
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
T3	Pearson Correlation	.009	-.071	-.159	.027	.275	1	-.142	-.235	-.344	-.171	.049	.255	.403	.180	.347	.247	.266	.173	-.128	.155	-.107	.488*
	Sig. (2-tailed)	.969	.765	.503	.909	.241		.551	.319	.137	.471	.836	.277	.078	.447	.134	.294	.257	.466	.591	.515	.655	.029
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
T4	Pearson Correlation	-.331	-.282	.076	-.092	.043	-.142	1	.122	.219	.303	.246	.282	.111	.084	-.040	-.295	.077	-.001	.177	-.140	-.320	.016
	Sig. (2-tailed)	.154	.228	.749	.699	.856	.551		.608	.354	.193	.296	.229	.643	.724	.867	.207	.745	.997	.455	.556	.169	.948
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
RBCcount	Pearson Correlation	-.172	-.067	.345	-.024	-.004	-.235	.122	1	.233	.717**	-.496-*	-.744-**	-.694-**	-.114	-.124	-.316	-.202	-.074	.306	-.088	-.036	-.596-**
	Sig. (2-tailed)	.470	.780	.136	.921	.986	.319	.608		.324	.000	.026	.000	.001	.631	.604	.174	.394	.758	.189	.712	.880	.006
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
HB	Pearson Correlation	-.015	-.342	-.137	-.017	.215	-.344	.219	.233	1	.513*	.347	.155	-.150	-.009	-.542-*	-.600-**	-.088	-.120	.297	-.453-*	-.023	-.411
	Sig. (2-tailed)	.949	.140	.565	.943	.363	.137	.354	.324		.021	.134	.514	.529	.969	.014	.005	.711	.614	.203	.045	.923	.072
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
HCT	Pearson Correlation	-.399	-.299	.106	-.196	.020	-.171	.303	.717**	.513*	1	.242	-.207	-.635-**	-.156	-.034	-.488-*	-.469-*	.163	.411	-.414	-.214	-.547-*
	Sig. (2-tailed)	.081	.201	.656	.407	.933	.471	.193	.000	.021		.304	.381	.003	.512	.886	.029	.037	.492	.072	.069	.366	.013
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
MCV	Pearson Correlation	-.241	-.309	-.353	-.220	-.008	.049	.246	-.496-*	.347	.242	1	.794**	.183	-.032	.072	-.193	-.301	.275	.118	-.456-*	-.232	.080
	Sig. (2-tailed)	.307	.185	.127	.351	.974	.836	.296	.026	.134	.304		.000	.439	.893	.762	.415	.198	.240	.619	.043	.326	.737
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

Appendix B

		Fas	FasL	TBARS	SepP	TSH	T3	T4	RBCcount	HB	HCT	MCV	MCH	MCHC	RDW	WBC	LYMPH	MONO	GRAN	PLT	MPV	PCT	PDW
MCH	Pearson Correlation	-.148	-.310	-.420	-.040	.021	.255	.282	-.744-**	.155	-.207	.794**	1	.738**	.017	.057	.142	.058	.051	-.049	-.297	-.059	.466*
	Sig. (2-tailed)	.532	.183	.065	.867	.930	.277	.229	.000	.514	.381	.000		.000	.944	.810	.549	.808	.831	.837	.204	.806	.038
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
MCHC	Pearson Correlation	.033	-.163	-.341	.184	.063	.403	.111	-.694-**	-.150	-.635-**	.183	.738**	1	.056	.029	.454*	.426	-.216	-.224	.060	.188	.697**
	Sig. (2-tailed)	.891	.491	.141	.437	.793	.078	.643	.001	.529	.003	.439	.000		.816	.903	.045	.061	.360	.342	.802	.427	.001
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
RDW	Pearson Correlation	.093	.253	.148	-.013	.418	.180	.084	-.114	-.009	-.156	-.032	.017	.056	1	.273	.244	.568**	.230	.093	.495*	-.074	-.131
	Sig. (2-tailed)	.697	.282	.533	.957	.067	.447	.724	.631	.969	.512	.893	.944	.816		.245	.301	.009	.329	.697	.027	.756	.581
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
WBC	Pearson Correlation	-.377	.009	-.109	-.160	-.090	.347	-.040	-.124	-.542-*	-.034	.072	.057	.029	.273	1	.465*	-.098	.701**	.119	.372	.155	.179
	Sig. (2-tailed)	.101	.971	.648	.500	.707	.134	.867	.604	.014	.886	.762	.810	.903	.245		.039	.681	.001	.618	.107	.514	.451
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
LYMPH	Pearson Correlation	-.207	.150	-.143	-.162	.057	.247	-.295	-.316	-.600-**	-.488-*	-.193	.142	.454*	.244	.465*	1	.268	-.014	-.260	.457*	.404	.448*
	Sig. (2-tailed)	.380	.528	.547	.494	.813	.294	.207	.174	.005	.029	.415	.549	.045	.301	.039		.254	.953	.268	.043	.078	.047
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
MONO	Pearson Correlation	.079	.234	.152	.095	.531*	.266	.077	-.202	-.088	-.469-*	-.301	.058	.426	.568**	-.098	.268	1	-.328	-.158	.456*	.181	.319
	Sig. (2-tailed)	.742	.322	.521	.692	.016	.257	.745	.394	.711	.037	.198	.808	.061	.009	.681	.254		.157	.505	.043	.444	.170
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
GRAN	Pearson Correlation	-.234	-.198	-.172	-.356	-.104	.173	-.001	-.074	-.120	.163	.275	.051	-.216	.230	.701**	-.014	-.328	1	.331	.054	-.020	-.150
	Sig. (2-tailed)	.321	.403	.470	.123	.662	.466	.997	.758	.614	.492	.240	.831	.360	.329	.001	.953	.157		.154	.822	.933	.527
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
PLT	Pearson Correlation	-.305	-.355	-.096	-.276	.093	-.128	.177	.306	.297	.411	.118	-.049	-.224	.093	.119	-.260	-.158	.331	1	-.418	-.310	-.606-**
	Sig. (2-tailed)	.190	.125	.686	.239	.695	.591	.455	.189	.203	.072	.619	.837	.342	.697	.618	.268	.505	.154		.067	.183	.005
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
MPV	Pearson Correlation	.075	.306	.097	.097	.332	.155	-.140	-.088	-.453-*	-.414	-.456-*	-.297	.060	.495*	.372	.457*	.456*	.054	-.418	1	.416	.350
	Sig. (2-tailed)	.755	.190	.683	.683	.153	.515	.556	.712	.045	.069	.043	.204	.802	.027	.107	.043	.043	.822	.067		.068	.130
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
PCT	Pearson Correlation	-.215	-.260	-.375	-.066	.098	-.107	-.320	-.036	-.023	-.214	-.232	-.059	.188	-.074	.155	.404	.181	-.020	-.310	.416	1	.178
	Sig. (2-tailed)	.364	.268	.103	.783	.680	.655	.169	.880	.923	.366	.326	.806	.427	.756	.514	.078	.444	.933	.183	.068		.452
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
PDW	Pearson Correlation	-.037	.116	-.128	.134	.087	.488*	.016	-.596-**	-.411	-.547-*	.080	.466*	.697**	-.131	.179	.448*	.319	-.150	-.606-**	.350	.178	1
	Sig. (2-tailed)	.876	.627	.590	.573	.716	.029	.948	.006	.072	.013	.737	.038	.001	.581	.451	.047	.170	.527	.005	.130	.452	
	N	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20

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

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

Appendix C
Correlation among studied biomarkers of patients with treatment group

		Fas	FasL	TBARS	SepP	TSH	T3	T4	RBCcount	HB	HCT	MCV	MCH	MCHC	RDW	WBC	LYMPH	MONO	GRAN	PLT	MPV	PCT	PDW
Fas	Pearson Correlation	1	.286	.179	.061	.082	-.016	-.247	.087	-.017	.141	.041	-.074	-.246	.029	-.082	.026	.164	-.106	-.051	-.016	-.151	-.043
	Sig. (2-tailed)		.074	.269	.707	.615	.921	.125	.595	.919	.387	.801	.648	.126	.857	.614	.873	.312	.517	.754	.922	.354	.793
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
FasL	Pearson Correlation	.286	1	.054	-.183	.038	.229	-.235	-.019	-.347-*	-.209	-.187	-.258	-.282	-.042	-.267	-.298	-.024	-.216	-.170	-.024	-.114	-.063
	Sig. (2-tailed)	.074		.741	.258	.815	.155	.145	.906	.028	.195	.248	.108	.078	.799	.095	.062	.882	.180	.294	.882	.485	.698
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
TBARS	Pearson Correlation	.179	.054	1	-.228	.203	-.202	.090	-.314-*	-.152	-.179	.257	.171	.011	-.031	-.081	-.002	-.244	-.177	.107	-.047	-.017	-.161
	Sig. (2-tailed)	.269	.741		.157	.209	.211	.581	.048	.349	.269	.110	.292	.947	.852	.620	.989	.129	.275	.510	.774	.917	.322
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
SepP	Pearson Correlation	.061	-.183	-.228	1	-.180	-.116	.100	.118	-.146	-.089	-.263	-.233	-.116	-.100	-.010	.164	.111	-.016	.003	-.033	.087	.088
	Sig. (2-tailed)	.707	.258	.157		.266	.477	.539	.470	.370	.584	.102	.148	.476	.540	.951	.313	.495	.923	.983	.842	.593	.588
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
TSH	Pearson Correlation	.082	.038	.203	-.180	1	-.126	.026	-.078	.173	.118	.182	.166	.115	-.035	-.025	-.074	-.109	.001	-.005	.106	-.208	.021
	Sig. (2-tailed)	.615	.815	.209	.266		.438	.873	.632	.287	.467	.261	.305	.481	.831	.880	.648	.505	.995	.976	.514	.197	.897
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
T3	Pearson Correlation	-.016	.229	-.202	-.116	-.126	1	.029	.287	-.170	.045	-.353-*	-.396-*	-.381-*	.168	-.100	-.224	.140	.008	-.079	.108	-.108	-.020
	Sig. (2-tailed)	.921	.155	.211	.477	.438		.858	.073	.294	.784	.025	.011	.015	.301	.538	.164	.390	.962	.629	.508	.508	.904
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
T4	Pearson Correlation	-.247	-.235	.090	.100	.026	.029	1	-.067	-.156	-.128	-.052	-.067	-.080	-.074	.049	.058	-.058	.075	.082	-.136	-.045	.032
	Sig. (2-tailed)	.125	.145	.581	.539	.873	.858		.681	.337	.430	.752	.680	.625	.650	.765	.722	.721	.648	.617	.402	.782	.846
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
RBCcount	Pearson Correlation	.087	-.019	-.314-*	.118	-.078	.287	-.067	1	.295	.683**	-.653-**	-.662-**	-.556-**	-.124	.077	-.030	.092	.066	.339*	-.012	-.063	-.214
	Sig. (2-tailed)	.595	.906	.048	.470	.632	.073	.681		.065	.000	.000	.000	.000	.447	.636	.853	.572	.688	.032	.942	.701	.185
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
HB	Pearson Correlation	-.017	-.347-*	-.152	-.146	.173	-.170	-.156	.295	1	.832**	.421**	.506**	.460**	-.172	.479**	.417**	.005	.410**	.307	-.176	-.080	.064
	Sig. (2-tailed)	.919	.028	.349	.370	.287	.294	.337	.065		.000	.007	.001	.003	.290	.002	.007	.977	.009	.054	.278	.624	.693
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
HCT	Pearson Correlation	.141	-.209	-.179	-.089	.118	.045	-.128	.683**	.832**	1	.092	.043	-.107	-.255	.243	.233	-.048	.183	.339*	-.228	-.280	-.164
	Sig. (2-tailed)	.387	.195	.269	.584	.467	.784	.430	.000	.000		.573	.791	.512	.112	.131	.147	.768	.259	.032	.157	.081	.311
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
MCV	Pearson Correlation	.041	-.187	.257	-.263	.182	-.353-*	-.052	-.653-**	.421**	.092	1	.941**	.605**	-.100	.064	.230	-.241	.034	-.083	-.294	-.245	-.023
	Sig. (2-tailed)	.801	.248	.110	.102	.261	.025	.752	.000	.007	.573		.000	.000	.538	.694	.154	.135	.834	.612	.066	.128	.889
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40

Appendix C

		Fas	FasL	TBARS	SepP	TSH	T3	T4	RBCcount	HB	HCT	MCV	MCH	MCHC	RDW	WBC	LYMPH	MONO	GRAN	PLT	MPV	PCT	PDW
MCH	Pearson Correlation	-.074	-.258	.171	-.233	.166	-.396-*	-.067	-.662-**	.506**	.043	.941**	1	.837**	-.018	.256	.315*	-.111	.231	-.039	-.197	-.049	.120
	Sig. (2-tailed)	.648	.108	.292	.148	.305	.011	.680	.000	.001	.791	.000		.000	.912	.111	.047	.494	.152	.812	.223	.764	.460
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
MCHC	Pearson Correlation	-.246	-.282	.011	-.116	.115	-.381-*	-.080	-.556-**	.460**	-.107	.605**	.837**	1	.120	.468**	.351*	.107	.443**	.020	.041	.312	.356*
	Sig. (2-tailed)	.126	.078	.947	.476	.481	.015	.625	.000	.003	.512	.000	.000		.461	.002	.026	.512	.004	.901	.801	.050	.024
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
RDW	Pearson Correlation	.029	-.042	-.031	-.100	-.035	.168	-.074	-.124	-.172	-.255	-.100	-.018	.120	1	.113	.062	.649**	.127	.156	.473**	-.030	-.087
	Sig. (2-tailed)	.857	.799	.852	.540	.831	.301	.650	.447	.290	.112	.538	.912	.461		.487	.706	.000	.435	.337	.002	.856	.592
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
WBC	Pearson Correlation	-.082	-.267	-.081	-.010	-.025	-.100	.049	.077	.479**	.243	.064	.256	.468**	.113	1	.672**	.485**	.837**	.276	.244	.269	.439**
	Sig. (2-tailed)	.614	.095	.620	.951	.880	.538	.765	.636	.002	.131	.694	.111	.002	.487		.000	.002	.000	.085	.129	.093	.005
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
LYMPH	Pearson Correlation	.026	-.298	-.002	.164	-.074	-.224	.058	-.030	.417**	.233	.230	.315*	.351*	.062	.672**	1	.367*	.443**	.362*	.022	.149	.156
	Sig. (2-tailed)	.873	.062	.989	.313	.648	.164	.722	.853	.007	.147	.154	.047	.026	.706	.000		.020	.004	.022	.893	.357	.337
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
MONO	Pearson Correlation	.164	-.024	-.244	.111	-.109	.140	-.058	.092	.005	-.048	-.241	-.111	.107	.649**	.485**	.367*	1	.444**	.185	.321*	.142	.241
	Sig. (2-tailed)	.312	.882	.129	.495	.505	.390	.721	.572	.977	.768	.135	.494	.512	.000	.002	.020		.004	.254	.043	.382	.134
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
GRAN	Pearson Correlation	-.106	-.216	-.177	-.016	.001	.008	.075	.066	.410**	.183	.034	.231	.443**	.127	.837**	.443**	.444**	1	.160	.247	.384*	.401*
	Sig. (2-tailed)	.517	.180	.275	.923	.995	.962	.648	.688	.009	.259	.834	.152	.004	.435	.000	.004	.004		.324	.125	.014	.010
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
PLT	Pearson Correlation	-.051	-.170	.107	.003	-.005	-.079	.082	.339*	.307	.339*	-.083	-.039	.020	.156	.276	.362*	.185	.160	1	-.286	.106	-.456-**
	Sig. (2-tailed)	.754	.294	.510	.983	.976	.629	.617	.032	.054	.032	.612	.812	.901	.337	.085	.022	.254	.324		.073	.516	.003
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
MPV	Pearson Correlation	-.016	-.024	-.047	-.033	.106	.108	-.136	-.012	-.176	-.228	-.294	-.197	.041	.473**	.244	.022	.321*	.247	-.286	1	.247	.517**
	Sig. (2-tailed)	.922	.882	.774	.842	.514	.508	.402	.942	.278	.157	.066	.223	.801	.002	.129	.893	.043	.125	.073		.125	.001
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
PCT	Pearson Correlation	-.151	-.114	-.017	.087	-.208	-.108	-.045	-.063	-.080	-.280	-.245	-.049	.312	-.030	.269	.149	.142	.384*	.106	.247	1	.430**
	Sig. (2-tailed)	.354	.485	.917	.593	.197	.508	.782	.701	.624	.081	.128	.764	.050	.856	.093	.357	.382	.014	.516	.125		.006
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
PDW	Pearson Correlation	-.043	-.063	-.161	.088	.021	-.020	.032	-.214	.064	-.164	-.023	.120	.356*	-.087	.439**	.156	.241	.401*	-.456-**	.517**	.430**	1
	Sig. (2-tailed)	.793	.698	.322	.588	.897	.904	.846	.185	.693	.311	.889	.460	.024	.592	.005	.337	.134	.010	.003	.001	.006	
	N	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40

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

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

الخلاصـــة

مرض قصور الغدة الدرقية هو حالة مرضية عالمية شائعة يمكن أن يكون لها تأثير ذو دلالة على حياة المرضى. هناك العديد من المؤشرات على أن الموت المبرمج للخلايا يمثل احد مسببات هذا المرض لأنه يؤدي إلى ضرر دائم لخلايا الغدة الدرقية لذلك ركزت هذه الدراسة على تحليل المؤشرات الحيوية الذائبة في الدم الخاصة بالموت المبرمج لدى المرضى المصابين بقصور الغدة الدرقية. شملت هذه الدراسة 60 مريضا مصاب بقصور الغدة الدرقية، 20 من هؤلاء المرضى تم تشخيصهم حديثا بالمرض و40 منهم تمت معالجتهم بالليفوثايروكسين لفترات مختلفة. ايضا، شملت الدراسة 30 شخص سليم كمجموعة سيطرة. كان المرضى من كلا الجنسين ذكور واناث تراوحت أعمارهم بين 20 إلى 70 سنة. تم جمع عينات الدم من المرضى والاصحاء في هذه الدراسة ، تم فصل 3 مللتر من الدم بواسطة جهاز الطرد المركزي للحصول على مصل الدم لتحليل الهرمونات الخاصة بالغدة الدرقية (الهرمون المحفز للدرقية وثلاثي يود الثايرونين والثايروكسين) ومؤشرات الموت المبرمج (Fas و FasL و Bcl-2 و Cytochrome C) ومؤشرات الإجهاد التأكسدي (TBARS) والمؤشرات الحيوية المضادة للأكسدة (SepP1) في حين تم وضع الباقي من الدم في أنبوب مضاد للتخثر لقياس صورة الدم الكاملة.

اشارت نتائج هذه الدراسة بان معظم المرضى كانوا من الاناث في الاربعينيات والخمسينيات من العمر اللاتي يسكنَّ المدينة ومصابات بمرض المناعة الذاتية المعروف بالتهاب الغدة الدرقية هاشيموتو. ارتفع الهرمون المحفز للغدة الدرقية ارتفاعا معنويا في كلا مجموعتي المرضى مقارنة بالسيطرة بينما انخفضت مستويات هرمونات الدرقية (T3 وT4) انخفضت معنويا في مرضى قصور الدرقية المعالجين وغير المعالجين على التوالي مقارنة بالسيطرة. كذلك وضحت الدراسة ارتفاعا معنويا في المؤشرات الحيوية للموت المبرمج (Fas وBcl-2 وCox) لدى المرضى غير المعالجين مقارنة بالأشخاص في السيطرة بينما ازداد FasL معنويا في مجموعة المرضى المعالجين مقارنة بالسيطرة والمرضى غير المعالجين. بينت النتائج زيادة معنوية في الجهد التأكسدي عن طريق ارتفاع مستوى TBARS ونقصان معنوي في المؤشرات الحيوية المضادة للأكسدة SepP1 في مجموعة مرضى قصور الدرقية غير المعالجين مقارنة بالسيطرة.

علاوة على ذلك، سجلت بعض مؤشرات خلايا الدم الحمر (الهيموغلوبين Hb و الهيماتوكريت HCT وهيموغلوبين الكرية الوسطي MCH والتركيز الوسطي لهيموغلوبين

الكرية MCHC) نقصانا معنويا على التوالي عند المرضى في كلا مجموعتي المرضى مقارنة بالسيطرة. ومن جهة اخرى، وجدت زيادة معنوية في متوسط حجم الكرية MCV لدى مجموعة المرضى المعالجين مقارنة بالسيطرة. كذلك كان هناك نقصانا معنويا في عدد الخلايا اللمفاوية والخلايا الوحيدة في كلا مجموعتي المرضى مقارنة بالسيطرة. . فيما يخص اعداد الصفيحات الدموية و الحجم الذي تمثله الصفائح الدموية (PCT) و التغاير في حجم الصفائح الدموية (PDW) اظهرت نقصانا معنويا لدى مرضى قصور الدرقية مقارنة بالاشخاص السليمين في مجموعة السيطرة.

تبعا لنتائج هذه الدراسة، كان للإناث اختلافات خاصة بالجنس فيما يتعلق باختبارات وظائف الدرقية ومؤشرات الموت المبرمج والجهد التأكسدي ومضادات الاكسدة . لوحظ وجود علاقة طردية معنوية بين TBARS وFas في المرضى غير المعالجين بينما كانت العلاقة بين FasL والهيموغلوبين والعلاقة TBARS وعدد خلايا الدم الحمر علاقة عكسية معنوية في المرضى المعالجين. كانت قيمة المنطقة تحت منحني روك للمؤشر الحيوي للموت المبرمج (Fas) (0.864) مما يعكس احتمالية استخدام هذا المؤشر في تحديد شدة الاصابة بالمرض او تحديد تقدم المرض. واخيرا أظهر المؤشر الحيوي للموت المبرمج (Fas) خطرا نسبيا معنويا بقيمة (2.9) مقارنة ببقية المؤشرات الحيوية المدروسة.

وقد تبين من خلال هذه الدراسة ان مرضى قصور الدرقية لديهم زيادة في الموت المبرمج في الدم والذي انعكس بارتفاع مؤشرات الموت المبرمج في كلا مجموعتي المرضى مقارنة بالسيطرة وكذلك امكانية استخدام المؤشر الحيوي للموت المبرمج (Fas) في التنبؤ بتقدم المرض.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية العلوم/ قسم علوم الحياة

تقييم بعض مؤشرات الموت المبرمج ومعايير الدم لدى مرضى قصور الدرقية

اطروحة مقدمة الى

مجلس كلية العلوم/ جامعة بابل وهي جزء من متطلبات نيل درجة الدكتوراه فلسفة في العلوم/علوم الحياة/ فرع الحيوان

من قِبل

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