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CLINICAL STUDY OF INTERLEUKIN-6 AND SOME BIOCHEMICAL PARAMICAL IN HYPOTHYROIDISM PATIENTS

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CLINICAL STUDY OF INTERLEUKIN-6 AND SOME BIOCHEMICAL PARAMICAL IN HYPOTHYROIDISM PATIENTS

By Shams Azzam Abdullah ALBAKRI

December 2022

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

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ABSTRACT

CLINICAL STUDY OF INTERLEUKIN 6 AND SOME BIOCHEMICAL PARAMICAL IN HYPOTHYROIDISM PATIENTS

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Master of Science in Chemistry

Advisor: Prof. Dr. Volkan EYÜPOĞLU

December 2022

The aim is to study the relationship between chronic inflammation in hypothyroidism and some chemical parameters and to study changes in the levels of oxidative stress (ROS) and a decrease in the levels of antioxidants, in addition to studying the decrease in the levels of some trace elements. The patients in the study were randomly taken from hypothyroid patients coming to Baghdad Teaching Hospital, where the patient group included 120 patients with problems with hypothyroidism and compared with 120 other healthy people as a control group. Age had great importance in the development of hypothyroidism, as the results indicated the development of the disease at an advanced age. Cytokines had important indicators in patients with hypothyroidism, but relatively little clinical importance. Changes in the levels of trace elements may lead to greater chances of developing inflammatory diseases by reducing interleukin levels. The levels of the lipid profile were significantly affected in the patients, and changes in the lipid profile may be a reason for the development of hypothyroidism. Iron was not statistically significant when compared to the control group. There was great importance of Zinc in patients with hypothyroidism, which may contribute to aiding zinc absorption. Y and MDI had statistical significance, indicating the influence of antioxidants in patients with hypothyroidism.

2022, 55 pages

Keywords: Hypothyroidism, Interleukin 6, Thyroxine, Triiodothyronine

ÖZET

HİPOTİROİDİ HASTALARINDA İNTERLÖKİN 6 VE BAZI BİYOKİMYASAL PARAMİYALLERİN KLİNİK ÇALIŞMASI

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Amaç, hipotiroidizmdeki kronik inflamasyon ile bazı kimyasal parametreler arasındaki ilişkiyi araştırmak ve oksidatif stres (ROS) seviyelerindeki değişiklikleri ve antioksidan seviyelerindeki düşüşü incelemek, ayrıca bazı eser elementlerin seviyelerindeki düşüşü incelemektir. elementler. Çalışmaya dahil edilen hastalar, hipotiroidi sorunu olan 120 hastadan oluşan hasta grubunun bulunduğu Bağdat Eğitim Hastanesi'ne gelen hipotiroidili hastalardan rastgele alındı ve kontrol grubu olarak diğer 120 sağlıklı kişi ile karşılaştırıldı. Sonuçlar hastalığın ileri yaşta geliştiğini gösterdiğinden, yaşın hipotiroidi gelişiminde büyük önemi vardı. Sitokinler, hipotiroidizmi olan hastalarda önemli göstergelere sahipti, ancak nispeten az klinik öneme sahipti. İz element seviyelerindeki değişiklikler, interlökin seviyelerini azaltarak inflamatuar hastalıkların gelişme şansının artmasına neden olabilir. Hastalarda lipid profili düzeyleri önemli ölçüde etkilenmiştir ve lipid profilindeki değişiklikler hipotiroidi gelişiminin bir nedeni olabilir. Kontrol grubu ile karşılaştırıldığında demir istatistiksel olarak anlamlı değildi. Çinko emilimine yardımcı olabilecek hipotiroidili hastalarda çinkonun büyük önemi vardı. Y ve MDI, hipotiroidizmi olan hastalarda antioksidanların etkisini gösteren istatistiksel bir öneme sahipti.

2022, 55 sayfa

Anahtar Kelimeler: Hipotiroidizm, İnterlökin 6, Tiroksin, Triiyodotironin

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Shams Azzam Abdullah ALBAKRI

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LIST OF SYMBOLS

-	Minus
%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equal
>	Less than
±	Plus minus
≤	Greater or equal to
≥	Less or equal to
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m^2	Square meter
µg	Microgram
mIU	Milli-international units
min	Minute
mL	Milliliter
mmol	Millimole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
rpm	Revolutions per minute
µL	Microliter

LIST OF ABBREVIATIONS

BSF-2	B-cell stimulatory factor 2
CRP	C-reactive protein
HFG	Hybridoma growth factor
HSF	Hepatocyte-stimulating factor
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL	Interleukins
PRRs	Pathogen-recognition receptors
RA	Rheumatoid arthritis
RBC	Red blood cell
SAA	Serum amyloid A
TLRs	Toll-like receptors
TSH	Thyroid stimulating hormone

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1. INTRODUCTION

Interleukins, often known as IL, are a kind of cytokine that were, for a long time, thought to be something that could only be produced by leukocytes. On the other hand, it was later found out that interleukins may also be produced by a great number of different cells in the body. In addition to this, they have properties that both encourage and discourage inflammatory reactions in the body. As a result, the primary function of interleukins is to exert an effect on the processes of cell proliferation, differentiation, and activation throughout the course of immune and inflammatory responses (Meng *et al.* 2020).

Interleukins are complex proteins that bind to high-affinity receptors on the surface of cells. Their composition includes a diverse collection of proteins. Because of this, the cells and tissues are forced to go through a wide range of various reactions. In addition to the paracrine function that they perform, they also perform the autocrine function. Animal studies also make use of interleukins for the purpose of investigating various elements of clinical medicine (IL6). It has a widespread impact on immune system cells as well as cells unrelated to the immune system, and it commonly displays hormone-like features that have an influence on the homeostatic processes. In addition to this, it contains qualities that may either be pro- or anti-inflammatory depending on the circumstances (Khodakheir *et al.* 2017).

Researchers have found a variety of cytokines in regions of persistent inflammation, such as those produced by periodontitis, autoimmune illnesses, thyroiditis, and arthritis. These conditions are all associated with chronic inflammation. There is also evidence that people who have hypothyroidism have an increased production of the cytokine IL6 (Šenolt *et al.* 2017).

1.1 Aim of Study

The goal of this investigation is to identify the connection between hypothyroidism and illnesses that are characterized by chronic inflammation. Although it is possible to say

that hypothyroidism suffers from an increase in oxidative stress (ROS) and a decrease in the levels of antioxidants as well as a decrease in the levels of some trace elements, which most probably refers to an increase in the chances of chronic inflammatory disease through a decrease in the levels of interleukin, it is possible to say that hypothyroidism suffers from a decrease in the levels of some trace elements.

2. LITERATURE REVIEW

Interleukins (IL) are a kind of cytokine that were at one time assumed to be exclusively expressed by leukocytes. However, it was subsequently discovered that many other bodily cells also generate interleukins. Additionally, they contain features that both promote and inhibit inflammatory responses. Therefore, the fundamental purpose of interleukins is to influence growth, differentiation, and activation in the course of immunological and inflammatory responses (Keller *et al.* 2019).

Interleukins are made up of a wide set of proteins that attach to high-affinity receptors on the surface of human beings' cells. This causes the cells and tissues to undergo a variety of different responses. They also have an autocrine role in addition to a paracrine one. Interleukins are also used in animal studies to investigate aspects related to clinical medicine (IL6). It has a widespread impact on immune cells as well as cells that are not connected to the immune system, and it commonly has hormone-like features that influence the homeostatic processes (Schroeder *et al.* 2014).

Additionally, it possesses context-dependent pro- and anti-inflammatory properties. At the areas of persistent inflammation, such as those caused by periodontitis, autoimmune disorders, thyroiditis, and arthritis, researchers have identified a number of cytokines. In addition, there is some evidence that has been recorded indicating that sick individuals with hypothyroidism have increased production of IL6 (Hofmann *et al.* 2016).

In the 1970s, the first interleukins were isolated and characterized. Because researchers first assumed that leukocytes (white blood cells) were responsible for the production of interleukins and that these proteins acted largely on other leukocytes, they termed these proteins interleukins, which literally translates to "between leukocytes." It was previously believed that the sole role interleukins served was that of immune function modulators. This is due to the fact that leukocytes are engaged in the process of mounting immune responses. There are known to be fifteen distinct varieties of interleukins, and these types are denoted by the numbers 1 through 15 in the following order: IL1 through IL15 (Hussien *et al.* 2022).

The immunological roles of the majority of the interleukins are, to varying degrees, known to the scientific community. T and B lymphocytes are white blood cells that are fundamental to the process of bringing about an acquired immune response. IL1 and IL2 are largely responsible for activating T and B lymphocytes, with IL2 being a stimulant of T- and B-cell proliferation and maturation. They are also primarily responsible for activating natural killer (NK) cells. Inflammation may also be mediated by IL1, just as it can be mediated by IL6 (Rysz *et al.* 2006).

IL4 is responsible for an increase in the production of antibodies by B lymphocytes, whereas IL12 is responsible for an increase in the production of cytotoxic T cells and natural killer cells among the leukocytes. Which cells will mount a response to the infection and how certain clinical symptoms of the illness will appear are both influenced by the set of interleukins that are triggered when a particular infectious agent is present (Bech *et al.* 2016).

Diabetes and interleukin-6 even while inflammation has been linked to diabetes and obesity for a long time, the exact mechanism behind the onset of this proinflammatory condition is still unknown. Patients who are obese and insulin resistant have higher levels of IL6, in addition to higher levels of other proinflammatory cytokines (Šenolt *et al.* 2015).

Rheumatoid arthritis and interleukin-6; rheumatoid arthritis is characterized by inflammation, which causes damage to the joints; IL6 is a major effector in this process. However, cytokines, including IL6, are found in high amounts in the arthritic joints, blood, and synovium of persons who have this condition, even though the cause of RA is still unknown (Grevich and Shenoi 2017).

Cancer and interleukin 6 in chronic inflammation, inflammatory cells migrate towards a region where they may multiply, differentiate, and then fail to die because apoptosis is dysregulated. This causes the inflammation to continue for a prolonged period of time (De Vito *et al.* 2015).

This component of the transcription factor effects is significantly influenced by the transcription factor NF-kB. Neoplasia can only develop if certain conditions are met, including self-sufficiency in growth signals, non-responsiveness to signals that impede development, the capacity to evade apoptosis, an unconstrained ability to multiply, the capability to penetrate tissue and disseminate, and continuing angiogenesis. Neoplasia can only develop if these elements are present (Hsu *et al.* 2017).

The main aim of the current investigation is to find the possible links between hypothyroidism and chronic inflammatory conditions. Whereas it is possible to say that hypothyroidism suffers from an increase in oxidative stress (ROS) and a decrease in the levels of antioxidants as well as a decrease in the levels of some trace elements, which leads to an increase in the chances of chronic inflammatory disease through a decrease in the levels of interleukin.

Patients who have hypothyroidism and are admitted to the hospital will be selected at random for participation in the trial. Herein, it will be collected 120 patients with in Hypothyroidism patients and compare with other 120 person apparently healthy as control group.

2.1 Thyroid

The thyroid gland is a tiny organ that is shaped like a butterfly and is positioned in the front of the neck right below the voice box (larynx). Imagine that the centre of the butterfly's body is located on your neck, and that the wings are encircling your windpipe and embracing each other (trachea). The regulation of metabolism is the base function of the thyroid gland (Hyun *et al.* 2015).

The process by which your body converts the food you eat into the fuel it needs to carry out its functions is called metabolism. The metabolism is regulated by the thyroid, which produces the hormones T4 and T3, respectively. These hormones are responsible for communicating with cells throughout the body to determine how much energy they

should use. They are responsible for regulating both your core temperature and your heart rate (Delitala *et al.* 2017).

When your thyroid is functioning properly, it will continually produce hormones, release those hormones into the bloodstream, and then produce new hormones to replace the ones that have been utilized, this ensures that your metabolism and the rest of your body's functions continue to operate normally, the pituitary gland, which is situated in the middle of the skull, just below the brain, is responsible for regulating the quantity of thyroid hormones that are released into the circulation (Bekkering *et al.* 2019).

As the pituitary gland detects either a deficiency in thyroid hormone or an excess of it, it modifies its own hormone, which is known as thyroid stimulating hormone, or TSH, and then delivers it to the thyroid in order to achieve hormonal equilibrium. The whole body is influenced whether the quantity of thyroid hormones is too high (also known as hyperthyroidism) or too low (also known as hypothyroidism) (Rovet 2014).

2.1.1 Hypothyroidism

Hypothyroidism is a disorder in which there is an inadequate amount of thyroid hormone present in the circulation, which results in a sluggish metabolic rate. Hypothyroidism is diagnosed when the thyroid gland related to normal human beings doesn't have the ability to produce and secrete an adequate amount of thyroid hormone into the body. This causes your metabolism to slow down, which has repercussions throughout your body. Hypothyroidism, also known as underactive thyroid disease, is a condition that affects a significant number of people (Obregon 2014).

Myxedema, however, is the medical term that describes when your thyroid levels are exceedingly low. In most cases, hypothyroidism is a disorder that may be effectively treated. It is possible to get it under control by taking prescribed drugs as directed and keeping all of your follow-up visits with then doctor (Mondal *et al.* 2016).

When the thyroid gland produces less thyroid hormone than is required, a condition known as hypothyroidism may occur. When compared to what they should be, the levels of thyroid hormones in a person's body are too low, which will result in an impaired metabolism throughout the body (Moog *et al.* 2017).

Hypothyroidism can be caused by a number of different factors, including pregnancy, disorders of the hypothalamus or pituitary gland, radioactive iodine treatment, thyroid surgery, radiation therapy to the neck, certain types of medications, and Hashimoto's thyroiditis, which is the most common cause of hypothyroidism (Jara *et al.* 2017).

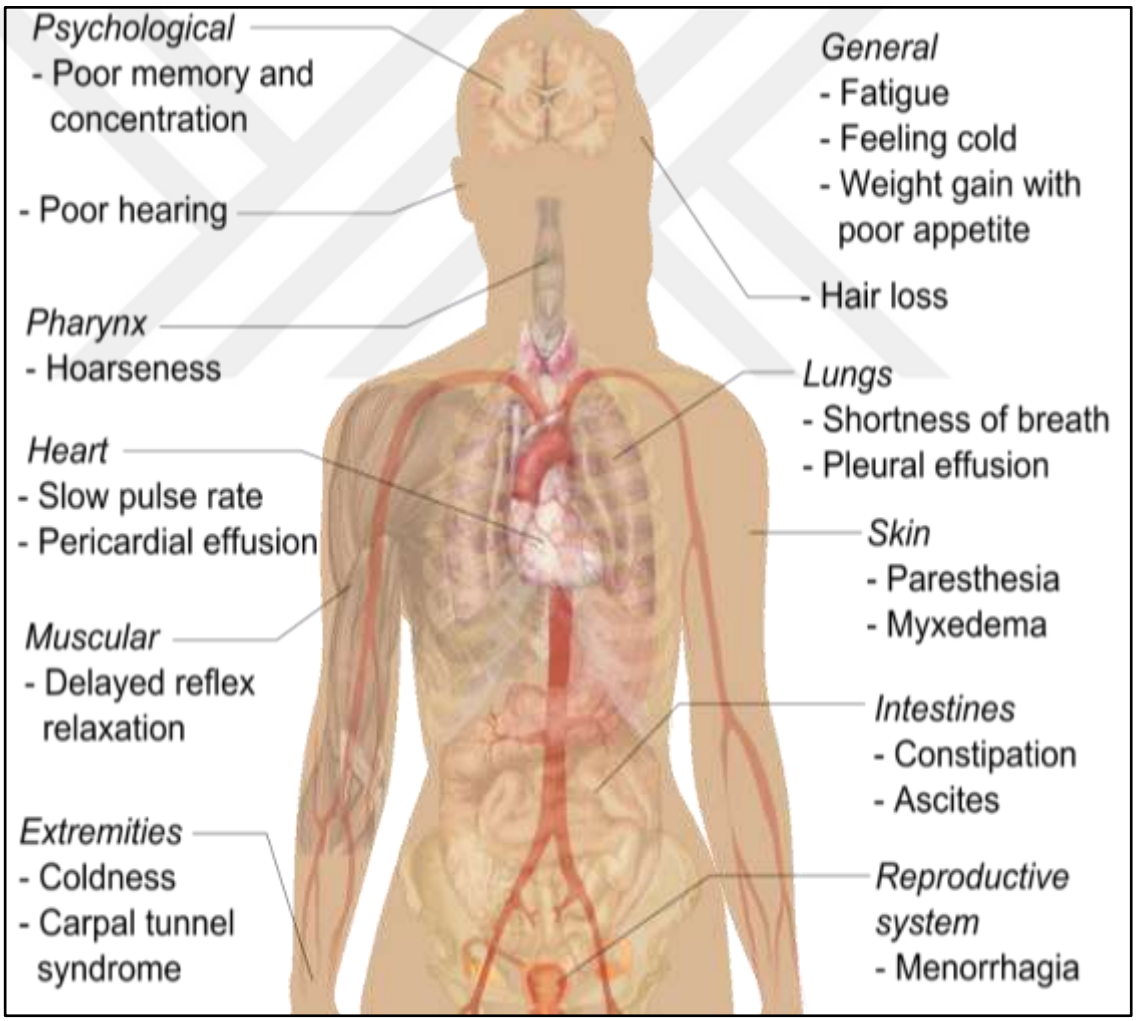

Figure 2.1 signs and symptoms of hypothyroidism (Bassett *et al.* 2016)

The diagnosis of hypothyroidism may be made quickly and readily using thyroid function testing. Treatment with synthetic thyroid hormone is advised in the event that the patient's thyroid level is much lower than the normal range (Gottwald-Hostalek and Schulte 2022). This form of treatment is not only successful but also quite easy to carry out. Despite the fact that males may also be affected by hypothyroidism beyond the age of 60, hypothyroidism is more likely to occur in women at that age (Duntas *et al.* 2018).

2.1.2 Hypothyroidism and hyperthyroidism

There are two possible origins of hypothyroidism: the original cause and the secondary cause. A disorder that has a direct influence on the thyroid and leads it to produce low amounts of thyroid hormones is one of the key causes of hypothyroidism. A secondary reason is anything that most probably refers to the failure of the pituitary gland, which results in the pituitary gland being unable to transmit thyroid stimulating hormone (TSH) to the thyroid in order to maintain hormonal equilibrium (Sinha *et al.* 2018).

Primary causes of hypothyroidism are found much more often than secondary causes. The autoimmune illness known as Hashimoto's disease is the key reason that manifests itself in the greatest number of patients. Hereditary thyroiditis is a disorder that may also be referred to as Hashimoto's thyroiditis or chronic lymphocytic thyroiditis (passed down through a family). Hashimoto's illness is characterized by the immune system of the body attacking and causing damage to the thyroid. Because of this, the thyroid is unable to produce and secrete an adequate amount of thyroid hormone (Salvatore *et al.* 2014).

Hypothyroidism is a condition in which the thyroid gland does not produce enough of the hormone thyroid hormone. The amount of thyroid hormone produced is what differentiates hypothyroidism from hyperthyroidism. Hypothyroidism is a condition in which the thyroid produces an abnormally low amount of the hormone thyroid hormone (Alvarez-Crespo *et al.* 2016).

On the other hand, hyperthyroidism refers to a condition in which a person's thyroid produces an abnormally high amount of thyroid hormone. The condition known as hyperthyroidism causes your metabolism to speed up because it results in greater quantities of thyroid hormones. Your metabolism will slow down significantly if you have hypothyroidism (Krashin *et al.* 2019).

Between these two states, there are a lot of things that couldn't be more different from each other. If you suffer from hypothyroidism, you can have a harder difficulty fending off the cold than other people. It's possible that you won't be able to manage the heat if you have hyperthyroidism. These two states of thyroid activity are polar opposites of one another. In a perfect world, you would be located somewhere in the center. The goal of treatment for each of these diseases is to get your thyroid function back to something as near to normal as is clinically achievable (Mancini *et al.* 2016).

2.1.3 Hypothyroidism symptoms

The initial symptoms of hypothyroidism that a person may acquire are fatigue and a lack of energy. A person with hypothyroidism will develop this condition. However, it is common for these symptoms to be dismissed as unimportant, and it may take weeks or even months before hypothyroidism is identified. This is because the condition is often not picked up on until other, more significant symptoms manifest (Razvi *et al.* 2018).

The metabolism and the production of other hormones are both profoundly affected by thyroid hormones. A sluggish metabolism and the reduced action of other hormones are often the causes of the symptoms of exhaustion and lack of energy that accompany hypothyroidism. In situations of hypothyroidism, a person's basal metabolism will be slower than normal, which will lead them to burn less calories than usual. A significant portion of these calories will be stored as fat, which is the reason for the weight gain. You will produce less heat in proportion to the number of calories that expend (Jabbar *et al.* 2017).

Brown fat, which is a distinct kind of body fat, may be negatively impacted when hypothyroidism is present in the body. This brown fat is responsible for the production of heat as well as the maintenance of a normal body temperature in chilly environments. If you have lately observed a cold intolerance that you did not have in the past, then you should consider about hypothyroidism and get checked out to make sure it is not present. Patients who suffer from hypothyroidism often report experiencing hair difficulties, which may sometimes be quite upsetting. Alterations in both the quality and the texture of the hair are to be expected, and a typical concern is also the loss of hair (Senese *et al.* 2014).

The regular absorption of nutrients from the digestive system will be hampered, which will result in the development of a number of nutritional deficiencies, which will lead to issues with the hair. Problems with hair growth are likely to manifest themselves in the event that, for instance, an iron deficit develops. Additionally, the hormones produced by the thyroid have a direct effect on the hair follicles, which in turn encourages the creation of new hair. The process of hair regeneration will be halted when hypothyroidism is present, which will, of course, result in a loss of hair. The good news is that once the thyroid hormone levels are regulated by replacement medication, both hair issues and hair loss will begin to improve (Jabbar *et al.* 2017).

Skin cells die and are replaced at a quick pace, and this process continues continually throughout an individual's lifetime. In situations of hypothyroidism, the process of skin cell regeneration will be hindered, which means that it may take the skin longer to repair (Hurlow and Bliss 2011).

Dry and pale skin are common complaints made by patients who have hypothyroidism; however, dry skin is frequently associated with other conditions, and it does not necessarily mean that it is caused by low levels of thyroid hormones. Patients who have hypothyroidism have low levels of thyroid hormone in their bodies (Augustin *et al.* 2019).

2.2 Interleukin 6

It is possible for the soluble mediator known as IL6 to have an effect that is pleiotropic, which means that it may have an effect on inflammation as well as the immune response and hematopoiesis. In the beginning, several different activities of IL6 were examined, and their distinct names were determined from the particular biological activities that they carried out (Kishimoto 2010). For instance, the capacity of the substance known as B-cell stimulatory factor 2 (BSF-2) to prompt the transformation of active B cells into antibody-producing cells led to the naming of the substance as BSF-2. Because of its influence on the rapid protein synthesis that occurs in hepatocytes during the acute phase, this molecule is known as hepatocyte-stimulating factor (HSF) (Liu *et al.* 2021).

The potential of HGF to stimulate the expansion of fusion cells that include both plasma cells and myeloma cells led to the naming of this protein as HFG. The name intersects with However, in 1986, when the BSF-2 cDNA was successfully cloned, it was found that the molecules with multiple labels explored by separate groups were in fact similar. As a result, the single name IL6 was given to the molecule rather than the multiple names that had been given to it previously (Cao *et al.* 2001).

The human IL-6 protein comprises a total of 212 amino acids, including a signal peptide that is just 28 amino acids long, and the location of its gene on chromosome 7p21 has been determined. The glycosylation process causes natural IL6 to be between 21 and 26 kDa in size, despite the fact that the core protein is only around 20 kDa (Caparrós *et al.* 2018).

Immediately and transiently created in response to infections and tissue damage, interleukin 6 (IL6) makes a contribution to the host's defense by stimulating acute phase responses, hematopoiesis, and immunological reactions. Dysregulated, continuous synthesis of IL6 has a harmful role in the development of chronic inflammation and autoimmune diseases, despite the fact that its expression is closely regulated by genomic and posttranscriptional processes (Niess *et al.* 2018).

2.2.1 Function

Synergistically working with the activity of IL3, the proliferation of murine pluripotent stem cells may be induced by IL6. While IL6 is responsible for initiating the entry of latent progenitor cells into the cell cycle, IL3 is responsible for ensuring that the cell cycle progression is maintained. Additionally, megakaryopoiesis may be stimulated by IL6 in conjunction with stem cell factor and thrombopoietin (Maxwell *et al.* 2015).

The megakaryocyte progenitors' level in IL6 knockout mice is much lower. In addition, the process of granulopoiesis is influenced by IL6. When both IL6 and G-CSFR are absent in mice, the resulting neutropenia is more severe than what is seen in animals that have been genetically altered to lack G-CSFR (Putoczki and Ernst 2010).

Additionally, the administration of IL6 into G-CSFR mutant mice results in an improvement in granulopoiesis. In both human (U937) and mouse (M1) myeloid cell lines, IL6 has been shown to be able to stop the cell cycle and promote differentiation. In response to IL6, murine M1 cells undergo a differentiation process that produces macrophages. Activation of Stat3 is what transmits this signal, which is caused by IL6 (Hofmann *et al.* 2016).

Interleukin 6 was first recognized as a lymphokine that is produced by T cells and is responsible for inducing the last maturation stage of B cells, which results in the production of antibodies. The synthesis of immunoglobulin M (IgM), immunoglobulin G (IgG), and immunoglobulin A (IgA) is induced by recombinant human IL-6 only in B cells that have been activated by Staphylococcus aureus Cowan I or pokeweed mitogen, but not in resting B cells (Campos-Rodríguez *et al.* 2010).

It was also shown that murine IL6 could operate on murine B cells that had been stimulated with anti-Ig or dextran sulfate. IL6 and IL1 work together to increase the proliferation and differentiation of murine B cells in a synergistic manner. Interleukin 6 might also enhance the proliferation of mitogen-stimulated thymocytes and peripheral T

cells, in addition to its ability to drive the growth and differentiation of T cells. When IL2 is present in the culture media, it was also shown that it has the potential to stimulate the formation of cytotoxic T cells in murine in addition to mortal thymocytes and splenic T cells (Robert and Miossec 2017).

2.2.2 Role

In response to both tissue injury and infections, the cytokine known as IL-6 is released. IL6 has pleiotropic effects (Tanaka *et al.* 2014). The synthesis of this cytokine is linked to a wide variety of cell types, including fibroblasts, keratinocytes, mesangial cells, vascular endothelial cells, mast cells, macrophages, dendritic cells, as well as T and B cells (Liu *et al.* 2020).

Following the engagement of its particular receptor, IL6 initiates a chain reaction of signaling events, the majority of which are associated with the JAK/STAT3 activation pathway, these events promote the transcription of multiple downstream genes that are involved in the processes of cellular signaling (Zenobia and Hajishengallis 2015).

In addition to this, it is responsible for controlling the creation of proteins that are involved in the regulation of gene expression. It's possible that the pleiotropic character of this interleukin may be explained by the large number of genes that are controlled by the activity of IL6 (Dienz *et al.* 2009).

As a result of this, the biological effects of IL6 production have been linked to both pro- and anti-inflammatory effects, which highlights the essential role that IL6 plays in the activation and control of the immune response (Zhao *et al.* 2020).

There have been two separate pathways reported that facilitate the suppression of Th1 polarization by IL6. These mechanisms are as follows: (2) IL6 has an effect on the secretion of IFN by CD4 T cells, which is a necessary interferon for promoting the polarization of Th1 cells. (1) IL6 encourages CD4 T cells to release IL4 and guide the

response to Th2, and (2) IL6 impacts the production of IFN by CD4 T cells. The suppression of IFN- production in Th1 cells has a comparable impact on CD8 T cell activation as the effect that is induced by these cells (Fauny *et al.* 2020).

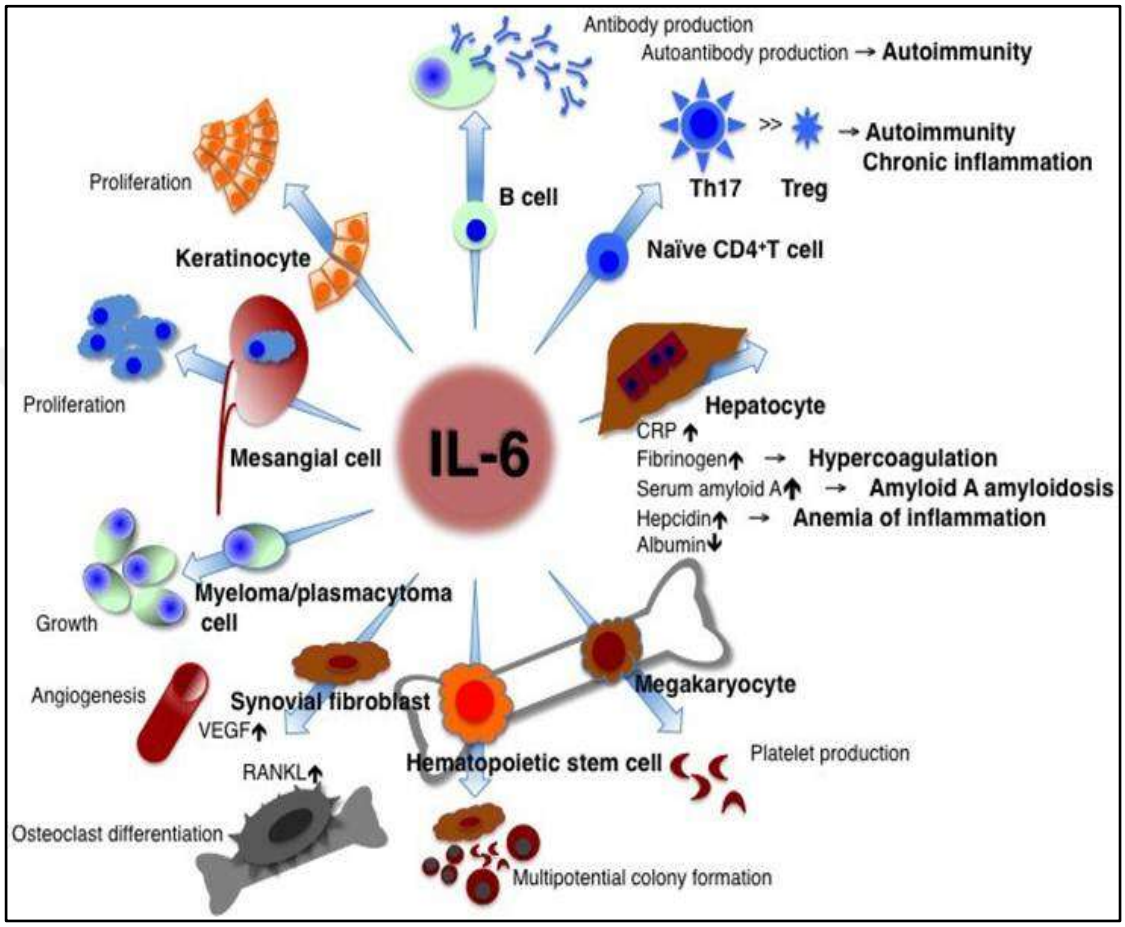

Figure 2.2 Roles of interleukin 6 (Fabre *et al.* 2018)

2.2.3 Biological effect

Following the synthesis of IL6 in a local lesion during the first stage of inflammation, it travels through the bloodstream to the liver. This is followed by the rapid induction of a wide variety of acute phase proteins such as; CRP, SAA, fibrinogen, haptoglobin, and 1-antichymotrypsin. However, IL6 inhibits the formation of fibronectin, albumin, and transferrin in the body. In the beginning, these biological effects on hepatocytes were investigated on the assumption that they belonged to HSF. Through the development of amyloid A amyloidosis, which occurs when high value of SAA are allowed to remain for

an extended period of time, a significant complication of various chronic inflammatory disorders is brought about (Wasilewska *et al.* 2016).

Deposition of amyloid fibrils, which leads to gradual damage in a variety of organs, is the consequence of this process. Additionally, IL6 is responsible for controlling the transporters of iron and zinc in the serum, making it a factor in the regulation of iron and zinc levels. In terms of serum iron, IL6 is responsible for inducing the formation of hepcidin, which in turn inhibits the function of the iron transporter ferroportin 1 on the gut and, as a consequence, lowers serum iron levels. In light of this information, we may deduce that the IL6-hepcidin axis is to blame for the hypoferremia and anemia that are linked with chronic inflammation. Inflammation is associated with hypozincemia, which is caused by IL6 because it increases the expression of the zinc importer ZIP14 on hepatocytes (Schön and Erpenbeck 2018).

It is ultimately due to the delivery of IL6 to the bone marrow since it encourages the maturation of megakaryocytes, which leads to the generation of platelets. For the goal of assessing the amount of inflammatory activity, the fluctuations in acute phase protein levels, as well as the changes in RBC and platelet counts, are analyzed in routine clinical laboratory examinations. [Clinical laboratory examinations] In addition, IL6 is the factor that drives naïve CD4+ T cells into a more differentiated state, which is a hallmark of the immune system (Hosokawa *et al.* 2014).

It is considered how an up-regulation of the Th17/Treg equilibrium is too responsible for the disruption of immunological tolerance, and as a consequence, it is pathologically involved in the emergence of autoimmune and chronic inflammatory disorders. In addition, it has been shown that in addition to being responsible for the synthesis of IL21, IL6 is also important for encouraging the growth of T-follicular helper cells. The creation of immunoglobulin (Ig) and, more particularly, IgG4 is under the control of IL21, which is responsible for controlling Ig synthesis (Hashizume *et al.* 2010).

Additionally, IL6 is responsible for causing $CD8^+$ T cells to differentiate into cytotoxic T cells. Continuous oversynthesis of IL6 causes hypergammaglobulinemia as well as the

production of autoantibodies. This is due to the fact that IL6 has the capability, which was identified when it was known by one of its former names, BSF-2, to drive the differentiation of active B cells into plasma cells that manufacture antibodies (Vitiello and Miller 2020).

In addition to its effects on hepatocytes and lymphocytes, IL6 is responsible for a variety of additional effects, many of which are found to be present in chronic inflammatory disorders. One of these effects is that the activation of RANKL, a protein that is necessary for the differentiation and activation of osteoclasts, occurs when IL6 is released in the stromal cells of the bone marrow. This is one of the effects. This, in turn, leads to the breakdown of bone and the condition known as osteoporosis. Inflammatory lesions are characterized by a number of pathogenic characteristics, including increased vascular permeability and enhanced angiogenesis (Ma *et al.* 2018).

These pathological characteristics can be observed, for instance, in the synovial tissues of rheumatoid arthritis (RA) or in the edema of remitting seronegative symmetrical synovitis with pitting edema (RS3PE) syndrome. Both of these conditions are characterized by symmetrical synovitis and pitting edema. IL6 is also responsible for inducing an excessive production of VEGF, which results in increased vascularization and improved angiogenesis. In conclusion, it has been demonstrated that IL6 promotes either the proliferation of keratinocytes or the production of collagen in dermal fibroblasts. Both of these processes, which may be responsible for the alterations seen in the skin of systemic sclerosis patients, have been shown to be induced by IL6 (Abbas *et al.* 2015).

2.2.4 Regulation

The cytokine IL6 functions as a mediator for the communication of information on the occurrence of various emergent events. An infected lesion will cause IL6 to be produced, which will then act as a warning signal to be sent throughout the cell's body. In an infected lesion, PRRs of immune cells like macrophages ın addition to monocytes are able to identify the distinctive molecular patterns that are associated with foreign pathogens.

These patterns are referred to as pathogen-associated molecular patterns (Isailovic *et al.* 2015).

This family of pattern recognition receptors includes not only TLRs, but also retinoic acid-inducible gene-1-like receptors, DNA receptors (PRRs), in addition to nucleotide-binding oligomerization domain-like receptors. They activate a number of signaling pathways as well as the transcription of the mRNA for inflammatory cytokines such as IL6, tumor necrosis factor (TNF), and IL1. In addition, they stimulate the transcription of the mRNA. One of which is NF-B. TNF- and IL1 both activate transcription factors, which ultimately results in the production of IL6. In the case that there is injury to the tissue, IL6 also sends out a warning signal (Maxwell *et al.* 2015).

DAMPs a term refer to molecules that may directly or indirectly increase inflammation. These molecules are generated from cells that have been injured or are dying as a result of noninfectious inflammations such burns or trauma. A rise in serum IL6 levels comes first during sterile surgical procedures; this is followed by an increase in both the body temperature and the concentration of serum acute phase protein. DAMPs derived from damaged cells include a wide array of components, including mitochondrial DNA (mtDNA), proteins called S100, and high mobility group box 1 (HMGB1) (Lockshin *et al.* 2018).

In trauma patients, the levels of serum mtDNA are hundreds of times higher than in controls. This is despite the fact that the interaction of HMGB1 to TLR2, TLR4, and the receptor of end products of advanced glycation (RAGE) may be responsible for inducing inflammation. This rise causes TLR9 to become stimulated, which in turn activates NF-B. However, there are about 25 members of the protein family known as S100, and some of those members interact with RAGE in order to bring about sterile inflammation (Zenobia and Hajishengallis 2015).

Many distinct types of cells, such as; mesenchymal cells, fibroblasts, immune-mediated cells, endothelial cells, and, are involved in the production of the cytokine IL6 in response to a wide variety of stimuli. This process is triggered by a wide range of stimuli. The

principal reasons for the strict regulation of IL6 formation that appears both during transcription factors and when it has been accomplished is that IL6 acts as a warning signal to indicate the presence of an emergency. This control occurs both before and after gene transcription has been finished (Qian *et al.* 2017).

It seems, a number of different transcription factors are accountable for controlling how much of the IL6 gene is expressed in cells. There are functional cis-regulatory elements in the 5' flanking region of the human IL6 gene. These elements include binding sites for nuclear factor B (NF-B), selectivity protein 1 (SP1), nuclear factor IL6 (NFIL6), and interferon regulatory factor 1. Activation of the IL6 promoter results from the stimulation of cis-regulatory elements by IL1, TNF, a signal mediated by TLR, and forskolin. The IL6 activations are promoter ultimately results in the synthesis of IL6 (Prinz *et al.* 2020).

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Studied groups

According to study objective and to do enhance results, In the experments in the lab within the field of this study, 120 samples of patients with hypotyroid , and healthy control (120 person) were chosen and divided into two groups, these groups of the present study are clearly shown in Figure 3.1.

Group A: Consists of 120 healthy person.

Group B: Consists of 120 hypothyroid patients with.

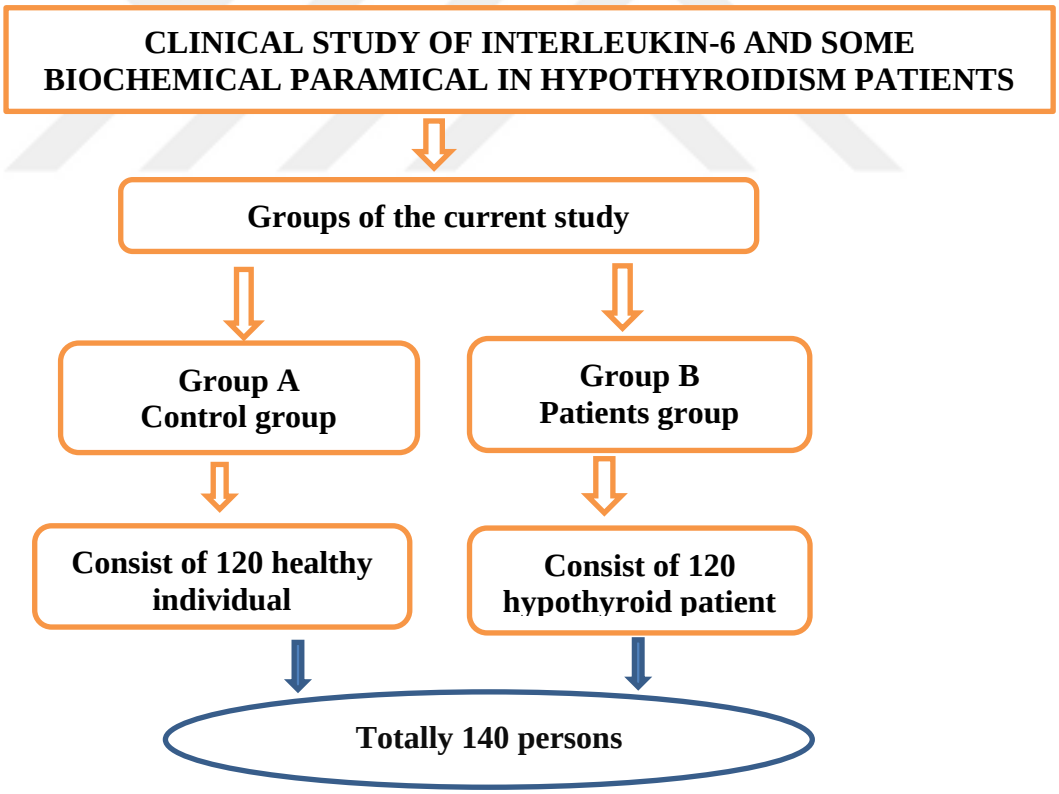

Figure 3.1 Studied groups

3.1.2 Tools and others devices

In Table 3.1, it has been shown some tools and others deviced which were used in vitro to complete and arrive the main goal of prresent study.

Table 3.1 Important material, devices, and tools during this current study

Devices and tools	**Origins**
Water distilled	GFL, Germany
Water path	Memmert, Germany
Centrifuge	Hettich, Germany
Refrigerator	Samsung, Korea
İncubator	Korea
ELISA analyzer	Germany
Precision pipette 50 µL, 100 µL, 1 mL	China
Microcentrifuge vial	China
Vortex mixer	UK
Microtiter plate readre	UK
Microplate	China

3.1.3 Blood samples

The blood samples for this study were obtained from the individuals and patients (3 mL) by using sterile syringes from brachium vein, and then placed in test tubes, and put the tubes at 25°C for 15 minutes in order to coagulate the blood, and reactor the tubes at 2000 rpm / min for 5 min to differentiate serum from other blood products, the serum will be isolated and withdrawn serum by micropipette, then placed in other tubes for conduct biochemical markers.

3.1.4 Patients chosen

After receiving informed permission from each participant, the patients who were selected to take part in this research were categorized in accordance with the Wagner classification system, and then they were assigned at random to one of the groups that will be discussed further below.

3.2 Methods

3.2.1 Thyroid function tests (TSH and T4)

TSH test

Incubate the wells at 37 degrees Celsius for an hour and a half after adding 100 microliters of the standard or the sample. Throw away the liquid, and then immediately add 100 L of the working solution for the biotinylated detection antibody to each well, it was incubated at lest for 60 min at 37°C. Repeat the process of aspirating and washing the plate three times. Include one hundred microliters of the HRP conjugate as working solution.

Incubate for 30 min at 37°C. 5 times through the process of aspirating and washing the plate. Add 90 L of the substrate reaction solution. Incubate for 15 min at 37°C. Include 50 L of the stop solution. Start reading the plate at 450 nm straight away. Electrochemiluminescence immunoassay will be used to calculate the results, and commercially available kits made by Roche Elecsys Modular Analytics Cobas e411 will be used to get the data for the test (Roche Di agnostics, Mannheim, Germany).

T4 test

Take out 300 L of the diluent, and then place it in the detection buffer together with the granule. After waiting until the granule has been thoroughly diluted, 75 L of the sample should be added to the detection buffer. Shake the sample mixture ten times to further combine the ingredients. The sample combination should be incubated for ten minutes at room temperature. After the allotted amount of time has passed in the incubation, 75 L of the sample mixture should be added to the cartridge.

Check to see whether there is any lateral flow happening on the membrane. Put the cartridge into the I chamber, and set the temperature and incubation duration to 25 degrees Celsius for ten minutes. After inserting the cartridge into the I chroma II, conduct the test, and make a note of the outcome.

3.2.2 Interleukin 6 (IL6) test

By using the Interleukin 6 derermination kit we calculation the IL6 concentration as below:

Insert the card into analyzer instrument and read the card, append 50 μL of blood sample to the buffer tube and mix well. Get 80 μL of sample and add to the casstte and then incubate at 25°C for 15 minutes. Insert the cassette into the analyzer and push "Test", the results will display on screen and the results will printed automatically.

3.2.3 Principle of total cholesterol test

To find and to arrive to the purpose of current investigation "determining the overall level of cholesterol in a blood sample", this method makes use of 3 kinds os enzymes, for example; peroxidase, cholesterol esterase, and cholesterol oxidase. A combination of phenol and 4- aminoantipyrine are adsorbent by hydrogen peroxide to conclude quinonemine dye and H_2O. The quinonemine dye represents the concentration of cholesterol in the sample. Cholesterol esterase is converted to cholesterol and fatty acids. The cholesterol is then oxidized to cholestenone and hydrogen peroxide. The methodology for calculating the total cholesterol that was used in this research may be seen in Equations (3.1), Equations (3.2), and Equations (3.3), respectively.

$$\text{Cholesteerol esterase} \xrightarrow{\text{Cholesterol esterase}} \text{cholesterol + fatty acids} \quad (3.1)$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol oxidase}} \text{cholestenone} + H_2O_2 \quad (3.2)$$

$$\text{4- aminoantipyrine +phenol} \xrightarrow{H_2O_2/\text{ peroxidase}} \text{Quinoneimine} + 4\ H_2O \quad (3.3)$$

3.2.4 Triglyceride test

This process involves the hydrolysis of serum triglycerides, which results in the formation of glycerol and fatty acids. The glycerol is then phosphor glycerided by ATP in the presence of glycerol kinase, which results in the formation kind such as; ADP in addition to glycerol-3-phosphate. Glycerol phosphate oxidase is responsible for the oxidation that results in the formation of DHAP and hydrogen from glycerol-3-phosphate.

Equation (3.4), Equation (3.5), Equation (3.6), and Equation (3.7) exhibit the congenital to parameter estimation the concentration of triglyceride in the sample. This is accomplished through the assistance of the enzyme peroxidase, which catalyzes the formation of quinoemine and water from 4-aminoantipyrine and phenol.

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + 3\ \text{Free fatty acids} \quad (3.4)$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{Glycerol-3- Phosphate} + \text{ADP} \quad (3.5)$$

$$\text{Glycerol-3- Phosphate} + O_2 \xrightarrow{\text{Glycerol peroxidase}} \text{DHAP} + H_2O_2 \quad (3.6)$$

$$\text{4- Aminoantipyrine} + \text{Phenol} \xrightarrow{\text{Peroxidase}} \text{Quinonemine} + H_2O \quad (3.7)$$

Procedure

After bringing all of the samples and reagents to room temperature, take one milliliter each from the tubes labeled "blank," "sample," and "cal standard" and place it in the R1 reagent tube. Next, place ten microliters of sample in the tube labeled "sample" and ten microliters of cal standard in the tube labeled "CAL standard." After thoroughly mixing all of the tubes and allowing them to rest for 15 minutes at room temperature, incubate the tubes at 25 degrees Celsius for ten minutes, and then compare the absorbance at 500

nanometers to the blank. For the purposes of determining serum triglyceride levels, the Equation (3.8) was used.

Triglycerides (mg/dL) = A sample / A standard × C standard (3.8)

3.2.5 High density lipoprotein (HDL) cholesterol test

Principle

This procedure is based on the precipitation of apoliprotein (VLDL, LDL) by phosphotungestic acid / $MgCl_2$, centrifuge to sediminate the precipitation and enzymatic analysis of the high density lipoproteins as residual cholesterol in the supernatant.

Reagent composition

R1: Precipitation reagent / Phosphotungestic acid 0.63 mmol / L, magnesium cholride 25 mmol / L.

Cal: Cholesterol standard / Cholesterol 50 mg/ dL.

R2: Cholesterol MR.

Procedrure

Bring all the reagents and samples to 25°C, place 0.2 mL of sample or standard into the tube containing 0.4 mL of R1 working reagent, admix well by vortix for 10 minutes at room teperature then centrifuge the tubes for 2 minutes at 12000 rpm, separate off the supernatant within 90 minute. Pipette into the R1 reagent tube 1 mL of each tube labelled blank, sample supernat and standard supernat also place 50 µL of sample supernat into the supernat tube, 50 µL of standard supernat into the standard tube, admix for 5 minutes at 37°C then read the absurbance at 500 nm. For the calculations HDL, it can be use the Equation (3.9).

HDL cholesterol (mg/ dL) = (A supernatant / A standard) × C standard (3.9)

3.2.6 Low density lipoprotein (LDL) cholesterol test

Principle

This method involves the precipitation of low density lipoproteins in blood using polyvinyl sulfate, followed by centrifugation of the precipitant and a test to determine the amount of cholesterol that is still present in the supernatant after the removal of VLDL and HDL.

Reagent compositions

R1: Precipitation reagent / polyvinyl sulfate 170 g / L.

CAL: LDL cholesterol standard / Cholesterol 50 mg / L .

Procedure

Bring all the reagents and samples at 25°C, add to labelled tube 0.2 mL of sample or standard with 0.1 mL of R1 working reagent and vortex for 10 minutes at 25°C then centrifuge for 2 minutes at 12000 rpm then remove an aliquot of supernatant and measure the total cholesterol of the sample by prepare two series of tests and measured the remaining cholesterol in the supernatant. Putinto the R1 tube 1 mL of each labelled blank, sample and standard supernat then add 50 µL of the sample supernat to the supernat tube, also put 50 µL of the standard supernat into the standard tube then read the absurbance at 500 nm. For the calculationsthe concentration, it can be use the Equation (3.10) in addition to Equation (3.11).

$$\text{Cholesterol supernat (mg/ dL)} = (\text{A supernatant} / \text{A standard}) \times \text{C standard} \quad (3.10)$$

$$\text{LDL cholesterol (mg / dL)} = \text{Total cholesterol-cholesterol supernatant} \quad (3.11)$$

3.2.7 Zink test

Procedure

Bring all the reagents and samples at room temperature, labell the microtube, put 475 µL of normal saline in 2 microtube then, add 25 µL of plasma to the microtube, hence the

dilution ratio as 1:20, mix well, label three tubes as blank, standard and test. Put 400 μL of R1 reagent to the three selected tubes, it was added about 100 μL of R2 reagent to all three tubes, add 100 μL of normal saline to the tube label blank, add 100 μL of R3 reagent to the tube label standard, shake it well then add 100 μL of diluted plasma to the tube label test, shake it well then incubate all the tubes at 25°C for 5 minutes, choose the programe "Zink" from the Androchem analyzer instrument and push "Run", the secreen display "use stored result", then choose "Yes" and the instrument will use stored "standard" results to calculate the result.

The screen display "Asprite sample" asprite sample, the screen display "Read blank", asprite blank then, the screen display "Read standard", asprite standard, asprite standard, the screen display Re- run standard, choose "No", the screen display "Read sample", asprite sample, then the screen display the results.

3.2.8 Iron test

Principle

The Fe^{+3} bound to serum ferritine dissociated by teepol and guanidium chloride to form Fe^{+3} and apotransferrin by hydroxylamine. The ferrous iron and ferrozine proportional to the concetration of iron present in the sample, for this putpose, it suiatable to use the Equation (3.12), Equation (3.13), and Equation (3.14).

$$\text{Transferrin} + Fe^{+3} \xrightarrow{\text{Teepol/ PH<5}} \text{Apotransferrin} + Fe^{+3} \quad (3.12)$$

$$Fe^{+3} + \text{Hydroxylamine} \longrightarrow Fe^{+2} \quad (3.13)$$

$$FE^{+2} + \text{Ferrozine} \longrightarrow \text{Fe (ferrozine)3}^{+2} \text{ complex} \quad (3.14)$$

Reagent composition

R1 / Buffer: Guanidine chloride 1 mol / L, hydroxylamine 0.6 mol/ L, acetate buffer 400 mol / L, PH 4.0 teepol.

R2/ Chromogen: Ferrozine 8 mol / L, sodium acetate 400 mmol / L.
Cal / Iron standard: Ferric ion 100 mg / dL.

Procedure

Bring all the reagents and samples to 25 °C, pipette into the label tubes, add 200 µL of reagent blank into the distlled water tube, 200 of sample blank and 200 µL of sample into the sample tube, 200 µL of cal standard into the cal standard tube, 1 mL of sample blank into the R1 tube, 1 mL of reagent blank and 1 mL of sample and 1 mL of cal standard into the working reagent tube, mix all the tubes and stand for 5 minutes at 25°C then read the absurbance at 560 nm of the sample blank against distilled water. Read the absurbance of the samples and standard at 560 nm against the blank, for the calculation; it can be use the Equation (3.15).

Iron concetration (mg /dL) = (A sample –A sample blank) / A standard (3.15)

3.2.9 Catalase test

Principle

This method is predicated on the interaction of catalase with a known amount of hydrogen peroxide; the reaction is inhibited by catalase after it has run its course for one minute. When hydrogen peroxide interacts with 3,5 of DHBS and AAP, a chromophore is produced that has a tensity that is proportional to the amount of catalase present in the sample. For the purpose of determining the levels, Equation (3.16) and Equation (3.17) can be applied.

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2 \quad (3.16)$$

$$2H_2O_2 + \text{DHBS} + \text{AAP} \xrightarrow{\text{peroxidase}} \text{Quinoneimine} + 4H_2O \quad (3.17)$$

Reagents

Buffer / 100 mM / L phosphate buffer. H_2O_2 500 mM /L

Chromogen inhibitor

Peroxidase > 2000 /L , 4- aminoantipyrine 2 mM/L.

Procedure

Dilute R2 reagent 1000 times before use, 10 uL of R2 reagent with 10 mL of distilled water, pipette the label tubes, add 0.05 mL of sample blank and 0.05 mL of sample into the sample tube, add 0.05 mL of sample blank and 0.1 mL of standard blank and 0.05 mL of standard into the distilled water tube, add 0.5 mL of sample blank and 0.5 mL of sample and 0.5 mL of standard blank and 0.5 mL of standard into the R1 tube, add 0.1 mL of sample and 0.1 mL of standard into the R2 tube, incubate all the tubes for 1 minute at room temperature then add 0.2 mL of each sample blank and sample, standard blank and standard into the R3 tube, add 0.5 mL of sample blank and 0.5 mL of sample and 0.5 mL of standard blank and 0.5 mL of standard into the R4 tube then incubate the tubes for 10 minutes at 37°C.

Read the absurbance at 510 nm of the sample against sample blank and standard against standard blank, for the calculation the levels (concentration); it can be use the Equation (3.18).

$$\text{Catalase activity (u/L)} = (\text{A standard} - \text{A sample}) / \text{A standard} \times 1000 \quad (3.18)$$

3.2.10 Glutathione (GSH) test

Serum preparation

Collect 5 mL of blood and allow it to clot for 20 minute at room temperature then centrifuge for 2000 g for 12 minutes at 4°C, collect the serum and stored it in a new vial, add 4 volium of cold reagent (5% w/v) to serum and store it for 15 minute at 4°C then

centrifuge the suspension at 12000 g for 10 minute at 4°C then collect the supernatant, the supernatant can be use for glutathion assay immediately.

Reagents

Glutathoine buffer 5 mL.

Glutathione reductase 8.7 µL.

NADPH solution 10 µL.

Procedure

Make dilutions GssG in vials using 400 µM of stock to achieve final concetration of 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 µM in 1x glutathione assay buffer, add 2.5 µL of 400 µM of oxidized glutathoine to 1 mL of 1x glutathione assay buffer then add 50 µL of GssG standards into the wells, diluting the sample 1:10 with 1x glutathione assay buffer then add 50 µL of sample into the wells, add 100 µL of assay mixture per well then incubate the plates at 25°C for 5 minutes, add 50 µL of working reagent per well and mix well.

Cover the plates and read the absurbance at 410 nm after 25 minutes of adding Eliman, s reagent, for the calculation the levels; it can be use the Equation (3.19).

Calculate the average absurbance of samples and standards against the blank. Calculate the total glutathione concetration for sample.

Total glutathione = OD at 410 nm – (Y-intercept/ slope × sample dilution ×2) (3.19)

3.2.11 Glutathione peroxidase (GPx) test

Principle

Glutathione peroxidase catalyzes the reduction of H_2O_2, oxidizing reduced glutathione to form oxidized glutathione, and then glutathione reductase reduced oxidized glutathione and B-nicotinamide adenine dinucleotide dinucleotide phosphate (NADPH) to form NADP+ and recycling the oxidizing reduced glutathione (GSH).

Reagents

Put the sample and place on ice, bring the GPx reagents to the temperature of the working room then setup the microplate reader at 340 nm, dilute the sample with buffer and place on ice, add 50 µL of diluted sample to suitable wells of microplate then add 50 µL of NADPH reagent to each well, add 50 µL of H_2O_2 to each well then equilibrate 50 seconds. Put microplate in plate reader, read the absurbance at 340 nm and calculate the results.

3.2.12 Malondialdehyde (MDA) test

Add 10 µL of BHt reagent to the microcentrifuge vial then add 250 µL of sample or calibrator to the vial, add 250 µL of acid reagent to vial then vortex and incubate at 60°C for 1 hour, centrifuge for 3 minutes at 10000 g, transfer the mixture to the suitable cuvette then record the absurbance from 400 to 700 nm.

4. RESULTS

Clinical markers of ınterleukin 6 and some biochemicals such as non-enzymatic antioxidants (GSH, MDA) and enzymatic antioxidants (catalase, GpX) were studied in hypothyroid patients. To Baghdad Hospital (120 hypothyroid patients were collected and compared with 120 other apparently healthy individuals as the control group), the totally number of the selected persons about 240, the results were as follows:

4.1 Age with Hypothyroid Patients

The mean of the age with the hypothyroid disease was (58.3000) in patients' group and (31.3000) in controls group which indicated to significant difference, and they may be important in early diagnosis for hypothyroid disease, as a shown in Table 4.1 and Figure 4.1 at P value= 0.000.

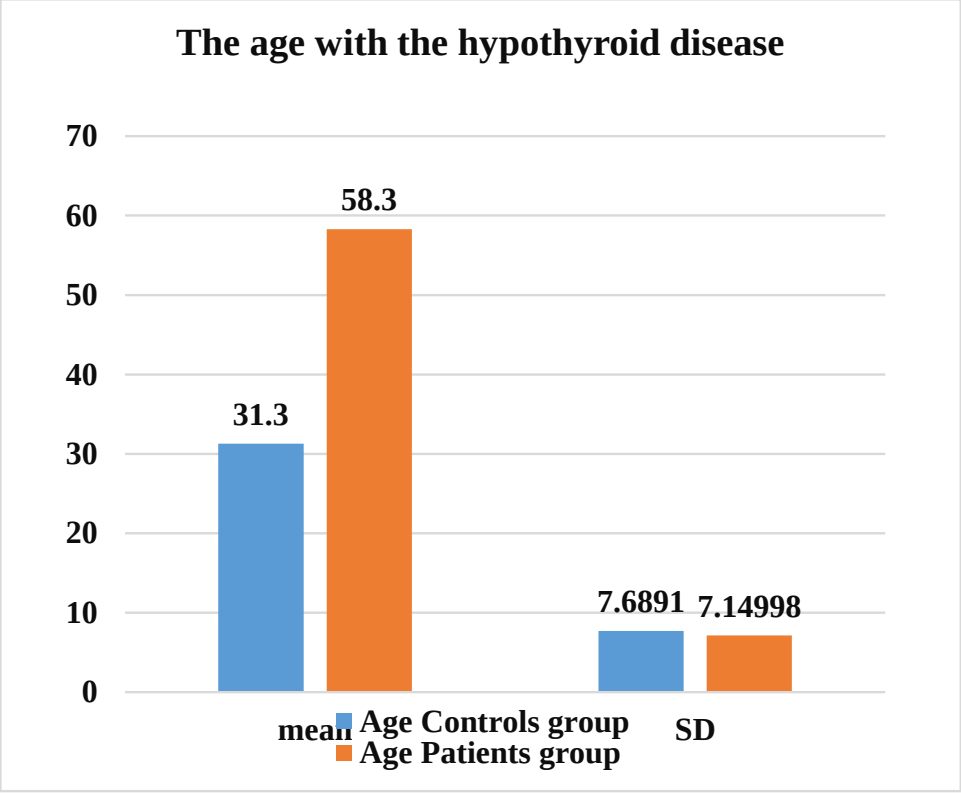

Figure 4.1 The mean of the age with the hypothyroid disease for patients and control groups

4.2 IL6 with Hypothyroid Patients

The IL6 mean was (1.2263) in patients' hypothyroid disease and in controls group (0.9676) which indicated to a non-significant difference, and they not important in diagnosis for hypothyroid disease, the results for both patients and control groups related to the IL6 value in patients' hypothyroid disease are clearly shown in Table 4.1 and confirmed in Figure 4.2, it seems from these results that the P value = 0.302.

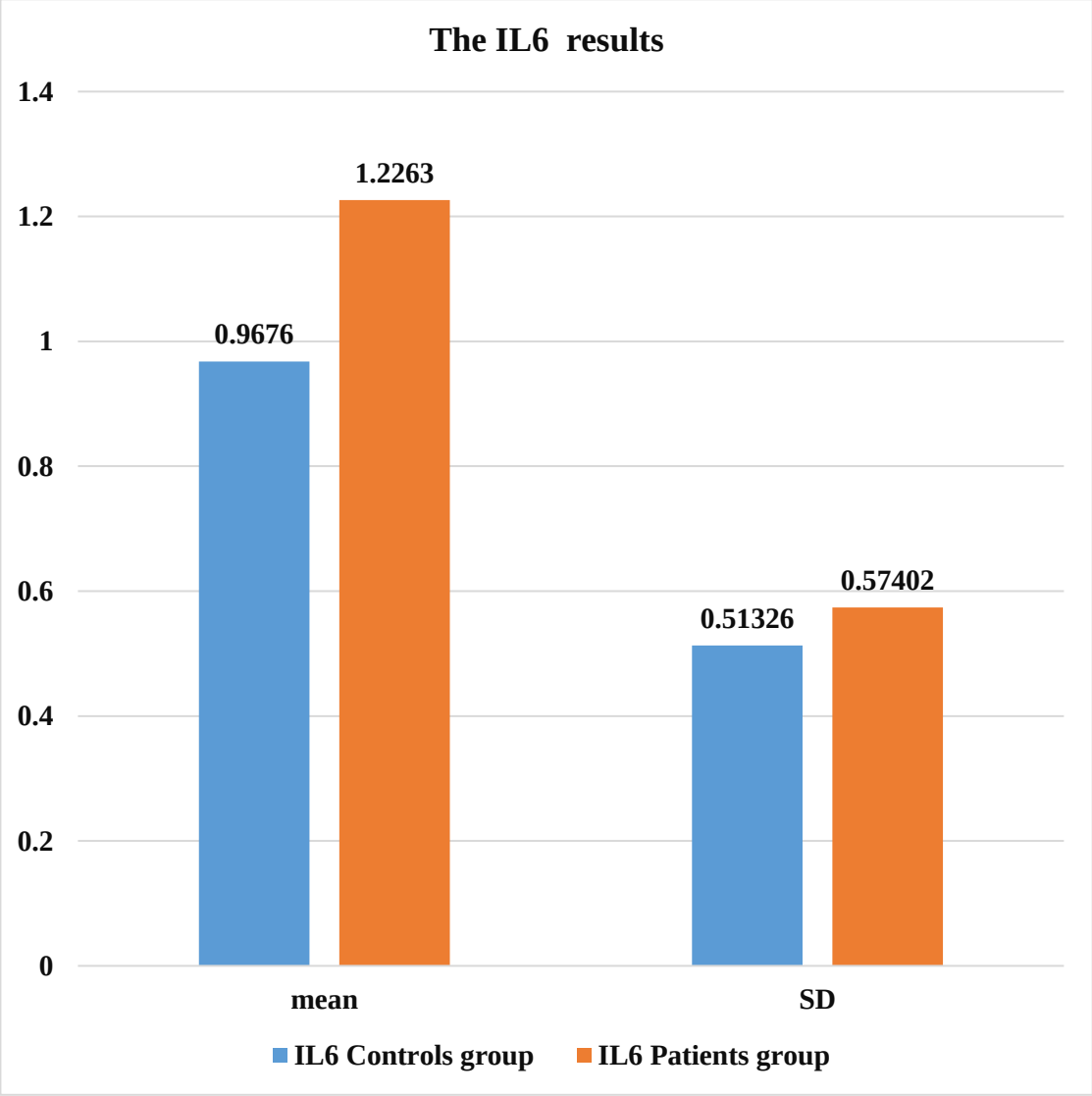

Figure 4.2 IL6 with hypothyroid patients

4.3 T4 and TSH with Hypothyroid Patients

The mean of the T4 and TSH with the hypothyroid disease was (2.6570 and 8.3741) in patients' group and (6.7092 and 3.5037 respectively) in controls group which indicated to significant difference, and they may be important in early diagnosis for hypothyroid disease, as a shown in Table 4.1 and Figure 4.3 at P value= 0.000.

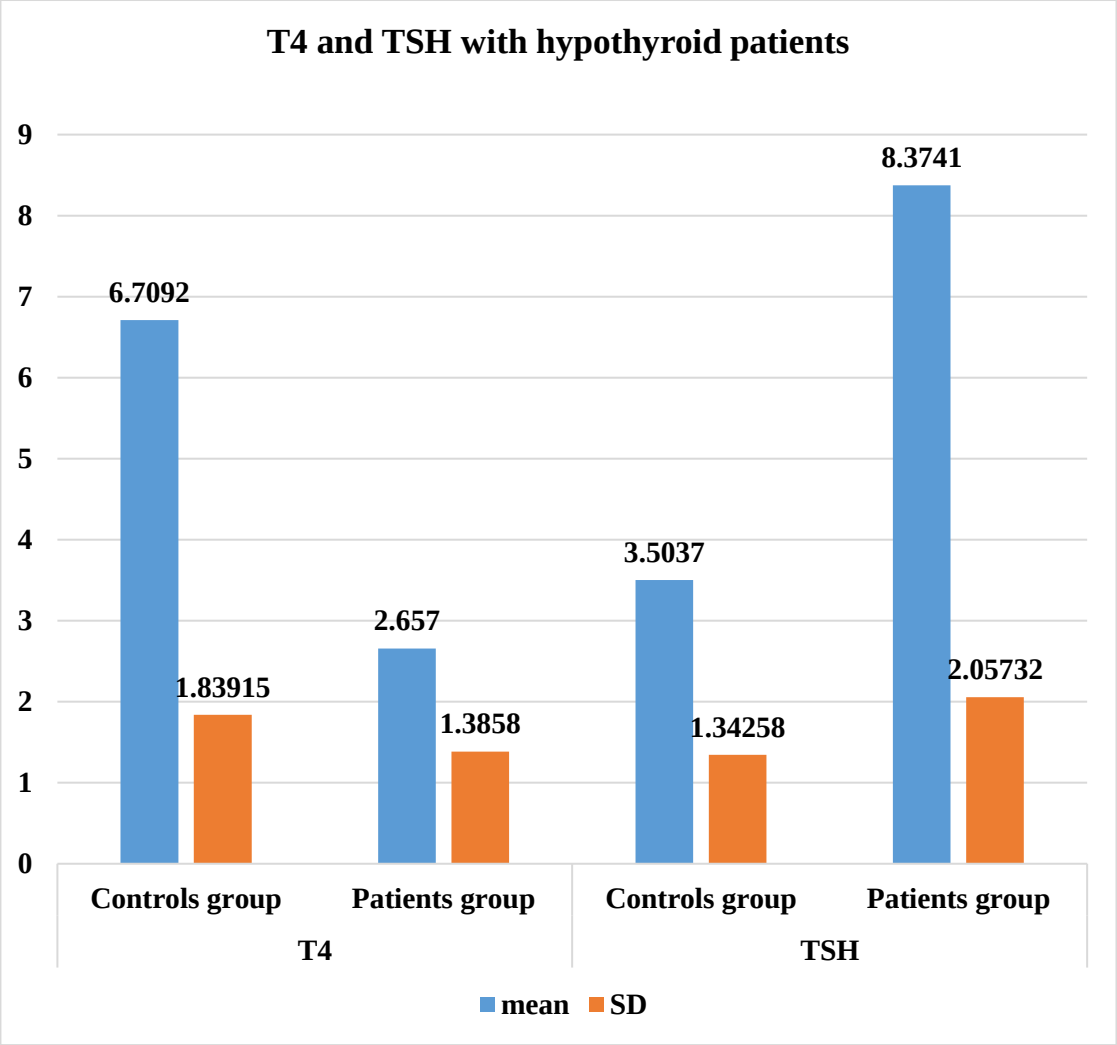

Figure 4.3 T4 and TSH with hypothyroid patients

4.4 Lipid Profile with Hypothyroid Patients

The lipid profile tests that were conducted for patients with hypothyroidism indicate that lipid levels were affected in people with severe hypothyroidism, where the averages for total cholesterol, triglycerides, HDL, LDL and VLDL were (222.200, 131.200, 43.5000, 152.460 and 26.2400 respectively) for the patients and were (175.900, 103.100, 54.4000, 86.3200 and 35.1800 respectively) for the for the control group, which indicates that there are significant statistically significant differences at P = < 0.05, as shown in Table 4.1 and Figure 4.3.

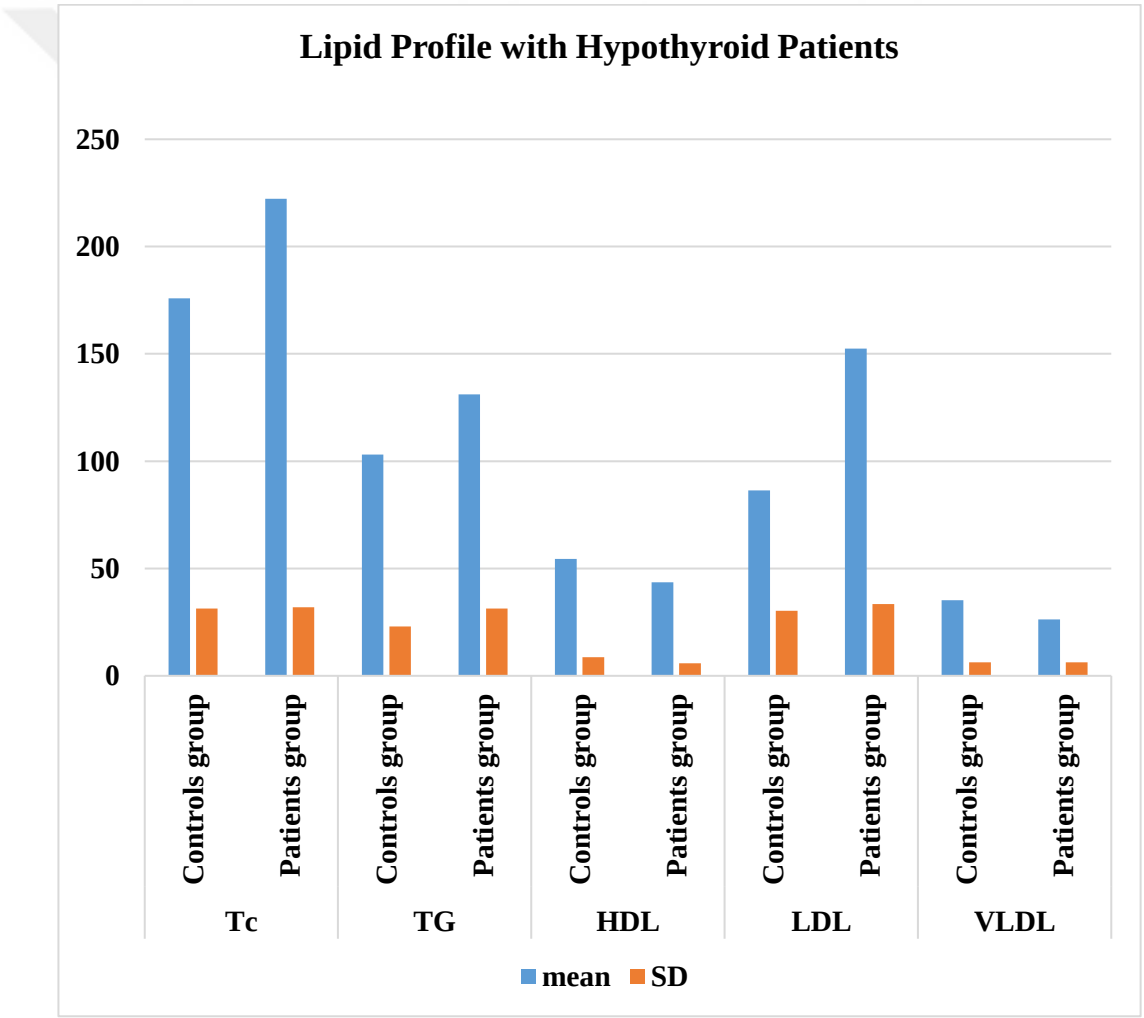

Figure 4.4 Lipid profile with hypothyroid patients

Table 4.1 The mean and SD for the stdied parameters

The group study						
Test	**Groups**	**N**	**Mean**	**SD**	**SEM**	**P-value**
Age	Controls group	120	31.3000	7.68910	2.43151	0.000
	Patients group	120	58.3000	7.14998	2.26102	0.000
IL6	Controls group	120	0.9676	0.51326	0.16231	0.302
	Patients group	120	1.2263	0.57402	0.18152	0.302
T4	Controls group	120	6.7092	1.83915	0.58159	0.000
	Patients group	120	2.6570	1.38580	0.43823	0.000
TSH	Controls group	120	3.5037	1.34258	0.42456	0.000
	Patients group	120	8.3741	2.05732	0.65058	0.000
Tc	Controls group	120	175.900	31.3207	9.90449	0.004
	Patients group	120	222.200	31.9888	10.1157	0.004
TG	Controls group	120	103.100	23.0142	7.27774	0.035
	Patients group	120	131.200	31.4034	9.93065	0.036
HDL	Controls group	120	54.4000	8.61781	2.72519	0.004
	Patients group	120	43.5000	5.79751	1.83333	0.004
LDL	Controls group	120	86.3200	30.3107	9.58509	0.000
	Patients group	120	152.460	33.4638	10.5822	0.000
VLDL	Controls group	120	35.1800	6.26415	1.98090	0.005
	Patients group	120	26.2400	6.28069	1.98613	0.005

*SD= Standard Deviation
*SEM= Standard Error Mean

4.5 Descriptive Testing of Samples

An independent samples test for age, IL6, T4, TSH, and lipid profile was conducted to find out the significance of the differences between the mean of the sample and the knowledge of the descriptive tests, and the results were as in Table 4.2.

Table 4.2 The test results for age, IL6, T4, TSH, and lipid profile

Independent Samples Test									
Parameters		**LT EV**					**t-test for equality of means**		
		F	**Sig.**	**t**	**df**	**MD**	**SED**	**95% CID**	
								Lower	**Upper**
Age	EVA	0.002	0.964	-8.13	18	-27.00	3.320	-33.97	-20.02
	EVnA			-8.13	17.9	-27.00	3.320	-33.97	-20.02
IL6	EVA	0.391	0.540	-1.06	18	-0.258	0.243	-0.770	0.2528
	EVnA			-1.06	17.7	-0.258	0.243	-0.770	0.2532
T4	EVA	2.097	0.165	5.56	18	4.052	0.728	2.5222	5.5820
	EVnA			5.56	16.7	4.052	0.728	2.5138	5.5904
TSH	EVA	2.293	0.147	-6.26	18	-4.870	0.776	-6.502	-3.238
	EVnA			-6.26	15.4	-4.870	0.776	-6.521	-3.219
Tc	EVA	0.137	0.715	-3.27	18	-46.30	14.15	-76.04	-16.55
	EVnA			-3.27	17.9	-46.30	14.15	-76.04	-16.55
TG	EVA	1.046	0.320	-2.28	18	-28.10	12.31	-53.96	-2.233
	EVnA			-2.28	16.5	-28.10	12.31	-54.13	-2.064
HDL	EVA	3.178	0.092	3.31	18	10.90	3.284	3.9995	17.800
	EVnA			3.31	15.7	10.90	3.284	3.9286	17.871
LDL	EVA	0.588	0.453	-4.63	18	-66.14	14.27	-96.13	-36.14
	EVnA			-4.63	17.8	-66.14	14.27	-96.15	-36.12
VLDL	EVA	0.106	0.748	3.18	18	8.940	2.805	3.0466	14.833
	EVnA			3.18	18.0	8.940	2.805	3.0466	14.833

*LTEV is a term refers to Levene's test for equality of variances; *EVA is a term too that refers to the aqual variances assumed; *EvnA is a term too, it is refers to equal variances not assumed; *MD a term refers to mean difference; *SED = Std. Error Difference; *95% CID = 95% confidence interval of the difference.

4.6 Zinc with Hypothyroid Patients

The mean of the zinc with the hypothyroid disease was (79.2000) in patients' group and (90.8000) in controls group which indicated to a significant difference when compared to control group which were without hypothyroid disease, as a shown in Table 4.2 and Figure 4.1 at P value= 0.938.

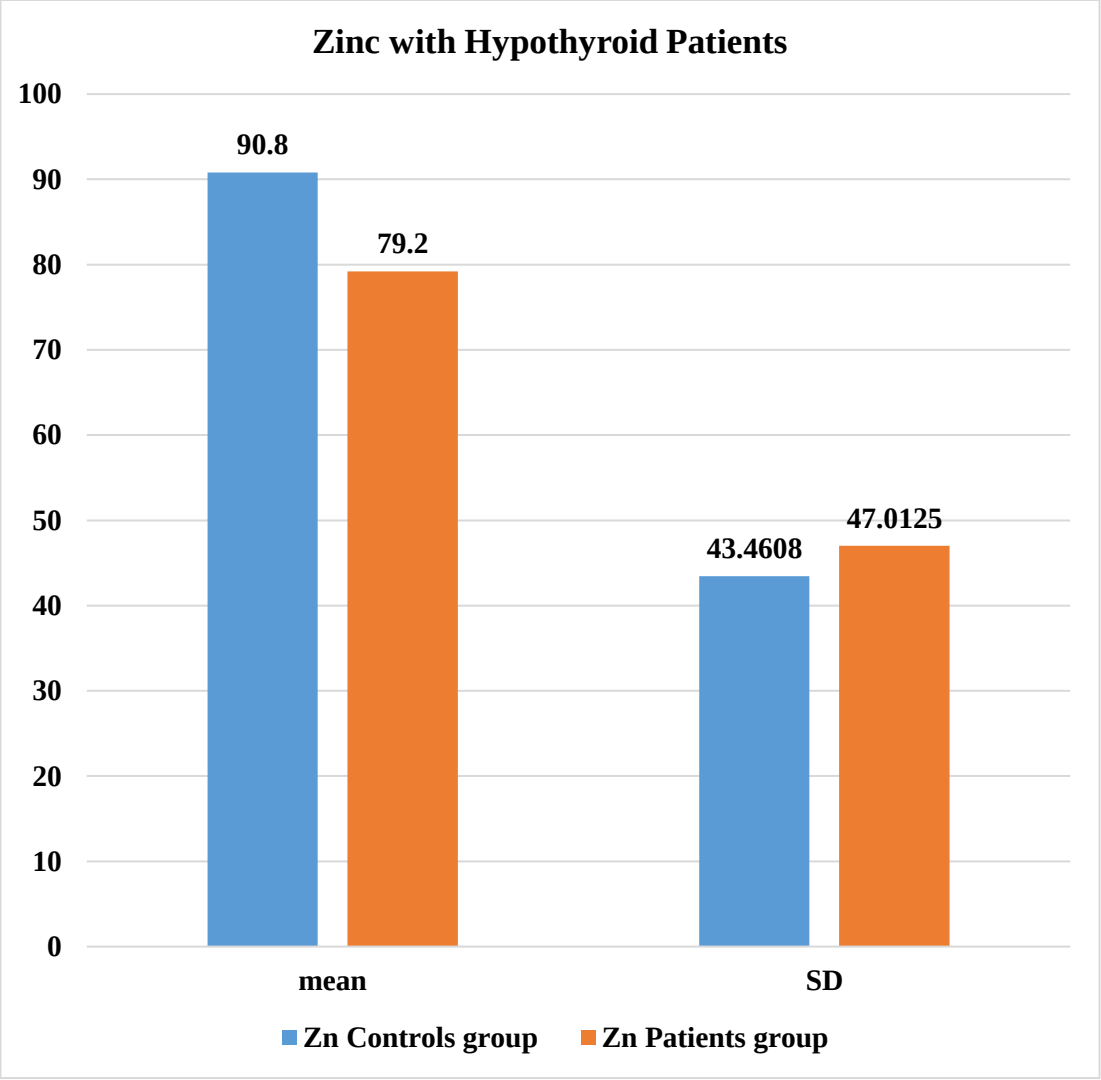

Figure 4.5 Zinc with hypothyroid patients

4.7 Iron with Hypothyroid Patients

The iron mean was (79.9000) in patients' hypothyroid disease and in controls group (87.7000) which indicated to a non-significant difference, and they not important in diagnosis for hypothyroid disease, as a shown in Table 4.2 and Figure 4.2 at P value = 0.372.

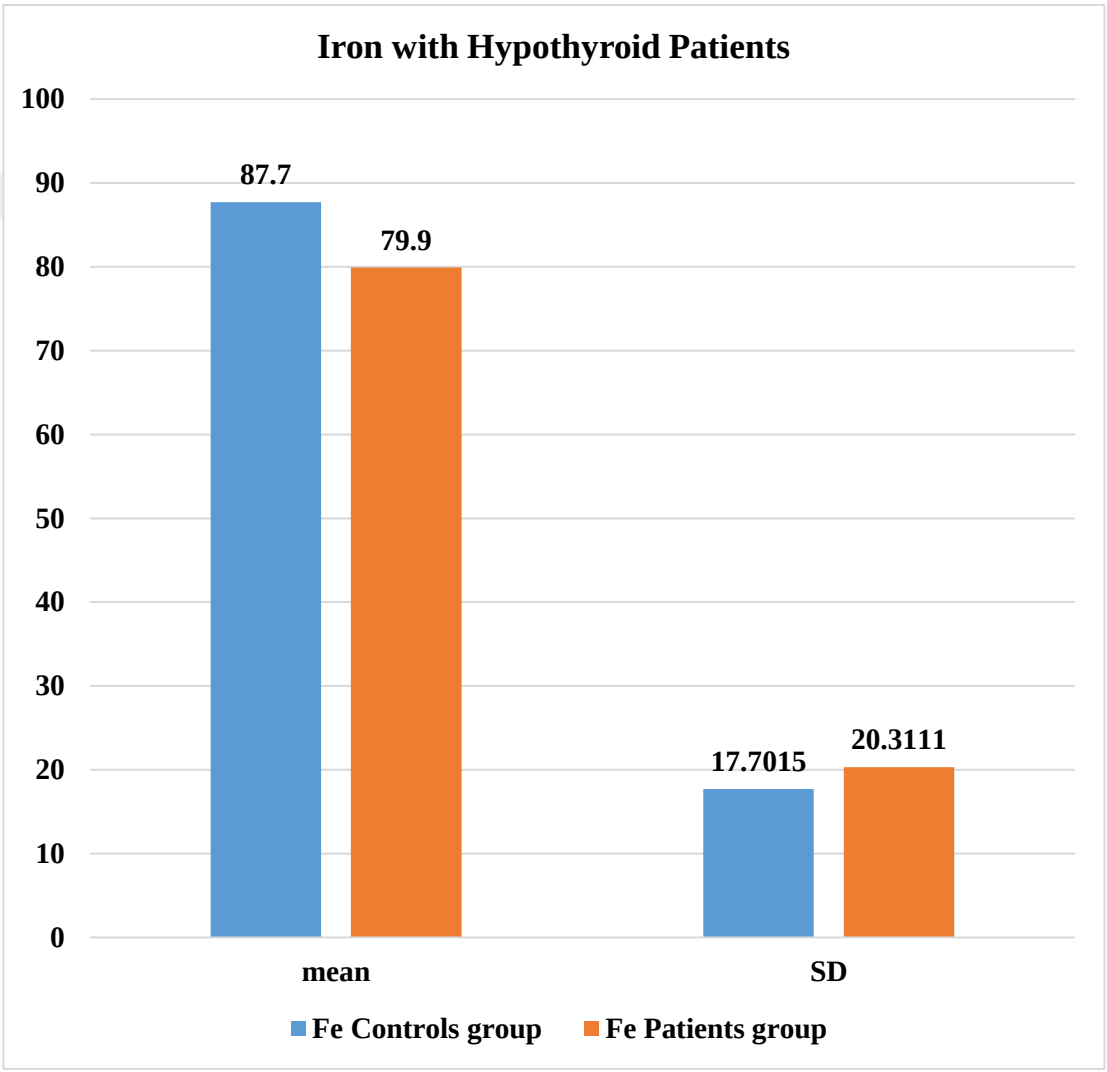

Figure 4.6 Iron with hypothyroid patients

4.8 Catalase and GSH with Hypothyroid Patients

The mean of the catalase and GSH with the hypothyroid disease was (89.0000 and 38.5000 respectively) in patients' group and (97.6000 and 55.5000 respectively) in controls group which indicated to significant difference for GSH, and they may be important in early diagnosis for hypothyroid disease, but showed to a non-significant difference for catalase, as a shown in Table 4.2 and Figure 4.3 at P value for catalase = 0.139 and for GSH= 0.001.

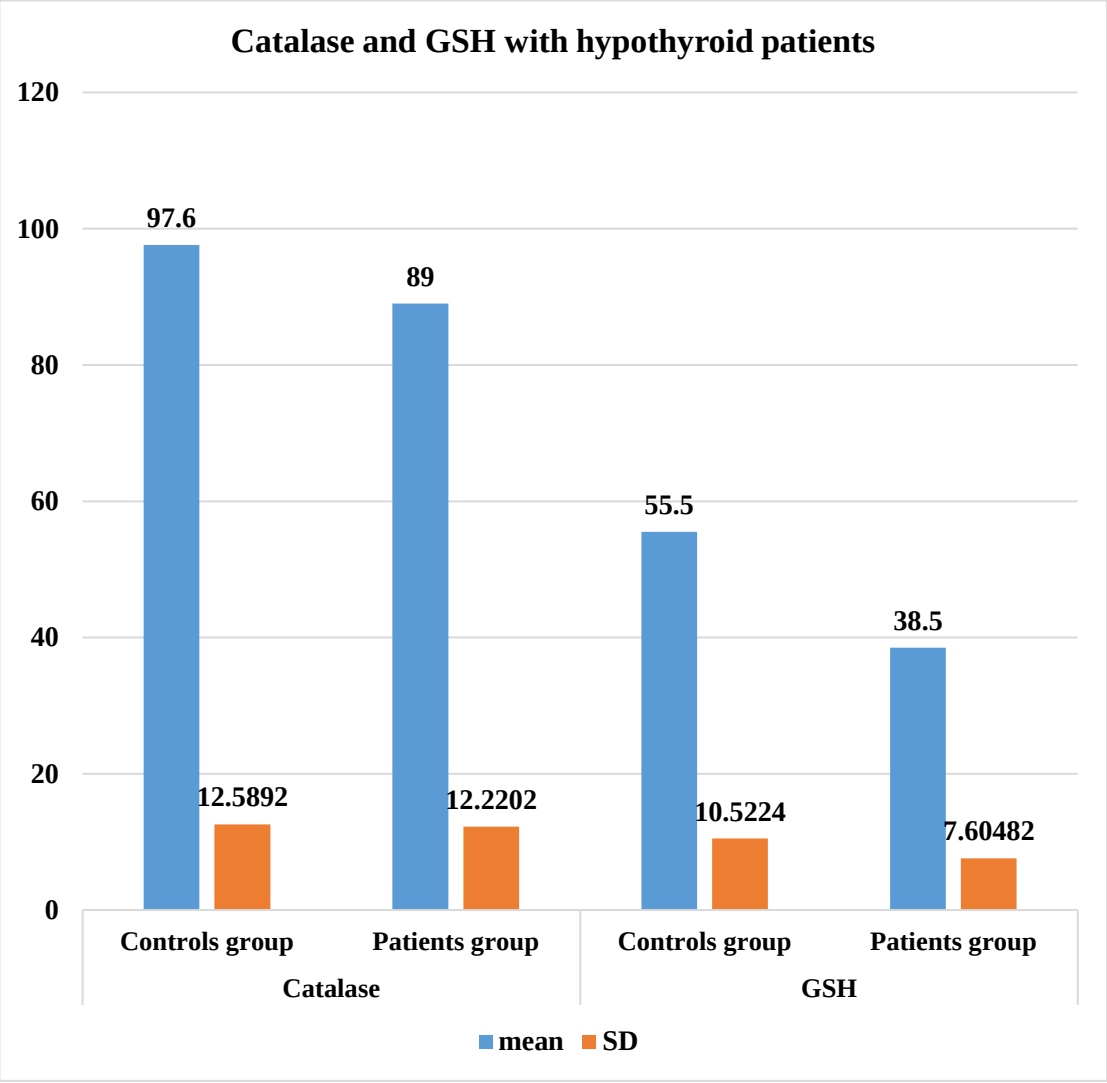

Figure 4.7 Catalase and GSH with hypothyroid patients

4.9 MDA and GPx with Hypothyroid Patients

The MDA and GPx tests that were conducted for patients with hypothyroidism indicate that MDA levels was high significant difference with severe hypothyroidism, where the averages for MDA and GPx were (156.000 and 11.8000 respectively) for the patients and were (187.200 and 14.5000 respectively) for the control group, which indicates that there are a non-significant statistically for GPx at P = 0.002 for MDA and P= 0.233 for GPx, as shown in Table 4.2 and Figure 4.3.

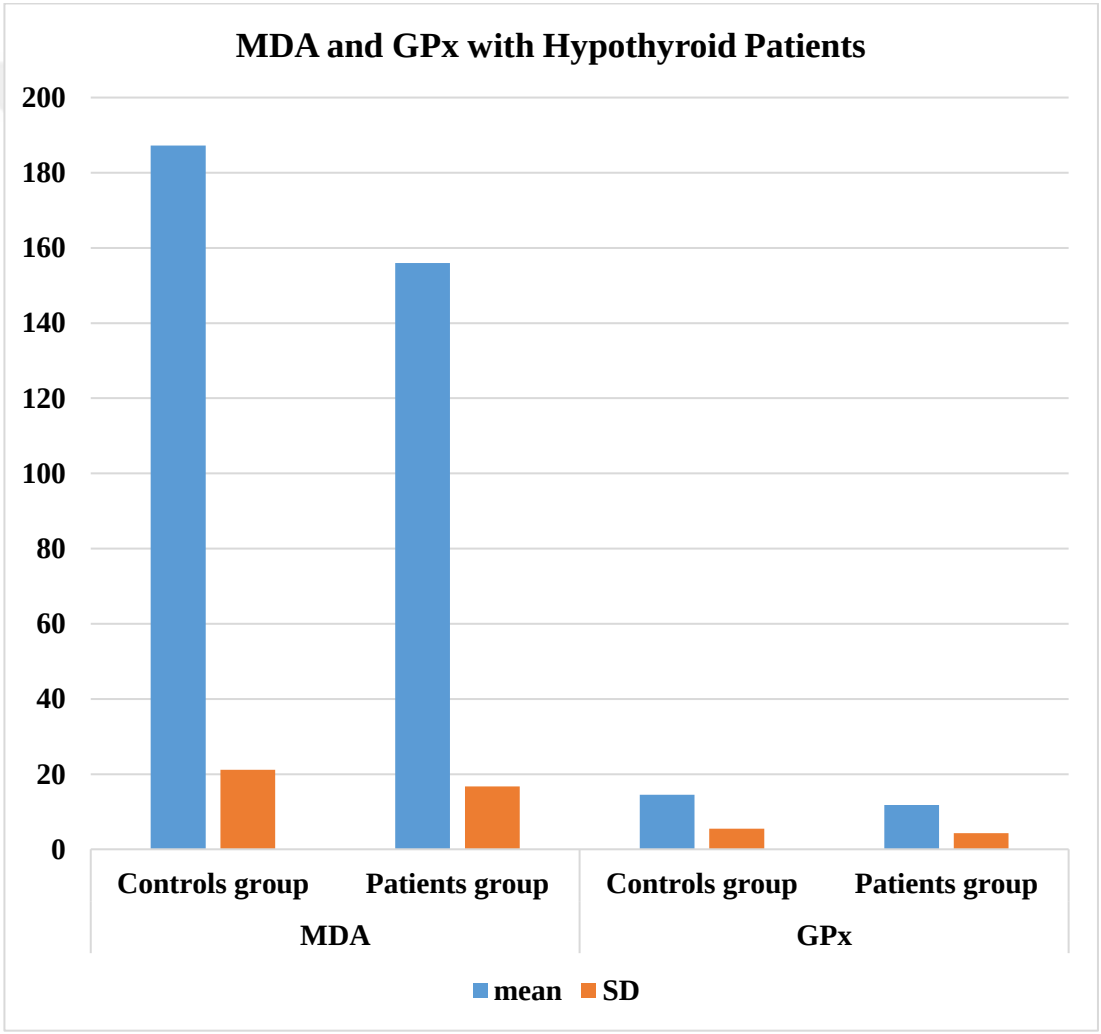

Figure 4.8 MDA and GPx with hypothyroid patients

Table 4.3 The results for the test for studied groups

The group study						
Test	**Groups**	**N**	**Mean**	**SD**	**SEM**	**P-value**
Zn	Controls group	120	90.8000	43.4608	13.74352	0.038
	Patients group	120	79.2000	47.0125	14.86667	0.038
Fe	Controls group	120	87.7000	17.7015	5.59772	0.372
	Patients group	120	79.9000	20.3111	6.42296	0.372
Catalase	Controls group	120	97.6000	12.5892	3.98107	0.139
	Patients group	120	89.0000	12.2202	3.86437	0.139
GSH	Controls group	120	55.5000	10.5224	3.32749	0.001
	Patients group	120	38.5000	7.60482	2.40486	0.001
MDA	Controls group	120	187.200	21.1229	6.67965	0.002
	Patients group	120	156.000	16.7265	5.28940	0.002
GPx	Controls group	120	14.5000	5.44161	1.72079	0.233
	Patients group	120	11.8000	4.26354	1.34825	0.234
*SD= Standard Deviation *SEM= Standard Error Mean						

4.10 Descriptive Testing of Samples

An Independent Samples Test for Zn, Fe, catalase, GSH, MDA and GPx was conducted to find out the significance of the differences between the mean of the sample and the knowledge of the descriptive tests, and the results were shown as in Table 4.4.

Table 4.4 Descriptive testing of samples

Independent samples test									
Parameter	**LT EV**						**t-test for equality of means**		
		F	**Sig.**	**t**	**df**	**MD**	**SED**	**95% CID**	
								Lower	**Upper**
Zn	EVA	0.069	0.796	0.07	18	1.600	20.24	-40.93	44.135
	EVnA			0.07	17.8	1.600	20.24	-40.95	44.154
Fe	EVA	0.241	0.629	0.91	18	7.800	8.519	-10.09	25.699
	EVnA			0.91	17.6	7.800	8.519	-10.12	25.723
Catalase	EVA	0.004	.950	1.55	18	8.600	5.548	-3.056	20.256
	EVnA			1.55	17.9	8.600	5.548	-3.057	20.257
GSH	EVA	1.161	0.296	4.14	18	17.00	4.105	8.3745	25.625
	EVnA			4.14	16.3	17.00	4.105	8.3132	25.686
MDA	EVA	0.782	0.388	3.66	18	31.20	8.520	13.299	49.100
	EVnA			3.66	17.1	31.20	8.520	13.231	49.168
GPx	EVA	0.972	0.337	1.23	18	2.700	2.186	-1.892	7.2927
	EVnA			1.23	17.0	2.700	2.186	-1.911	7.3116

*LTEV is a term refers to Levene's test, it is for equality of Variances; *EVA is a term too, it is refers to the equal variances assumed; *EvnA it is a rerm too, it is refers to the

equal variances not assumed; *MD it is a term too, it is refers to the mean difference; *SED = Std. Error Difference; * 95% CID= 95%, it is refers to the difference confidence interval level.

5. DISCUSSION

Patients that suffered from subacute thyroiditis and certain participants with amiodarone-induced thyrotoxicosis have recently been shown to have increased blood concentrations of interleukin 6 (IL6). This may be because injured thyroid cells produce cytokines that cause an increase in interleukin 6 levels.

There was a significant correlation (P 0.0001) between the rise in IL6 and the size of the nodule or goiter, but there was no correlation with the quantity of ethanol injected or the dosage of radioiodine that was given to the thyroid. After PIEI, RAI, or FNA, there was also a rise in serum thyroglobulin, although there was not a significant association between it and the increase in IL6 that could be proven. In the case of thyroid disease only, this disease depends on an increase in the serum concentration of cytokine, and the results of this study confirm the results achieved in previous studies, as it gives more credence to the theory that IL6 is a good and reliable indicator of the destructive processes of the thyroid gland in the human body. This theory is supported by the findings of the study (Bartalena *et al.* 1994).

IL6 is a pleiotropic cytokine that is postulated to be involved in the etiology of sick euthyroid syndrome. Despite the fact that the immediate in vitro effects of IL6 on human thyroid function are controversial, it is postulated that IL6 plays a role in the development of sick euthyroid syndrome. Serum IL6 is complexly associated with the soluble IL6 receptor (sIL6R) at gp 130, an IL6 signal transducer. Serum IL6 reduces thyroid activity independently and completely in the presence of physiological concentrations of IL6R (100 ng/mL). All this is accompanied by a decrease in the concentrations of 125I-T3 / 125I-T4, and this decrease is not only in the thyroid follicle.

In line with these results, a somewhat impaired thyroid function was seen when large quantities of sIL6R were administered. In addition, RT-PCR tests showed that human thyroid follicles produced messenger RNAs for IL6 and gp130, but only a negligible amount of messenger RNA for IL-6R. These data show that IL6 alone has very little effect

on function of the thyroid in thyroid follicles when IL6R gene expression is very little. This was investigated in vitro.

However, due to the high levels of sIL6R that are found in serum, IL6 in vivo has the potential to limit the production and release of T4 and, to a larger degree, T3 from the thyroid gland. This is because serum contains a lot of sIL6R. These results obtained in vitro are at least somewhat connected to the onset of ill euthyroid syndrome (Yamazaki *et al.* 1996).

Forty-five individuals diagnosed with hypothyroidism were investigated when they were in the first four to thirty-six months of their thyroxine replacement medication. Over the course of three to six months, all of the patients achieved clinical euthyroidism, with 23 patients becoming euthyroid while taking 0.1 mg/d, 14 patients becoming euthyroid while taking 0.15 mg/d, 7 patients becoming euthyroid while taking 0.2 mg/d, and 1 patient becoming euthyroid while taking 0.25 mg/d.

After that, the patients were split into two groups for the purpose of data analysis. Group I had normal serum T3 levels, normal (or increased) serum T4 levels, and normal serum TSH levels. The individuals in Group II had high blood TSH levels while having normal serum T3 levels and normal (or higher) serum T4 levels. Group II was then separated into a group of 13 cases called group IIa, whose dosage of thyroxine was purposefully raised until the blood TSH level was corrected. Five of these patients developed clinical and biochemical signs of hyperthyroidism as a result of this treatment. Group IIb comprised of eight patients who had normal blood T3 and T4 levels but high serum TSH levels. These patients were followed up despite not having any attempts made to regulate their serum TSH levels. None of them developed thyrotoxicity, and the levels of TSH in their blood hardly changed at all. Based on these data, it seems that measuring serum TSH levels on their own is not sufficient to determine the appropriate amount of thyroxine replacement treatment (Cai *et al.* 2019).

Changes in lipid profile are a common symptom of hypothyroidism. People with both clinical and subclinical hypothyroidism have a different lipid profile compared to patients

with euthyroidism. Due to the fact that thyroid state may cause changes in lipid profile, monitoring of thyroid status is particularly essential. This kind of dyslipidemic condition is important not only for the treatment of thyroid problems, but also for the prevention and treatment of common illnesses like coronary atherosclerosis and obesity in the population (Humerah *et al.* 2016).

One year following the TSH adjusted intervention, reevaluation was performed on 11 sick individuals who had received the levothyroxine (LT4) medication and 15 sick individuals who had received the placebo. A reduction in atherogenic variables was found in the L-T4-treated group, with significance for total cholesterol (-20.0 mg/dL as opposed to +16.1 mg/dL in the placebo group) and LDL-c (-21.7 mg/dL as opposed to +17.2 mg/dL). We came to the conclusion that SH causes a lipid profile that is intermediate between those observed in persons with euthyroidism and those found in patients with manifest hypothyroidism. We also discovered that a considerable lipid profile ensure good quality one year following L-T4 replacement medication (Teixeira *et al.* 2008).

The generation of thyroid hormone requires a sufficient amount of zinc. It is essential for the maintenance of the metabolic rate of thyroid hormones as well as the normalization of the resting metabolic rate. In order for the body to absorb zinc, thyroid hormone is also necessary. Hypothyroidism often manifests itself with a generalized thinning of the hair. A lack of copper, selenium, or zinc may lead to hypothyroidism since these minerals all play a role in the creation of thyroid hormones. Copper, selenium, and zinc are all examples. Concurrently, the production of thyroid hormone is essential for zinc absorption in the body. Therefore, an acquired zinc deficit may occur when there are reduced amounts of thyroid hormones, as is the case with hypothyroidism. Even while thyroid hormone supplements like thyroxine are advised for correcting hypothyroidism, addressing symptoms like hair loss with them alone is not enough (Severo *et al.* 2019).

Oxidative stress contributes to the development of hypothyroidism. Hypothyroidism is a condition that is characterized by elevated levels of oxidative stress. According to the findings of this research, a high level of MDA may be seen in individuals with primary hypothyroidism who have not yet received any medication. After receiving therapy with

L-thyroxine, there is a discernible decrease in the level of the stress marker. MDA is a helpful biomarker that may be used in the measurement and monitoring of oxidative stress. In spite of adding an antioxidant in the form of selenium, it was not possible to determine what impact it played (Chakrabarti *et al.* 2016).

When compared to the control group, the breast tissue of those with hypothyroidism had significantly reduced levels of catalase, GPX, and DUOX activities, while the concentrations of 4-hydroxynonenal (HNE) were much greater. In addition to this, the hypothyroid group demonstrated an increase in the ratio of H2Ax to H2Ax. The twenty-one day hypothyroid group exhibited higher levels of catalase and SOD activity, but there were no significant changes in the levels of oxidative stress indicators or DNA damage across the groups. MCF10A cells that had been treated with TSH had increased levels of ROS generation at all three levels: extracellular, intracellular, and mitochondrial. In addition, a larger comet tail DNA percentage and more 53BP1 foci were seen in these cells, which is evidence of enhanced DNA damage. In conclusion, we discovered that treatment with TSH did not affect the vitality of the cells (Peixoto *et al.* 2021).

6. CONCLUSIONS

Age had a great importance in the development of hypothyroidism, as the results indicated the development of the disease at advanced age. Cytokines were important indicators in patients with hypothyroidism, but were of great clinical importance. The levels of the lipid profile were significantly affected in the patients, and changes in the lipid profile may be a reason for the development of hypothyroidism. Iron was not statistically significant in reference to the control group. There was a great importance of zinc in patients with hypothyroidism, which may contribute to aiding zinc absorption. GSH and MDA had statistical significance, indicating the influence of antioxidants in patients with hypothyroidism.

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