Some Hematological and Biochemical Changes in Patients Suffering from Hyperthyroidism

A Thesis Submitted From

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To

The council of the college of science of the university of Babylon in partial fulfillment of the requirements of the degree of doctor of philosophy of science in Biology/Zoology

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بعض التغيرات الدموية والكيموحيوية في مرضى فرط الدرقية

اطروحة مقدمة من

داخل غاني عمران الوطيفي

إلى

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

اشراف

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certification

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بسم الله الرحمن الرحيم

أَوَلَمْ يَرَ الْإِنسَانُ أَنَّا خَلَقْنَاهُ مِن نُّطْفَةٍ فَإِذَا هُوَ خَصِيمٌ مُّبِينٌ ﴿٧٧﴾ وَضَرَبَ لَنَا مَثَلًا وَنَسِيَ خَلْقَهُ قَالَ مَن يُحْيِي الْعِظَامَ وَهِيَ رَمِيمٌ ﴿٧٨﴾ قُلْ يُحْيِيهَا الَّذِي أَنشَأَهَا أَوَّلَ مَرَّةٍ وَهُوَ بِكُلِّ خَلْقٍ عَلِيمٌ ﴿٧٩﴾

صدق الله العظيم

الآية (٧٧-٧٩)

من سورة (يس)

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Dakhel, ٢٠٠٧ .

Some Hematological and Biochemical Changes in Patients Suffering from Hyperthyroidism

Summary

The present study was designed to investigate changes occurring in body temperature and some hormonal, hematological, and biochemical changes in subjects suffering from hyperthyroidism. A total number used was ١٧٠, patients and healthy subjects of both sexes, males and females. The total number of patients was ١٣٠; ١٠٠ females and ٣٠ males, while the number of control subjects was ٤٠.

The ages of all subjects ranged between ٢٠ years to ٥٥ years. It was found that hyperthyroid subjects have a significant increase (P<٠.٠١)of triiodthyronine ($T^{٣}$)and tetraiodothyronine ($T^{٤}$) levels and a significant decrease (P<٠.٠١) of thyroid stimulating hormone (TSH) levels when compared with those of control groups . It was also found that ratio of affected females (٧٦.٩٢٪) more than that of affected males (٢٣.٠٧٪). In addition, the susceptibility to such thyroid disorder was directly proportional to age, since, the most susceptible age group was ranged between (٢٠ – ٣٠)years of age .

Concerning haematological changes, studying red blood corpuscles count (RBCs), hemoglobin concentration (Hb) ,and packed cell volume (PCV) showed a significant increase (P<٠.٠١) when compared with healthy subjects. Red blood corpuscle indices which involve mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC)were recorded non significant decrease of MCV values and non significant difference of MCH and MCHC values in a comparison with those for healthy subjects. In addition, values of hemoglobin electrophoresis

which included adult hemoglobin (HbA), adult hemoglobin٢ (HbA٢),and fetal hemoglobin (HbF) showed non significant difference when compared with control subjects. Results of erythrocyte sedimentation rate (ESR) and white blood cells count (WBCs) were also recorded non significant difference in a comparison with those healthy subjects. Results of the study indicated a significant increase (P<٠.٠٥) in body temperature of hyperthyroid patients in comparison with control groups.

Biochemical changes in serum, showed a significant decrease (P<٠.٠١) in the levels of serum reduced glutathione (GSH) in hyperthyroid patients in comparison with healthy subjects. Total serum protein (TSP)and serum albumin levels pointed out significant decrease (P<٠.٠١) in hyperthyroid subjects, while serum globulin values showed non significant decrease when compared with those control subjects. Levels of serum alkaline phosphates (ALP) showed a significant increase(P<٠.٠١) in hyperthyroid subjects when compared with those control subjects. Levels of calcium in both serum and urine samples showed significant increase(P<٠. ٠١)in hyperthyroid subjects. Values of phosphorus in serum and urine samples showed significant increase(P<٠. ٠٥) of hyperthyroid subjects when compared with those healthy subjects. More over, the present study was also involved determination of sodium (Na^+) and potassium (K^+) levels in both serum and urine samples of hyperthyroid patients. Sodium values showed significant increase (P<٠.٠١) in both serum and urine samples. While , results of potassium showed non significant decrease in both serum and urine samples of hyperthyroid subjects when compared with control subjects. Total serum cholesterol levels showed significant decrease (P<٠.٠١) in comparison with control subjects. In addition, results of serum triglycerides showed significant decrease (P<٠.٠١) when compared with those healthy subjects . Finally, trace elements results (serum copper and zinc) showed significant decrease(P<٠.٠١) of copper levels and a significant increase (P<٠.٠٥) of zinc levels in hyperthyroid subjects when compared with those healthy control subjects. In view of the changes summarized, the increase or decrease in some hematological and biochemical parameters may be attributed to hypermetabolic state which arise due to higher production of thyroid hormones which, in turn, affect most of body tissues.

بعض التغيرات الدموية والكيموحيوية في مرضى فرط الدرقية

الخلاصــة

تضمنت هذه الدراسة معرفة تأثير فرط إفراز هرمونات الغدة الدرقية على درجة حرارة الجسم وبعض صفات الدم والتي تضمنت المتغيرات الدموية والكيميوحيوية.

شملت هذه الدراسة فحص (١٧٠) شخص من المصابين بفرط افراز هرمونات الغدة الدرقية وغير المصابين (الأصحاء) ومن كلا الجنسين الذكور والإناث . حيث كان عدد الاناث المصابات (١٠٠) انثى وعدد الذكور المصابين (٣٠) ذكراً اما اشخاص السيطرة (الاصحاء) فقد كان عدد الاناث الاصحاء (٢٠) انثى وعدد الذكور الاصحاء (٢٠) ذكراً. حيث تراوحت أعمارهم بين (٢٠) سنة إلى (٥٥) سنة .

تميز الأشخاص المصابين بفرط إفراز هرمونات الغدة الدرقية بارتفاع مستوى هرموني الثايرونين ثلاثي اليود (T٣) والثايرونين رباعي اليود (T٤) بصورة معنوية بمستوى ($P<0.01$) وانخفاض مستوى الهرمون المحفز للدرقية (TSH) بصورة معنوية بمستوى ($P<0.01$) عند مقارنتها بالأشخاص الأصحاء.

بينت نتائج هذه الدراسة بأن نسبة الاناث المصابات (٧٦.٩٢٪) اكثر من نسبة الذكور المصابين (٢٣.٠٧٪) وكذلك تبين ان اكثر الاعمار تأثراً بهذا المرض انحصر بين ٢٠ سنة الى ٣٠ سنة.

اما بالنسبة للتغيرات الدموية فقد لوحظ ارتفاع معنوي بمستوى ($P<0.01$) لكل من العدد الكلي لكريات الدم الحمر (RBCs) وتركيز خضاب الدم(Hb) وحجم الخلايا المضغوط (PCV) للأشخاص المصابين عند مقارنتها بالأشخاص غير المصابين .

أما بالنسبة لدلائل كريات الدم الحمر (RBCs indices : MCV,MCH,MCHC) فأنها لم تسجل فروقاً معنوية عند مقارنتها بالأشخاص الاصحاء .

كذلك لوحظ بأن قيم أنواع خضاب الدم (HbA,HbA٢,HbF) والعدد الكلي لخلايا الدم البيض (WBCs) ومعدل سرعة ترسيب كريات الدم الحمر (ESR) لم تسجل فروقاً معنوية عند مقارنتها بالأشخاص الأصحاء.

لوحظ ارتفاع درجة حرارة الجسم (Body temperature) بصورة معنوية بمستوى (P<٠.٠٥) في الاشخاص المصابين بفرط الدرقية عند مقارنتها بالأشخاص الاصحاء.

أما بخصوص التغييرات الكيموحيوية فقد لوحظ انخفاض الكلوتاثيون المختزل (GSH) بصورة معنوية بمستوى (P<٠.٠١) بالمقارنة مع الأشخاص الاصحاء .

أشرت قيم البروتين المصلي الكلي (TSP) والألبومين (Albumin) انخفاضاً معنوياً بمستوى(P<٠.٠١) عند مقارنتها بالأشخاص الأصحاء كذلك لوحظ انخفاض غير معنوي في قيم الكلوبيولين عند مقارنتها بالأشخاص الأصحاء

لوحظ بان قيم خميرة الفوسفتاز القاعدي (ALP) قد سجلت ارتفاعاً معنوياً بمستوى (P<٠.٠١) عند مقارنتها بالأشخاص غير المصابين .

أما بخصوص قيم الكالسيوم ($Ca^{+٢}$) والفسفور ($PO_{٤}^{-٣}$) في عينات المصل والإدرار للأشخاص المصابين فقد لوحظ ارتفاعاً معنوياً بمستوى (P<٠.٠١) في قيم الكالسيوم وارتفاع معنوي بمستوى (P<٠.٠٥) في قيم الفسفور عند مقارنتها بالأشخاص الأصحاء .

كذلك لوحظ بان قيم الصوديوم (Na^{+}) قد سجلت ارتفاعاً معنوياً بمستوى (P<٠.٠١) في عينات المصل والأدرار عند مقارنتها بالأشخاص الأصحاء .

أما قيم البوتاسيوم (K^{+}) فأنها قد سجلت انخفاضاً غير معنوي في عينات المصل والإدرار في الاشخاص المصابين عند مقارنتها بالأشخاص الأصحاء .

أما بخصوص العناصر النادرة (Trace elements) فقد لوحظ ارتفاعاً معنوياً بمستوى (P<٠.٠٥) في قيم الزنك (zinc) وانخفاضاً معنوياً بمستوى (P<٠.٠٥) في قيم النحاس عند مقارنتها بالأشخاص الأصحاء .

أن التغييرات الحاصلة في المعايير الدموية والكيموحيوية يمكن إعزاؤها بصورة رئيسة إلى ارتفاع معدل الايض بسبب الإفراز العالي لهرمونات الغدة الدرقية .

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List of Abbreviation

ALP	Alkaline phosphatase
ANP	Atrio-natriuretic peptide
ATP	Adenosine triphosphate
C	Centigrade
$Ca^{+٢}$	Calcium ion
$Cu^{+٢٠}$	Copper ion
DDW	Double distilled water
DIT	Diiodotyrosine
DNA	Deoxy ribonucleic acid
DTNB	Dinitro benzoic acid
ECF	Extracellular fluid
ELISA	Enzyme linked immuno-sorbent assay

EIA	Enzyme immunoassay
ESR	Erythrocyte sedimentation rate
F	Fahrenheit
fL	Femto liter
GSH	Reduced glutathione
GSSG	Oxidized glutathione
H^+	Hydrogen ion
H_2O_2	Hydrogen proxide
Hb	Hemoglobin
hCG	human chorionic gonadotropin
HDL	High density lipoprotein
I	Iodine
K^+	Potassium ion
LDL	Low density lipoprotein
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MIT	Monoiodotyrosine
mRNA	Massenger ribonucleic acid
Na^+-K^+ ATpase	Sodium-Potassium adenosine triphosphatase
NADPH	Nicotinamid adenine dinucleotide phosphate (reduced)
PCV	Packed cell volume
PG	Picogram

PO_4^{-3}	Phosphate
RBCs	Red blood corpuscles
SE	Standard error
T^3	Triiodothyronine
T^4	Tetraiodothyronine
TCA	Trichloro acetic acid
TG	Triglyceride
TRH	Thyrotrophic releasing hormone
TSH	Thyroid stimulating hormone
TSP	Total serum protein
UC P	Uncoupling protein
VLDL	Very low density lipoprotein
WBC	White blood cell
Zn^{+2}	Zinc ion

Summary

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VLDL	Very low density lipoprotein
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$Zn^{+۲}$	Zinc ion

Introduction

Hyperthyroidism is the most endocrin disorder in human and animals, manifest itself in exposure of body tissues to excessive levels of thyroid hormones, tri-iodothyronine ($T^{٣}$) and tetra-iodothyronine or thyroxine ($T^{٤}$),the two main hormones of thyroid gland (Dahlen,٢٠٠٢).

This disorder had also been found to affect women five times more than men(Mayne,١٩٩٨).Hyperthyroidism is also called thyrotoxicosis, a general term, refers to hypermetabolic state that results because of excess thyroid hormones (Intenzo, ٢٠٠٣). There are several causes of hyperthyroidism one of which an auto immune disorder, its etiology involves the production an antibodies against the thyroid stimulating hormone(TSH) receptors, that result in excess production of $T^{٣}$ and $T^{٤}$(Ali *et al.,* ٢٠٠٢).

Moreover, high level of thyroid hormones can also result from autonomous production by solitary or multiple thyroid nodules, which secret thyroid hormones autonomously (Kosugi and Shiji,٢٠٠٢).Also, excessive TSH secretion by pituitary tumors resulted in higher stimulation to thyroid gland (Beck *et al.,* ١٩٩٦). The over production of human chorionic-gonadotropin hormone during pregnancy competes with TSH in binding with TSH receptors(AL–Rawi *et al.,*٢٠٠٣). Thyroiditis, an inflammatory state of thyroid gland also resulted in libration of stored hormones ($T^{٣}$ and $T^{٤}$) into blood circulation (Summaria *et al.,* ١٩٩٩).Finally, the other miscellaneous causes represent a little incidence in hyperthyroidism

such as metastatic thyroid carcinoma, and struma ovarri (Greenspan and Gardner,٢٠٠١).

In general, thyroid hormones play essential roles in regulating vast physiological states in the body (Kirsten, ٢٠٠٠). Thyroid hormones play essential roles in regulation the metabolism of major nutrient biomolecules such as carbohydrates, lipids, and proteins (Weber *et al*., ٢٠٠٣). Protein synthesis and breakdown is stimulated by thyroid hormones. The influence of thyroxine on normal body growth is derived largely from the stimulation of protein synthesis (Karytko and Cutter,١٩٩٧).Thyroid hormones stimulate the rate of carbohydrates absorption, gluconeogenesis, and insulin secretion (Dimitriadis and Raptis, ٢٠٠١). Synthesis, mobilization, and degradation of lipids are controlled by thyroid hormones, because lipids are the major calirogenic molecules (Nogueira *et al*.,٢٠٠٢). Moreover, thyroid hormones are important factors for the other physiological phenomena such as growth, puberty, and mental development (AL- Anssari, ١٩٩٩; Monden *et al*., ٢٠٠٦). On the other hand, over production of thyroid hormones disturbs the normal body status causes retardation of growth, body wasting, heat intolerance, increase metabolic rate, and infertility (Krassas, ٢٠٠٠; Kadhim, ٢٠٠٣). However, when thyroid gland enables to produce adequate amount of $T^{٣}$ and $T^{٤}$, consequently lead to hypothyroidism, a disorder which has the opposite features of hyperthyroidism **(**Abramowics *et al*.,١٩٩٧; Norigoshi *et al*., ٢٠٠١).

<u>The aim of the present work was designed to clarify the following points :-</u>

١- Determination of the levels of T٣, T٤, and TSH.

٢- Estimation of some blood parameters and their relationships with hyperthyroidism.

٣- Determination the metabolic activity of higher levels of T٣ and T٤ through measurement of body temperature, some serum lipids (cholesterol and triglycerides), and serum proteins.

٤- Investigation the effects of hyperthyroidism on the serum anti-oxidants levels.

٥- Investigation the effects of hyperthyroidism on the available minerals (calcium, phosphorus, sodium, and potassium)in serum and urine samples.

٦- Determination of some trace elements such as copper and zinc and their relation with hyperthyroidism.

Literature Review

١- Thyroid gland

a)History

Thyroid gland was described by Galen (١٣٠-٢٠٠AD) but the name was applied to it first Wharton (١٦٥٦) named it thyroid from its proximity to thyroid cartilage. The cartilage was named thyroid (shield like),by Galen because of its characteristic shape (Ahmed *et al.*, ٢٠٠٤). Hyperthyroidism was defined in Dorland's medical dictionary as a condition caused by excessive production of iodinated hormones (Burtis and Ashwood , ١٩٩٩).

b) Embryology

Thyroid gland originates as out pouching in the floor of the pharynx ,which grows downward anterior to the trachea , and can be identified in human embryo by the third week (Friedberg, ١٩٨٩).Colloid spaces may be seen in the gland by the end of ninth week; follicular elements from and can be shown to contain colloid. By the fourteenth week the thyroid of the fetus is essentially an adult gland (Sadler, ٢٠٠٠).

c) Anatomy and histology

In the adult ,the thyroid gland consists of two lobes connected by an isthmus ,a bridge of tissues. The thyroid gland is located in the neck below the larynx, on either side of and anterior to the trachea. This gland is highly vasculature and supplied by the superior thyroid artery and the inferior thyroid artery. The parathyroid gland is located at each end of the upper and lower poles of thyroid and may be located imbedded in the tissues of thyroid gland (Gartner and Hiatt, ٢٠٠٠). On microscopic examination, the thyroid gland is found to consist of a series of follicles of varying size. Each spherical follicle is surrounded by single layer of epithelial cells based on basement membrane, and filled with pink staining material called colloid (Steven and Lowe,٢٠٠٢)

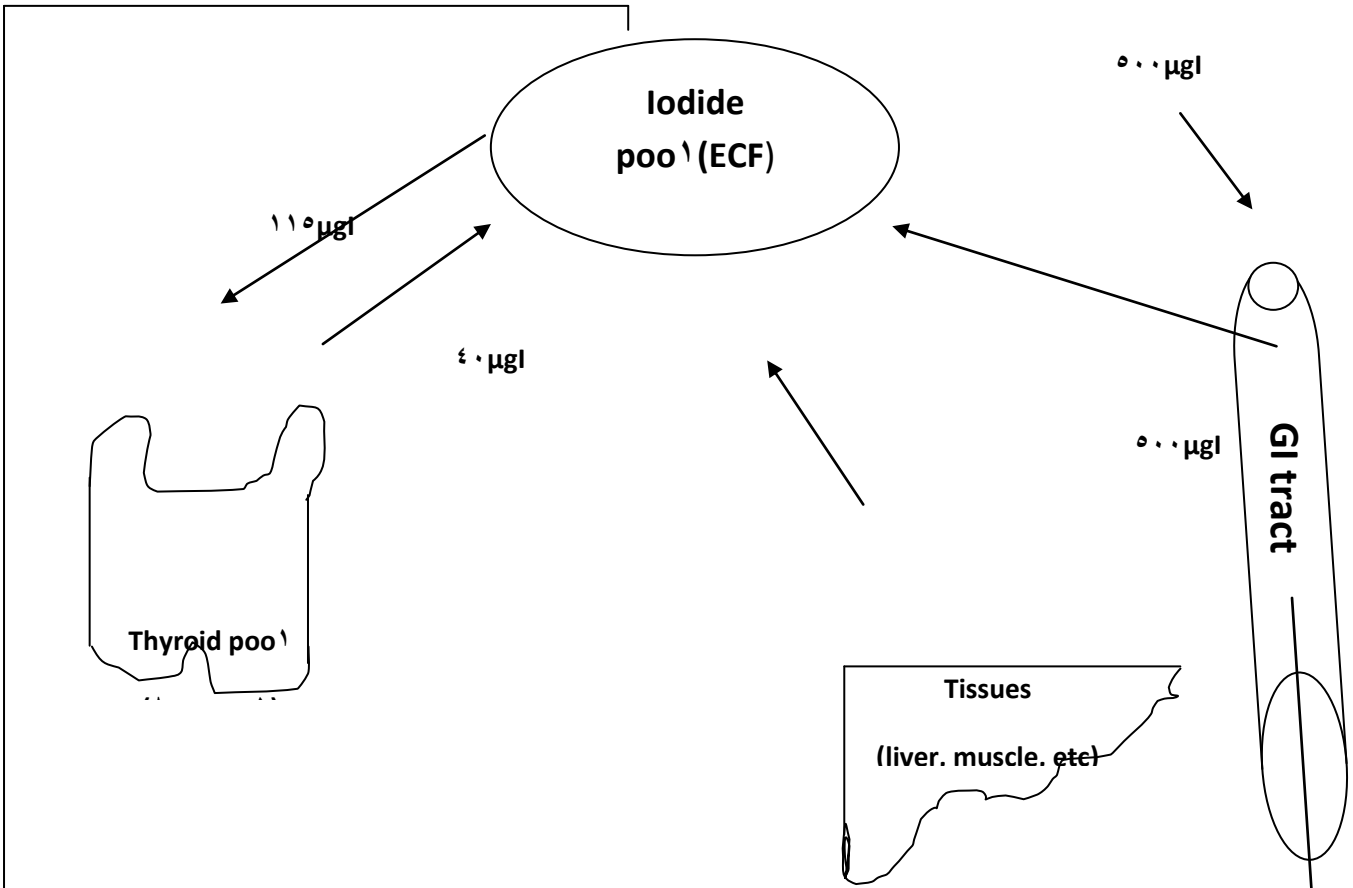
d)Physiology of thyroid gland

The follicular cells of thyroid gland produce two powerful metabolically active modified amino acids, T٣ and T٤, (Schonfeld *et al.*, ١٩٩٧; Hulbert, ٢٠٠٠). Follicular cells have the ability to trap or concentrate iodide by mechanism called "Iodide trapping" through active transport and passive diffusion(Yamada and Sato, ١٩٩٩).

Iodide, is the main source in the synthesis of thyroid hormones, once inside the cells, iodide is rapidly metabolized and bound to thyroglobulin by process of iodination (Dabon and Surks, ١٩٩٨; Huang *et al.*, ٢٠٠١). Thyroglobulin, a major glycoprotein manufactured in thyroid cells has about ١٠-١٢٥ tyrosyl residues, some of which are accessible for iodination to give monoiodotyrosine (MIT) and diiodotyrosine (DIT) (Ringel, ٢٠٠١). Of

all endocrine gland, thyroid is one able to store large quantities of hormones in their follicular lumen. T٣ andT٤ bind with thyroglobulin by peptide linkage and extruded into lumen by exocytosis (Al-Rawi *et al.*, ٢٠٠٣).

However, As seen in figure (١) of iodine metabolism, dietary iodine reduced in gastrointestinal tract into iodide(I^-)and absorbed into blood circulation ,one third of iodide removed by thyroid cells, and the remainder is excreted by kidneys(Guan *et al.*, ٢٠٠١).Iodide enter the follicular cells through the iodide trapping mechanism, is an example of secondary active transport, sodium(Na^+) and iodide (I^-) are co-transport into the thyroid cells, and then Na^+ is pumped out of the cells by Na^+-K^+ ATP ase (Liou *et al*., ٢٠٠٠).Inside the follicular cells, iodide is oxidized to iodine. This oxidation is catalyzed by thyroid peroxidase, an enzyme which use hydrogen peroxide ($H_٢O_٢$)as an electron acceptor. After that, thyroperoxidase catalyzes incorporation of iodine into tyrosyl residues of thyroglobulin to produce monoiodotyrosine (MIT) and diiodotyrosine (DIT) (Shet *et al*.,١٩٩٩)



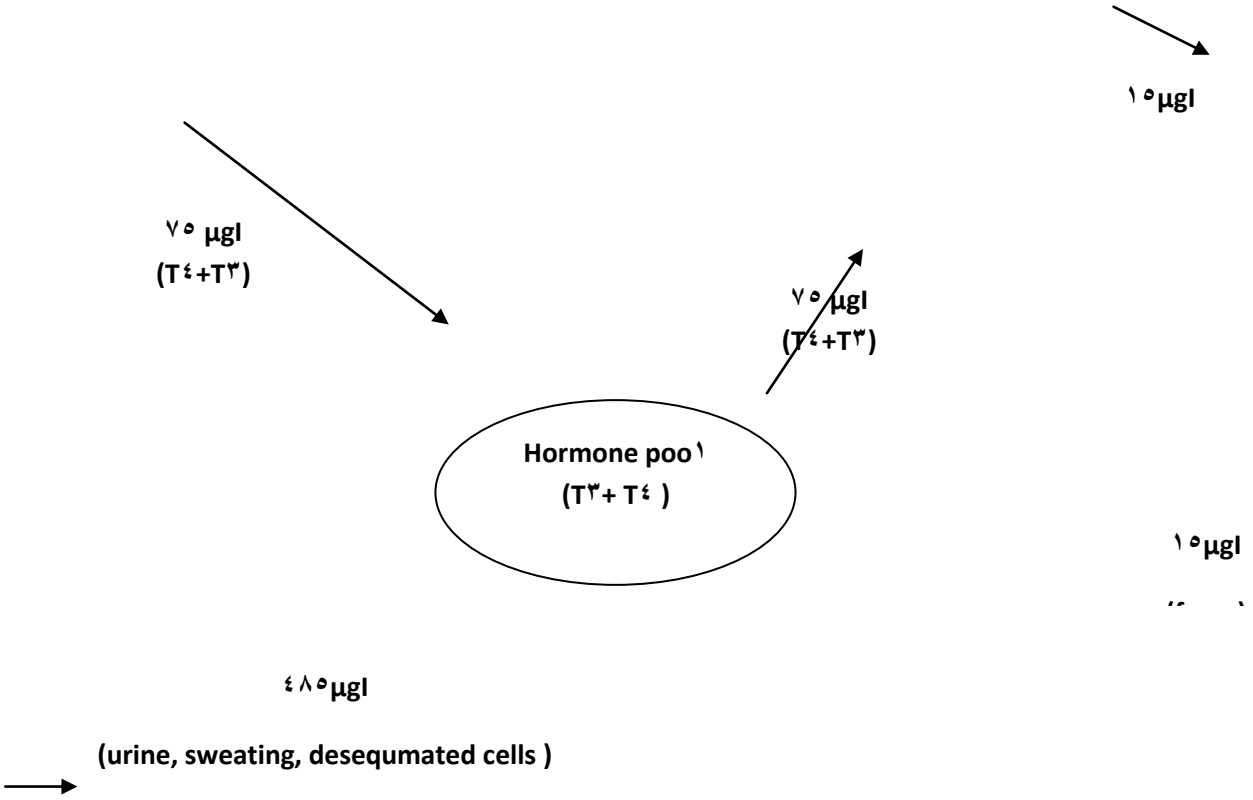
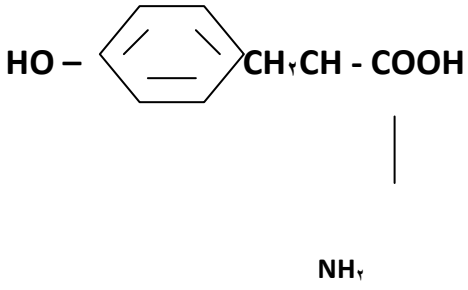


Figure (١):- Iodine Metabolism

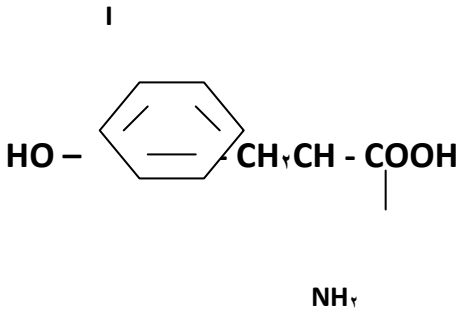
(Modified from Greenspan and Gardner , ٢٠٠١)

Moreover, as seen in figure (٢) of thyroid hormones structure, thyroperoxidase catalyzes condensation of iodotyrosine molecules to produce T٣ and T٤. Since, it condensate one molecule of MIT with one molecule of DIT to produce T٣, or condensate two molecules of DIT to produce T٤. (Samules, ١٩٨٨). Deiodenases, the other enzymes

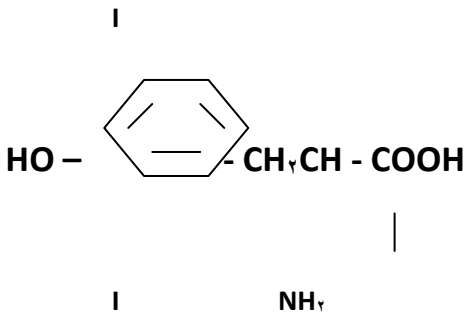
present in peripheral tissues are responsible for elimination of iodine from T٣ and T٤. Some of iodine is reutilized in synthesis of new T٣ and T٤ and the other iodine is extruded by kidneys(Fereidoun, ١٩٨٥; Susan *et al.,* ١٩٩٣).



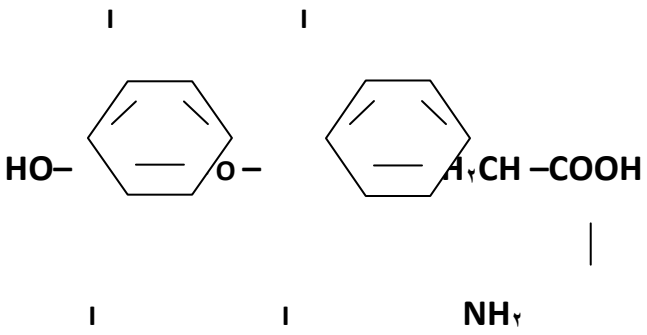
Tyrosine



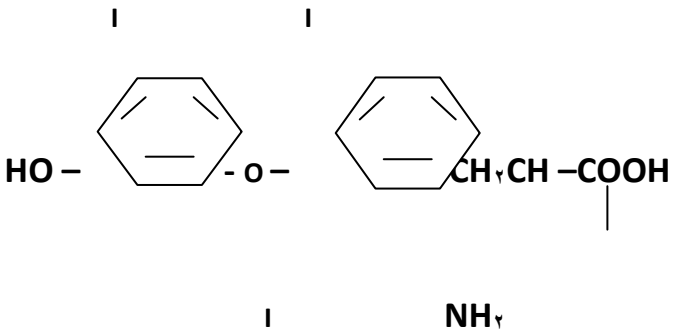
٣-Monoiodo tyrosine (MIT)



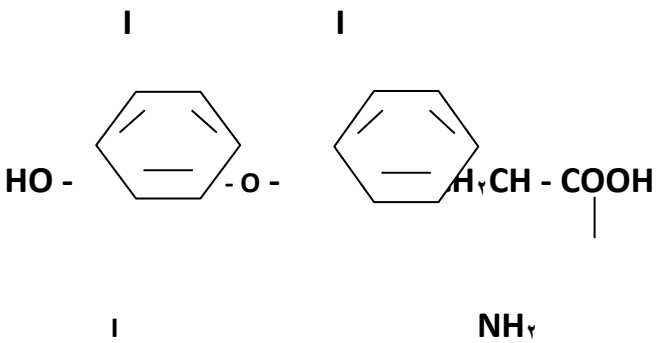
٣,٥ – Diiodo tyrosine (DIT)



٣,٥,٣´,٥´ - Tetraiodothyronine (Thyroxine) [T٤]



٣,٥,٣´ - Triiodothyronine (T٣)



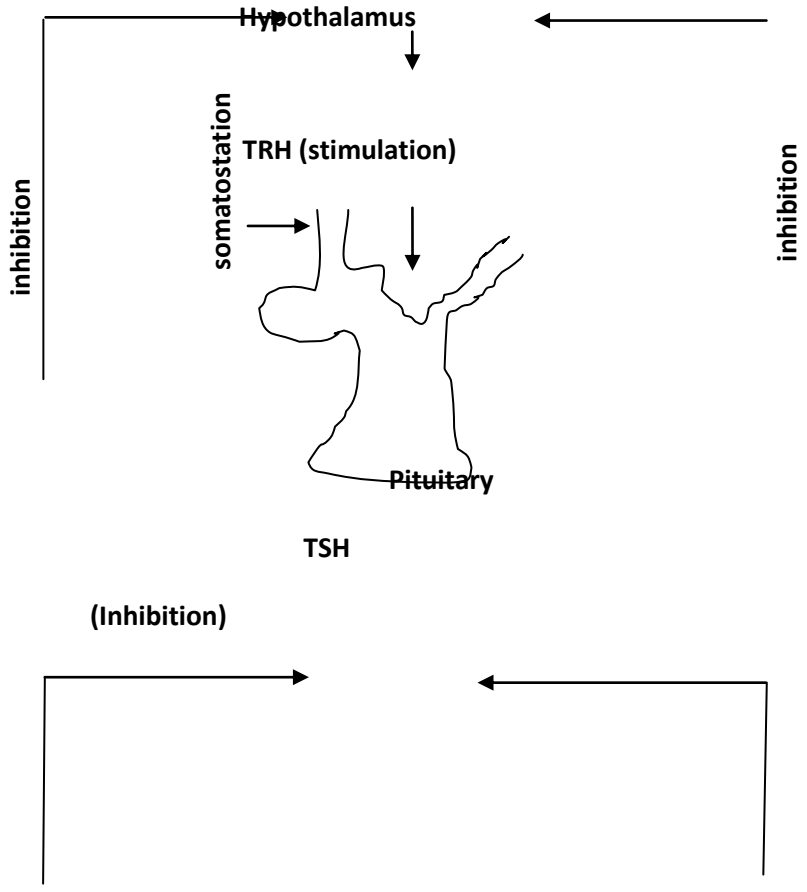
٣,٣´,٥´ - Triiodothyronine (reverse T٣ [rT^3])

Figure (٢):- Structure of thyroid hormones and related compound . (Modified from Murry *et al .,* ٢٠٠٠)

e) Regulation of thyroid gland activity

Hypothalamic-pituitary axis is the system that regulates the production and secretion of thyroid hormones (Ross,٢٠٠١) as seen in figure (٣).

The regulation of thyroid begins with hypothalamus, thyrotropin -releasing hormones (TRH) is a tripeptide released by the hypothalamus, and effects anterior lobe of pituitary gland, where it stimulates manufacture and release of thyrotropin stimulating hormone (TSH).Which in turn stimulates thyroid gland to produce T٣ and T٤(Mayne, ١٩٩٨; Kirsten, ٢٠٠٠). The secretion of TSH seems to be regulate an interplay of negative feedback with circulating free T٣ and T٤ (Hirsnberg *et al.*, ٢٠٠٠).



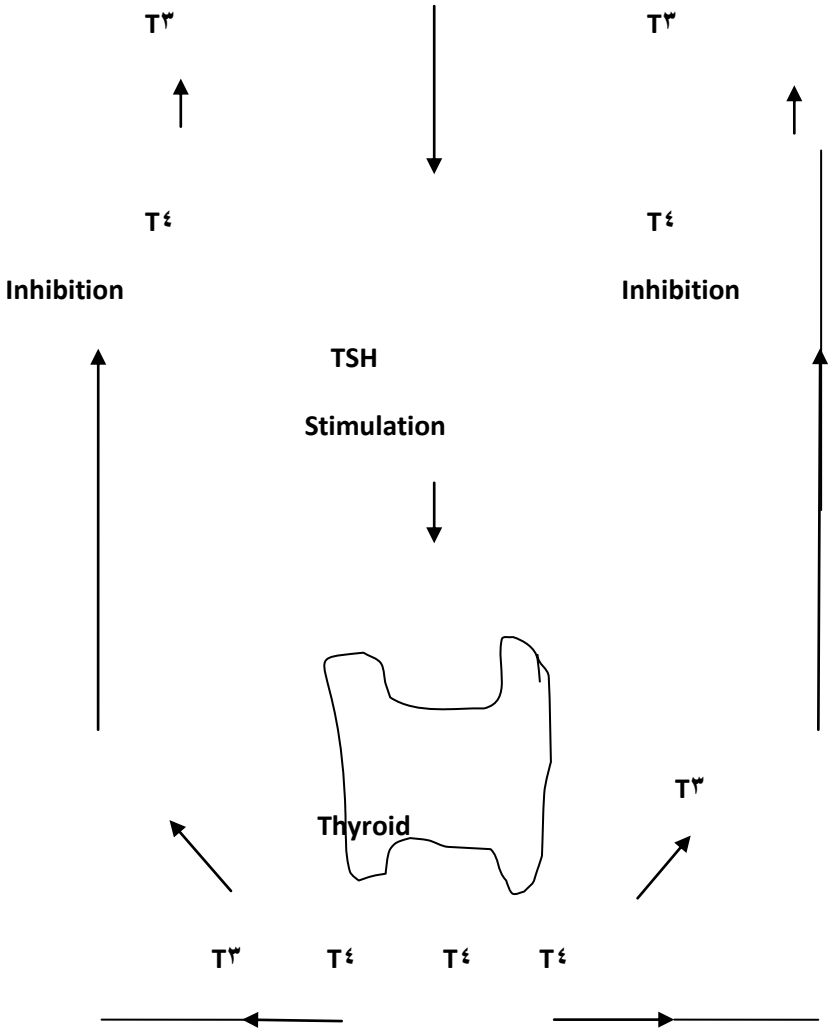


Figure (٣):- The negative feed back system regulating thyroid function .

(Modified from Bishop *et al .,* ٢٠٠٠)

f)Thyroid stimulating hormone (TSH)

TSH is a glycoprotein, secreted from anterior lobe of pituitary gland in response to TRH of hypothalamus (Larsen, ١٩٨٢) It consists of two subunits, alpha and beta, linked non covalently. The beta chain has the specificity of this hormone, while the alpha chain is

similar to other glycoproteins hormones like luteal hormone, follicle stimulating hormone, and human chorionic gonadotropin (Beck *et al*., ١٩٩٦). TSH, the main stimulus for thyroid gland to enhance iodide uptake and stimulates the manufacture and release of T٣ and T٤.(Takeda and Kyokao,٢٠٠٢).

g) Secretion and transport of thyroid hormones.

When thyroid gland becomes stimulated by TSH,the secretion of thyroid hormones is initiated by recovery by the cells of stored thyroglobulin (Burrow, ١٩٨٨). Microvilli, a small projections at apical membrane of the epithelial follicular cells which envelop a small pieces of colloid through endocytosis process to form intracellular droplets, and then fused with intracellular lysosomes, which have proteolytic (proteinase)and deiodinase enzymes. Proteinase hydrolyzes the peptide linkage between iodinated tyrosyl residues and thyroglobulin (Burch and Wart , ١٩٩٣). As a result T٣, T٤, MIT, and DIT are produced. T٣ and T٤ diffuse directly into blood stream, whereas MIT and DIT metabolized by deiodinase ,and iodide then used in next synthesis of T٣ and T٤ (Brent, ١٩٩٤).

Almost all circulating T٣ and T٤ are bound to serum proteins: thyroxine binding globulin, prealbumin, and albumin. Only small amounts of free T٣ and T٤ are physiologically active thyroid hormones (Bartaline, ١٩٩٤). T٣ and T٤ diffuse across the plasma membrane of target tissues and bind with their nuclear receptors to initiate the biological actions, some of their receptors are situated in cytoplasm of the target cells (Motomura and Brent, ١٩٩٨). Much of

T_4 is converted into T_3 in peripheral tissues by the function of deiodinase enzyme activity (Depalo *et al*.,١٩٩٤).

T_3 is more active than T_4 because it is not tightly bound to the serum proteins as is T_4, and has greater affinity to target tissue receptors. When thyroid hormones bind with their nuclear receptors to form hormone-receptors complex, which in turn stimulates transcription of messenger ribonucleic acid (mRNA) from target gene, and then mRNA translated in the cytoplasm to produce special metabolic enzyme (Openheimer,١٩٨٥). Thyroid hormones actions include calorigenesis, oxygen consumption, and regulation of carbohydrates, lipids, and protein metabolism (Cachefo *et al*., ٢٠٠١). T_3 and T_4 then undergo three steps to be metabolize into inactive form by deiodination, conjugation, and side chain modification processes (Chopra, ١٩٧٨; Hagag *et al*., ١٩٩٨).

h) Hyperthyroidism:

Hyperthyroidism occurs when tissues expose to excess amount of the thyroid hormones. This disorder is also called thyrotoxicosis, which refers to hypermetabolic state and describes the clinical syndrome (Damjanov *et al.,* ١٩٩٨).

Hyperthyroidism resulted from several causes such as:

(١) Graves' disease (difuse goiter) is an auto immune thyroid disease and comprises ٨٠٪ of hyperthyroidism. In this auto immune disease, auto antibodies bind with TSH receptors to activate

thyroid. TSH becomes suppressed because of negative feed back mechanism of T٣ and T٤ (Al-Jubori, ١٩٩٨).

(٢) Toxic nodules, these nodules either single or multiple. Nodules tend to secret thyroid hormones autonomously. TSH secretion is also suppressed by negative feed back mechanism of T٣ and T٤.

(٣) Well differentiated thyroid carcinoma such as papillary and follicular carcinoma (Al-Hindawi *et al.,* ١٩٨١).

(٤) TSH-secreting pituitary tumor. In these tumors, pituitary gland tends to secret a high amount of TSH regardless of T٣ and T٤ levels.

(٥) Other miscellaneous causes which represent a little incidence in onset of hyperthyroidism such as iodide induced hyperthyroidism, pregnancy, post partum, struma ovarri, and stress factors (Young *et al.,* ٢٠٠١).

Clinical features of hyperthyroidism include nervousness, tachychardia, weight loss, profuse sweating, heat intolerance, diarrhea, muscular weakness, emotional excitement, and irregular menses (Mohamimad and Al-Dabbagh, ١٩٩٩).

٢)Hematological parameters study

a) Red blood corpuscles (RBCs)

They are biconcave discs, that are manufactured in the bone marrow in response to erythropoietin stimulation via erythropoiesis

process (Ogawa, ١٩٩٣). In mammals, they lose their nuclei and mitochondria before entering in to blood circulation, and survive for average of ١٢٠ days. The vital function of RBCs is to carry oxygen to different parts of the body because they contain hemoglobin by which $O^{٢}$ is transported (Broudy,١٩٩٧). The mature red blood corpuscle derives its energy in the form of adenosine triphosphate (ATP) from anaerobic (glycolysis) conversion of glucose, to lactate.

ATP generated by glycolysis is essential for integrity and stability of the cell membrane, in part this achieved by maintaining the concentration gradients of cations across cell membrane through Na^{+}–K^{+} adenosine triphosphatase (Deluise and Flier, ١٩٨٣; McCarrol, ١٩٩٥). ٢,٣–diphosphoglycerate is an intermediate metabolite of glycolysis, it is important compound because it binds with hemoglobin and dissociates of oxygen (Zere and Tanaka, ١٩٨٩). Pentose phosphate pathway in which reduced nucleotides such as (reduced) nicotinamide adenine dinucleotide-phosphate (NADPH)are produced, and used in reduction reactions that occurring in RBCs (Hsia, ١٩٩٨).

b)Erythropoietin

Erythropoietin is a single polypeptide chain. It is produced by cells close to proximal renal tubules. In fetus, erythropoietin is produced in the liver as well as in the kidneys, but there after most erythropoietin production occurs in kidneys (Krantz, ١٩٩١).The main function of erythropoietin is to regulate erythropoiesis through its actions on erythroid progenitor cells in bone marrow. A decrease of partial pressure of oxygen in blood stimulates erythropoietin secretion (Jelkmann,١٩٩٢).

c)Hemoglobin(Hb)

Hb is oxygen transporter in erythrocytes. Vertebrate hemoglobin consists of four polypeptide chains, two of one kind and two of another, the four polypeptide chains are held together by non covalent attractions. Each contains a heme group, which consists of porphyrin ring with iron resides in the center(Todd, ١٩٨٠; Turgeon, ٢٠٠٥).

Human hemoglobin occurs in several forms. The major for adult hemoglobin (HbA), consists of two alpha and two beta chains, hemoglobin A٢ (HbA٢), the other type of Hb, which comprises two alpha and two delta chains and constituents a less amount of normal HbA throughout life. (Stryer, ١٩٩٥).

The other of hemoglobin is of fetal hemoglobin (HbF), which contains two gamma polypeptide chains instead of beta chains. HbF, the main hemoglobin during fetal life. (AL-Awamy, ٢٠٠٠).

Structurally, each globin chain has its own genetic locus, the genes for globin chains can be divided into two major groups: the alpha genes, which are located on chromosome١٦, and non alpha genes, which are located on chromosome١١ (Bilto,١٩٩٨).

d)Red blood corpuscles indices (MCV, MCH, MCHC)

Values which are obtained for red blood corpuscles count, packed cell volume, and hemoglobin concentration can be further by studying red blood corpuscles indices, which define the size, Hb content of average of red blood cells .

MCV is the average volume of RBCs in femtoliters (fL). The mean cell hemoglobin (MCH)is the average weight of hemoglobin content in a RBC, is calculated from the hemoglobin concentration and RBC count, its unit is picogram (pg). The third index MCHC, is defined as the average concentration of Hb in each individual RBC, its unit is expressed as gram per deciliter g/dL(Pittiglio and Sacher, ١٩٨٧; Dacie and Lewis, ١٩٩٥).

e)Packed cell volume (PCV)

PCV, is the ratio of blood volume of RBCs to the volume of whole blood. PCV is usually expressed as a percentage (Dacie and lewis, ١٩٩٥).

f)Erythrocyte sedimentation rate (ESR)

ESR is the speed in millimeters that red corpuscles fall per unit of time, which is usually one hour. The rate of sedimentation is directly proportional to the red cell mass and inversely proportional to plasma viscosity (Dacie and Lewis, ١٩٩٥).

g)White blood cells (WBCs) or leukocytes

Leukocytes are nucleated cells of blood tissue. Of these, the granulocytes(polymorphonuclear leukocytes)are the most numerous, since, these cells have multilobed nuclei as the cell grow older (Borregaard and Cowland, ١٩٩٧). Most of them contain neutrophilic granules (neutrophils). But a few contain granules that stain with acidic dyes (eosinophils)and some have basophilic granules (basophils) (Metcalf, ١٩٩١). The other two cell types found normally in peripheral blood are: lymphocytes, which have large round nuclei and scanty cytoplasm; and monocytes,which have kidney– shaped nuclei (Pittiglio and Sacher, ١٩٨٧). By acting together, these cells provide the body with powerful defenses against tumors, viral, bacterial, fungal, and parasite infections (Gabig and Babior, ١٩٨٤;Hoff brand *et al*., ١٩٩٩).

٣- Body temperature

Man is able to maintain his central body temperature; this is temperature of the brain , thoracic cavity, and abdominal cavity, at a constant value which is independent of the temperature of the surrounding environment(Simon, ١٩٩٣). Heat is continually being produced in the body as a by product of metabolism, and body heat is also continually being lost to the surrounding (Guyton, ١٩٨٦). A variety of basic chemical reactions contribute to body heat production at all times are:(١) Basal rate of metabolism of all the cells of the body; (٢)Increase in rate of metabolism caused by muscle contraction; (٣) Increase in metabolism caused by effect of thyroxine ;epinephrine, norepinephrine on cells (٤) Ingestion of food; and (٥)

Brown fat, which has a high rate of metabolism, this tissue is located around axilla and aorta (Yen, ٢٠٠١). The heat is lost from the body by radiation, conduction, convection, and evaporation. (Ganong, ١٩٩٧) .

The balance between heat production and heat loss determines the body temperature because the speed of chemical reaction varies with temperature and because the enzyme system of the body has narrow temperature ranges in which their function is optimal, and normal body function depend upon a relatively constant body temperature (Loke and Maclennan, ١٩٩٨).

Biochemical studies :

a)Total serum proteins(TSP)

Proteins, the structural and functional components of a cell. The functional components serve as biocatalysts (enzymes), regulator of metabolism, (hormones) (Devlin,٢٠٠٢).Plasma proteins and tissue proteins share the same amino acid pool (building blocks of proteins), and thus alterations in one group will eventually affect the other. So, the plasma proteins can be hydrolyzed to amino acids, which can be used for production of energy (Kent, ١٩٨٨; Hershko and Ciehanover, ١٩٩٢). The origin of plasma proteins, circulating antibodies in the gamma globulin fraction of plasma proteins are manufactured in plasma cells. Most of the other plasma proteins are synthesized in the liver (Deutcher, ١٩٩٠)

b) Albumin

Albumin is the protein present in highest concentration in the serum. It is synthesized in the liver. Albumin has two well– known functions, one is the contribution albumin makes to the colloid osmotic pressure of intravascular fluids, due to its high concentration, which maintains the appropriate fluids in the tissues (Geveart and Vandekerchohove,٢٠٠٠). The other prime function is its propensity to bind various substances in the blood for example, albumin binds billirubin, fatty acids, hormones, metals, and ions (Murray *et al.,* ٢٠٠٣**).**

c) Globulins

The globulin fraction is subdivided into numerous components. One classification divide it into alpha١, alpha٢, beta ١, beta ٢ and gamma globulins. The immunoglobulin are synthesized in the reticuloendthelial system, and other globulins are synthesized in the paranchymal liver cells (Pepys and Hirchfield, ٢٠٠٣).

The alpha-globulins carry metals, lipids , and carbohydrates, whereas glycoproteins are a group of carbohydrate containing proteins which are important constituents of the alpha-glycoproteins, (Brockhausen,١٩٩٣).

Beta–globulin, much of the beta- globulin are as apolipoproteins, is combined with cholesterol and other lipids, and also, as individual binding globulins, carries fat soluble vitamins and hormones, (Myers *et al.,* ١٩٩٤). Most antibodies are gamma-and most of them have antibody activity which is formed in the immunologically competent cells (Burton, ١٩٩٠).

d)Cholesterol

Cholesterol is unsaturated steroid alcohol of high molecular weight, consisting of a perhydrocyclopentathioline ring and a side chain of eight carbon atoms. In its esterified form ,it contains one fatty acid molecule(Javitt,١٩٩٥).The high solubility of cholesterol in blood is due to plasma lipoprotein mainly low–density lipoprotein (LDL)and very low density lipoprotein (VLDL) that have the ability to bind and thereby solubilize large amount of cholesterol (Gordon and Rifkind, ١٩٨٩; Sundaram *et al.,*١٩٩٧). LDL delivers cholesterol to various tissues that require cholesterol for membrane structure or steroid hormone synthesis. Where as HDL, which is rich in cholesterol and has a low triglyceride content, is the main vehicle for carrying excess cholesterol from peripheral tissues to the liver, where it can be excreted in bile either directly in the form of cholesterol or after conversion to bile (Sassolas and Cartier, ١٩٩٩).

e)Triglycerides (TG)

Triglycerides molecule comprises one molecule of glycerol with their fatty acids including both saturated and unsaturated fatty acids. The source of triglycerides in the body can be either dietary or synthesized in liver and other tissues (Warnik, ١٩٩١). Chylomicrons are the largest of the lipoprotein particles and represent the major carriers of exogenous(dietary)triglycerides. Very low density lipoprotein (VLDL), like, chylomicrons, are also rich in triglycerides. They are the major carriers of endogenous (Liver synthesized)triglycerides (Harris *et al*., ١٩٨٨ Hussain ١٩٩٦). Triglycerides may be enzymatically hydrolyzes to release the fatty acids and glycerol. Lipoprotein lipase hydrolyzes triglycerides in plasma proteins, this enzyme is located on the surface of endothelial cells of capillaries. Hormone–sensitive lipase, the other enzyme, acts inside fat cells to release free fatty acids from triglycerides. This enzyme is stimulated by epinephrine,cortisol, and thyroxine hormone (Valdemarsson *et al*.,١٩٨٤; Cachefo *et al*., ٢٠٠١) .

f) Reduced glutathione (GSH)

Reduced glutathione(GSH)is a tripeptid that is important in detoxification of endogenously generated peroxides and exogenous chemical compounds (Cheung *et al*., ٢٠٠٠). Glutathione is synthesized by formation of the dipeptide gamma glutamyl cysteine and the subsequent addition of glycine. The synthesis of glutathione is largely regulated by cysteine availability, The liver plays a major role in the synthesis of reduced glutathione (GSH) from glutamate, cysteine, and glycine (Meister and Anderson, ١٩٨٣; Fernandez and Videla, ١٩٩٦).

The reduced form of glutathione, a tripeptide with free sulfhydryl group, serves as a sulfhydryl buffer that maintains the cysteine residues of hemoglobin and other cell proteins in the reduced state. The reduced form also plays a role in detoxifycation by reacting with hydrogen peroxides (H_2O_2) (Fernandez *et al*., ١٩٩١). Glutathione in turn cycles between a reduced thiol form (GSH) and an oxidized form (GSSG) in which two tripeptides are linked by a disulfied bond. Glutathione peroxidase, an enzyme, uses sulfhydryl groups of glutathione (GSH) as a hydrogen donor with formation of the disulfide form of oxidized glutatuion. The oxidized glutathione in turn reduced to GSH by glutathione reductase, a flavoprotein that uses NADPH as electron source (Karplus and Schulz, ١٩٨٩).

g) Alkaline phosphatase (ALP)

The phosphatases are a group of enzymes which promote the hydrolysis of organic phosphates with liberation of phosphate ions. These enzymes classification depending on whether the enzyme has maximum activity in alkaline or in acid medium(Bikle, ١٩٩٤). ALP has a major concern with bone metabolism , and produced in many cells of the body, and one important site of secretion is the osteoblasts. The main other alkaline phosphatase isoenzymes come from liver, placenta, and sometimes from intestinal mucosa (Archer and Taylor, ١٩٩٦; Biscoveanu and Hasinsk, ٢٠٠٠).

h)Calcium ($Ca^{+٢}$)

More than ٩٩٪ of calcium in the body is part of bones and teeth. The remaining ١٪ is mostly in the blood and other extra cellular fluids. Very little amount is in the cytosol of most cells (Bushinsky and Monk,١٩٩٨).The plasma calcium is found in two states partly bound to protein and partly diffusible. It is the free, ionized calcium in the body fluids, that is vital second messengers and is necessary for blood coagulation, muscle contraction, and nerve function. Calcium is absorbed from small intestine by $Ca^{+٢}$–H^{+} ATPase pump and passive diffusion (Wandrup,١٩٨٩). Three hormones probably operate to maintain the constancy of the calcium level in the body are parathyroid hormone, vitamine–D٣,and calcitonin (Glenn, ١٩٩٥; Nissenson, ٢٠٠٠) .

i)Phosphorus($PO_{٤}^{-٣}$)

The compounds of phosphate are everywhere in living cells and participate in many of the most important biochemical processes. The genetic materials deoxy–nucleic acid (DNA)and ribonucleic acid (RNA)are a complex phosphodiesters. Most enzymes are esters of phosphouric acid or pyrophosphoric acid. The most important reservoir of biochemical energy are ATP, creatin phosphate, and phospho-enol pyruvate. (Bender and Kadenbach, ٢٠٠٠; Shiber and Mattu, ٢٠٠٢).

Most of phosphorus in the blood exists as phosphates or esters. Phosphorus is absorbed in small intestine by active transport and passive diffusion. Many stimuli that regulate calcium level ,including vitamin-D٣,growth hormone, parathyroid hormone also regulate phosphorus level (King *et al.*, ١٩٨٧)

j) Sodium(Na^+)

Sodium, a blood electrolyte, is the most abundant cation and the chief base of the blood. Its primary functions in the body are to chemically maintain osmotic pressure, acid - base balance, and to transmit nerve impulses (Kumar and Tomas, ١٩٩٨). Sodium is absorbed from intestinal tract via sodium glucose cotransport, and then into interstitial fluid by Na^+–K^+ pump . The Na^+ concentration in the blood plasma is relatively high, whereas in the cytoplasm is low. On the other hand, the concentration of potassium in cytoplasm is more than in the plasma. (Crook, ٢٠٠٢).

Maintenance of these gradients a cross plasma membrane depends upon the input of ATP. The RBCs and other cells of the body contain specialized enzyme called Na^+–K^+ transporting adenosine triphosphatase, which functions as an enzyme and as a molecular pump. It catalyzes pump three Na^+ ions out ward of the cell in exchange with pump two K^+ ions in ward of the cells (Skou , ١٩٩٢;Chan *et al.,*٢٠٠١).

A multiple regulatory mechanisms have been evolved in terrestrial animals to control excretion of this ion. Sodium concentration is under control of the kidneys and the central nervous system acting through the endocrine system (Adrogue and Medians, ٢٠٠٠). Adrenal mineralocorticoids such as aldosterone increase tubular reabsorption of Na^+ in association with excretion of K^+ and H^+ (White, ١٩٩٤). Atrionatriuretic peptide (ANP),a peptide hormone, secreted by atrial myocytes enhances excretion of Na^+ by the kidneys. (Maack, ١٩٩٢).

k)Potassium (K^+)

Potassium is the principal cation in the cells, and it is present in relatively low concentration in the extracellular fluids. Potassium plays important role in nerve conduction and muscle function. Moreover, it helps maintain acid- base balance and osmotic pressure (Gennari , ٢٠٠٢).

Potassium is absorbed from intestinal lumen by Na^+–K^+– ٢Cl^- co-transport into epithelial cells, and then into interstitial space by K^+ channels. The potassium uptake from extra cellular fluids into cells is performed by Na^+–K^+ pump and passive diffusion (Hass, ١٩٩٤, Harry, ٢٠٠٥). The kidneys are important in the regulation of K^+ balance. Initially, the proximal tubules reabsorbed nearly all the K^+ ions, then, under the influence of aldosterone, additional K^+ is secreted in to urine in exchange for Na^+ ions in both distal tubules and colleting ducts (Aronson, ١٩٨٣; Whany and Sims, ٢٠٠٠).

l)Trace elements

A few elements are present in the body in small quantities that they are called trace elements. Usually, the amount of these in the foods are also minute. Those believed to be essential for life at normal levels. Conversely, some of them can be toxic when present in excess (Nielsen, ١٩٨٤; Standstead, ١٩٩٥).

i) Copper (Cu^{+2})

Copper is an essential trace element. It is required in the diet because it is the metal cofactor for a variety of enzymes including ceruloplasmin, cytochrome oxidase, superoxide dismutase, dopamine beta-hydroxylase, ascorbate oxidase, lysyl oxidase, and tyrosinase (Walshe, ١٩٩٥). Copper is a absorbed in the stomach and upper small intestine, after diffusion across luminal membrane, copper is bound to metallothionein in the cytosol of enterocytes. Metallothionein also found in other cells of the body to provide protection of this metal available to generate free radicals (Harris,٢٠٠٠).In liver, part of copper is excreted in the bile, and the other part leaves the liver attached to ceruloplasmin, which is synthesized in that organ. Ceruloplasmin is an alpha٢-globulin and has a high copper content and carries ٩٠٪ of the copper and the remainder ١٠٪ is carried with albumin (Olivares,١٩٩٦;Saari and Schuschke , ١٩٩٩).

ii) Zinc($Zn^{+٢}$)

The physiological functions of zinc are based largerly on its presence as an essential component of many enzymes involved in virtually all aspects of metabolism(King,١٩٩٠).Over ١٠٠ enzymes are known to requir zinc as part of their prosthetic groups. These include alkaline phosphatase, super oxide dismutase, carbonic anhydrase, alcohol dehydrogenase, and DNA polymerase (Standstead , ١٩٩٥).

Zinc enzymes are essential to growth, wound healing, reproductive function, the immune system,and protection from free radical damage(Prasad,١٩٩٦).Absorption of zinc is inhibited by increased dietary content of phytate (inositol phosphate). Moreover, zinc absorption appear to induce the synthesis of metallothionein in the intestinal mucosa, and its absorption proportionate to level of metallothionein, and competes for absorption with iron and copper (Hurgunow, ٢٠٠٠).Zinc is transported in the circulation by albumin and alpha٢ macroglobulin. It is found primarily in prostate, RBCs, glands, muscles, bones, and liver, (Dursun *et al*., ١٩٩٥).

Materials and Methods

A) Materials

١- Subjects of study:-

This study was carried out over a thirteen months period, from April ٢٠٠٥ to May ٢٠٠٦ in Hilla teaching hospital. The subjects of the study were ١٧٠ ; patients and healthy subjects of both sex, males and females. The total number of patients were (١٣٠) ١٠٠ females and ٣٠ males. Forty subjects were used as control group. Their ages ranged between ٢٠ years to ٥٥ years. This study was carried out before doing treatment for all patients. Women of this study were non pregnant and no contraceptive drugs were used. All patients were attending to the hospital for laboratory diagnosis and

treatment, while the (٤٠) control subjects were attending to the public health laboratory in the hospital.

٢- Chemicals :- The chemicals materials sources used in this work were as follow :-

No.	Chemicals materials	Sources
١	EDTA (Ethylene diamine tetra acetic acid).	Fluka, AG, Switzerland.
٢	Sodium chloride.	Merk, Germany.
٣	Potassium chloride.	Merk, Germany.
٤	Copper sulfate anhydrous.	Fluka.
٥	Methanol.	Fluka.
٦	Hydrochloric acid.	Fluka.
٧	Glutathione.	Biochemical.
٨	٢- nitro benzoic acid. (DTNB).	Sigma chemical.
٩	Glacial acetic acid.	Chem Supply.
١٠	Trichloroacetic acid.	Hopkin and Williams.
١١	Total protein kit.	Spinreact.
١٢	Albumin kit	Spinreact.
١٣	Total cholesterol kit.	Biomaghreb.
١٥	Total calcium kit.	Montgat (Spain)
١٦	Total phosphorus kit.	Spin react, S. A.
١٧	Alkaline phosphatase kit.	Teco diagnostics, Anheim, CA.
١٨	TSH kit.	Biocheck, Inc
١٩	T٤ kit.	Biocheck, Inc
٢٠	T٣ kit	Biocheck, Inc

٣-Instrument:- The following table shows the main instrument used in this work and their sources.

No.	Instrument	Sources
١	Hb Electrophoresis.	Seba, profil, Austria.
٢	Hematocrit centrifuge.	H. Jurgens, Co. Hettich, zentrifuger D- ٧٢٠٠, Tuttingen.
٣	Hemocytometer.	OSAKA, Japan.
٤	Microscope.	Olympus, CH., Japan.
٥	Water bath.	Memmert, ٨٥٤ Schwa Bach, Germany.
٦	Centrifuge.	Heraeus Christ, Vamed, Comp. Germany.
٧	Coulter mixer.	Colter electronic, LTd, England.
٨	Hb meter.	Optima, ٢٠٢, Japan.
٩	Spectronic type ٢١.	Molton Roy(Switzerland).
١٠	Vortex mixer.	Karlkole (Germany).
١١	Oven.	Hearson (England).
١٢	Atomic absorption Spectrophotometer.	PYE Unicam SP٩.
١٣	Centrifuge tube.	Mes (١.ogm)Fisonsm (England).
١٤	Plaine tube.	AFma – Dispo, Jordan.
١٥	EDTA tube.	Merk,Germany
١٦	Micro – pipette ٢٠ µL.	Gilson, France.
١٧	Micro – pipette ١٠٠ – ١٠٠٠ µL.	Gilson, France.
١٨	Micro – pipette ١٠٠ µL.	Oxford sampler micro – pipetting system (USA).
١٩	Micro – pipette ٤٠-٢٠٠ µL.	Oxford sampler micro – pipetting system (USA).
٢٠	Micro – pipette ٥٠ µL.	Nichiryo (Japan).
٢١	Micro – pipette ١٠٠٠ µL.	Socorex (Swiss).
٢٢	Micro – pipette ٥٠٠ µL.	Nichiryo (Japan).

٢٣	Disposable syringe.	Becton Dickinson,(Spain).
٢٤	Flame photometer.	Halsteed essex,England.
٢٥	Thermometer.	Hemedia (India).

B) Methods

١- Blood collection:-

The collection of blood was performed in Hilla teaching hospital. The vein on the front of the elbow is almost employed. The arm should be warm to improve blood circulation and distended the vein. A tourniquet was applied directly on the skin around the arm(usually from the left arm), approximately (٦-٨cm) above the site of collection. The skin over the vein will be sterilized with a small pad of cotton wool soaked with ٧٠٪ ethyl alcohol. Needles used were ٢١, ٢٢, ٢٣ gauge. The site was air dried with clean gauze, to prevent hemolysis by alcohol. Two groups of labeled tubes were used (Bishop *et al*., ٢٠٠٠); the first tubes contain EDTA as anti- coagulants to prevent clotting of blood to be used for hematological studies. The second group of tubes were without anti-coagulant as plain tubes, for blood to be used for preparing sera for subsequent biochemical tests. The blood is allowed to clot for ١٥ minutes, the clot shrinks and serum can be obtained by centrifugation and precautions were taken to avoid hemolysis. The serum samples were liquated in sterile test tubes using micropipette with sterile disposable tips. Each sample was labeled and given a serial number together with the patient name, the serum samples were frozen at(-٢٠°C) for biochemical analysis.

٢- Urine analysis :

The collection of urine samples was done on random samples of urine freshly voided by patients. A plastic containers were used to collect of urine samples and then directly transferred to refrigerator to avoid bacterial decomposition. Urine samples were diluted with distilled water (١:٥) and then urinary Ca^{+2}, P, K^{+}, and Na^{+} concentrations were done as described in their corresponding estimation in serum. Estimation was carried out by using colorimetric and flame photometric method. (According by Phosphorus kit from spinreact).

٣- Hormonal studies :

a)Determination of thyroid stimulating hormone (TSH)

Principle of the method :-

The ultra sensitive TSH enzyme linked immuno-sorbent assay (ELISA) is based on the principle of solid phase enzyme linked immuno-sorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti TSH antibody is used for solid phase immobilization. A goaut anti-TSH antibody is in the antibody enzyme (horse radish peroxidase) conjugate solution. The test sample is allowed to react

simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme linked antibodies. After two hour of incubation at room temperature, the wells were washed with water to remove unbound labeled antibodies. A solution of TMB reagent is added and incubated for twenty minutes, resulting in the development of a blue color. The color development is stopped with addition of stop solution change the color to yellow. The concentration of TSH is directly proportional to the color intensity of the sample test.

Procedure :-

١- The desired number of coated wells was taken by using the holder.

٢-١٠٠ micoliter (µL) of standards, samples, and controls were distributed into appropriate wells.

٣- ١٠٠ µL of enzyme conjugate reagent was distributed into each well.

٤- The wells were thoroughly mixed for ٣٠ seconds. It was very important to mix them completely.

٥- The wells were incubated at room temperature (١٨–٢٥C°) with shaking at ١٧٥ rpm, for ١٢٠ minutes.

٦-The incubation mixture was removed by flicking plate contents into waste container.

٧- The wells were rinsed and flicked ٥ times with deionized water.

٨- The wells were striked sharply onto absorbent paper towels to remove all residual water droplets.

٩- ١٠٠ µL of TMB reagent was distributed into each well, and gently mixed for ١٠ minutes.

١٠–The wells were incubated at room temperatures for ٢٠ minutes.

١١– The reaction was stopped by adding ١٠٠ µL of stop solution to each well.

١٢–The wells were gently mixed for ٣٠ seconds. It was important to make sure that all the blue color changes to yellow color completely.

١٣–The absorbance was read at ٤٥٠ nanometer(nm)with a micro titer well reader within ١٥ minutes.

١٤-Standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentrations on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X-axis. The concentrations of TSH standard curve were (٠, ٠.١, ٠.٥, ٢, ٥, and ١٠ µIU/ml). (according by TSH kit from Biocheck, Inc.)

b)-Measurement of total tetraiodothyronine (T_4) or thyroxine

Principle of the method :-

The enzyme immunoassay (EIA) is used to determine T٤.In the T٤ EIA, a certain amount of anti–T٤ antibody is coated on microtiter wells. A measured amount of patient serum, and constant amount of T٤ conjugated with horse radish peroxidase are added to the microtiter wells. During incubation, T٤ and conjugated T٤ compete for the limited binding sites on the anti – T٤ antibody. After ٦٠ minutes incubation at room temperature, the wells are washed ٥ times by water to remove unbound T٤ conjugate. A solution of TMB reagent is then added and incubated for ٢٠ minutes, resulting in the development of the blue color. The color development is stopped with the addition of stop solution. The intensity of the color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T٤ in the sample.

Procedure

١-The desired number of coated wells was taken by using the holder.

٢- ٢٥ µL of standard, samples, and controls was pipetted into appropriate wells.

٣–١٠٠ µL of working conjugate reagent was distributed into each well and thoroughly mixed for ٣٠ seconds. It was very important to mix completely.

٤–The mixture was incubated at room temperature (١٨-٢٥ C°) for ٦٠ minutes.

٥-The incubated mixture was removed by flicking plate contents into a waste container..

٦-The micro-titer wells were rinsed and flicked ٥ times with de-ionized water.

٧–The wells were sharply striked onto absorbent paper or paper towels to remove all residual water droplets.

٨-١٠٠ µL of TMB reagent were dispensed into each well and gently mixed for ١٠ seconds.

٩ -The wells were incubated at room temperature in the dark for ٢٠ minutes.

١٠-The reaction was stopped by adding ١٠٠ µL of stop solution to each well and gently mixed for ٣٠ seconds. It was important to make sure that all the blue color changes to yellow color completely.

١١- The absorbance was read at ٤٥٠ nm with a microtiter well reader within ١٥ minutes.

١٢-Standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentrations on graph paper, with absorbance values on the vertical axis and concentrations on the horizontal axis. The concentration of T٤ standard curve were (٠,٢,٥,١٠,١٥, and ٢٥ µg/dL). (according by T٤, kit from Biocheck, Inc.)

c) Measurement of total triiodothyronine (T٣)

Principle of method:-

Enzyme immunoassay EIA is used to determine T٣. In the T٣ EIA a second antibody(goat anti-mouse IgG) is coated on micro- titer wells. A measured amount of patient serum, a certain amount of mouse monoclonal anti-T٣ antibody, and a constant amount of T٣ conjugated with horse raddish peroxidase are added to the microtiter wells. During incubation, the mouse anti-T٣ antibody is bound to the second antibody on the wells, and T٣ and conjugated T٣ compete for the limited binding sites on the anti –T٣ antibody. After ٦٠ minutes incubation at room temperature, the wells were washed ٥ times by water to remove unbound T٣– conjugate. A solution of TMP reagent is then added and incubated for ٢٠ minutes, resulting in the development of blue color. The color development is stopped with addition of stop solution. The intensity of color formed is proportional to the amount of enzyme present and is inversely related to the amount of unlabeled T٣.

Procedure :-

١- The required number of coated wells was taken by using the holder.

٢- ٥٠ µL of standards, samples, and controls were pipetted into appropriate wells.

٣- ٥٠ µL of the antibody reagent was dispensed into each well and thoroughly mixed for ٣٠ seconds.

٤- ١٠٠µL of working reagent was added into each well and thoroughly mixed for ٣٠ seconds. It was important to have a complete mixing in this step.

٥- The mixture was incubated at room temperature for ٦٠ minutes.

٦- The incubated mixture was removed by flicking plate contents into a waste container.

٧- The microtiter wells were rinsed and flicked ٥ times with deionized water.

٨- The wells were sharply stricked onto absorbent paper to remove residual water droplets.

٩- ١٠٠ µL of TMB reagent was dispensed into each well, and gently mixed for ١٠ seconds.

١٠- The wells were incubated at room temperature in the dark for ٢٠ minutes without shaking.

١١- The reaction was stopped by adding ١٠٠ µL of stop solution to each well and gently mixed for ٣٠ seconds. It was important to make sure that the blue color changes to yellow color completely.

١٢- The optical density was read at ٤٥٠ nm within ١٥ minutes.

١٣- Standard curve was constructed by plotting the mean absorbance obtained from each reference standard against its concentrations on graph paper, with absorbance values on the vertical axis and concentrations on the horizontal axis . The concentrations of T٣ standard curve were (٠,٠.٧٥,١.٥,٣.٠,٦.٠, and ١٠.٠ ng /mL). (according by T٣ kit from Biocheck, Inc.)

٤-Hematological studies

a)Red blood corpuscles count (RBCs count):-

Blood was diluted with formal citrate solution (١٪ formalin in ٣٨ gram /liter(gm /L)tri-sodium citrate).A twenty microliter of blood was added into four ml of diluting fluid, the diluted blood was mixed in a mechanical mixture; the counting chamber (Neubaur hemocytometer) was filled by using the Pasteur pipette before being examined under ٤٠X objective lens of the microscope to count RBCs in the four corners and center tertiary squares of the RBCs counting area of the chamber on both sides (Dacie and lewis,١٩٩٥).

b)White blood cells count (WBCs count):-

Blood was diluted with ٠.٤ ml of Turk's solution (١ ml glacial acetic acid, ٢ ml of gentian violet, and ١٠٠ ml distilled water).A twenty microliter of blood was mixed in a mechanical mixture ; the counting chamber a (Neubaur hemocytometer*)* was filled by using the Pasteur pipette before being examined under ٤٠X objective lens of the microscope to count WBCs in the four corners secondary squares(Dacie and lewis,١٩٩٥).

c) Erythrocytes sedimentation rate (ESR):-

Westergren method was used to determine the ESR. Blood was diluted with tri-sodium citrate solution ٣.٨٪. ٠.٥ ml of diluted solution was used for ٢ ml of blood, the mixture was mixed in a mechanical mixture, and then drown into Westergren tube up to the zero mark and tube set upright in a stand position with a spring clip on top and rubber at bottom. The level of the top of the red blood corpuscles column is read at the end of one hour (Pittiglio and Sacher, ١٩٨٧).

d)Determination of packed cells volume (PCV):-

Microhematocrit method was used to determine PCV. Heparinized capillary tubes used, and blood was permitted be fill to approximately three quarters of their lengths then the unmarked end is closed with modeling clay and put in the microhematocrit centrifuge. After centrifugation for ١٥ minutes, the red blood corpuscles were separated from plasma and remain a band of buffy coat at the interface between them consisting of leukocytes and blood platelets (Dacie and Lewis, ١٩٩٥**).**

e)Estimation of hemoglobin (Hb):-

A cyanomethemoglobin method was used to estimate the hemoglobin contents of the blood.The principle of this method was as follow:-

Procedure:-

Reagents	**Standard**	**Blank**	**Sample**
Drabkin`s solution	_	٥ ml	٥ ml
Standard	٥ ml	_	_
Sample	_	_	٢٠ µL

Drabkin`s solution was prepared by mixing one container of Drabkin`s reagent with ٩٨٠ ml of deionized water, and then ٠.٥ ml of ٢٥٪ Brij-٣٥ solution ٢, added into solution with mixed well. ٢٠ µL of blood was added for ٥ ml of Drabkin`s solution with mixing, and incubated for at least ٥ minutes at ٣٧°C and then the results were estimated by using Hb meter at ٥٤٠ nm wave length (Markarem,١٩٧٤).

F)Measurement of RBCs indices:-

I)The mean corpuscular volume (MCV)was calculated as the following:-

$$MCV = \frac{\text{Packed cell volume \%}}{\text{Red corpuscles count } 10^{-12}} \text{ Femtoliters}$$

II)The mean corpuscular hemoglobin (MCH) was calculated as the following :-

$$MCH = \frac{\text{Hemoglobin in g/dL}}{\text{RBCs count } 10^{12}} \text{X picogram}$$

III)The mean corpuscular hemoglobin concentration (MCHC) was calculated as following :-

$$MCHC = \frac{\text{Hemoglobin gm/dL}}{\text{PCV L/L}} \text{ gm/dL}$$

(Dacie and Lewis,١٩٩٥)

g)Hemoglobin electrophoresis

Principle of method:-

Cellulose acetate electrophoresis method was used to determine hemoglobin quantitation (HbA, HbA٢, and HbF). Electrophoresis is the name given to the movement of charged particles through on electrolyte subject to an electric field. If these are differently, they will move in apposite direction. The rate of migration of particles of like charge will depends among other thing on the number of charges each carrier. Hb will migrate from cathode to the anode in the following order. First hemoglobin constant spring, then HbA٢,C,and E migrate in the same band, next HbS and Lepore, again in the same band next hemoglobin F followed by Hb A then Hb Bart`s and last HbH (Pittiglio and Sacher, ١٩٨٧).

i)Procedure

a)Washing stage :-

Two milliliters of blood sample were added to ١٠ ml of normal saline(isotonic solution ٠.٩٪NaCl).The diluted mixture was centrifuged at ٢٠٠ rpm for ٥ minutes and then removed supernatant after centrifugation, and this process was repeated three times to

ensure remove serum and plasma. The precipitation was taken to perform the next stage.

b)Lysate stage :-

In this stage, ١ml of precipitation(RBCs)was added to ٢ml of distilled water to hemolyze RBCs and release of hemoglobin. The mixture was mixed gently and then centrifuged at ١٥٠٠ rpm at for half hour. After complete centrifugation, supernatant was transferred into tube to complete electrophoresis. Of the different techniques, semi–micro electrophoresis was used because the larger quantity of sample used renders the interpretation of the results easier and more reliable. The following step by step procedure was applied to semi – micro electrophoresis method :-

١- The phorestrip membranes were immersed in tris–veronal buffer for ١٥ minutes.

٢- Tweezers supplied with the system, were used to remove the membranes from the buffer and held them vertically for a few second to allow excess buffer to drib off.

٣- Membranes were placed between two sheets of filter paper to blot excess buffer.

٤- Membranes were also applied on the bridge by using the tweezers. The absorbent dull surface of the membranes were correctly facing upward.

٥- Hemoglobin samples were applied on the membranes by using sample applicator.

٦- The power supply was ٢٢٠ volt for ٩٠ minutes, it was applied to electrophoretic tank.

٧- At end of migration, the power supply was stopped and disconnected the tank.

٨- The membrane were removed from bridge, and then they were placed in staining solution. The membranes were immersed with making sure the dull surface where the samples were applied is facing downward into the staining solution for ٥ minutes.

٩- The membranes were removed from staining solution by using tweezers, and they were held vertically for a few seconds to let excess staining solution drip off. This process was repeated until a perfectly clear white back ground was obtained .

١٠- Membranes were placed in dehydration solution to dehydrate the membranes for ٥ minutes.

١١- Tweezers were used to remove membranes from dehydration solution, and then were placed in the clearing solution for ١ minutes.

١٢-Membranes were removed from clearing solution and they were held vertically for few seconds to allow excess clearing solution to drip off.

١٣-Membranes were applied on a perfectly clean glass plate (with the surface upon which the sample were applied against the glass surface)with avoiding the formation of air bubbles, and the membranes were left for one hour to dry at room temperature.

١٤- The membranes were removed from the glass plate and use normal densito– metric scanning at ٥٣٠ nm (wave lengthfor Panceau red stain) to obtain the quantitative evalution of the results.

٥- Measurement of body temperature

The clinical thermometer was used to determine body temperature, this thermometer records the temperature in degrees centigrade. The clinical thermometer has an arrow usually at ٣٧°C or ٩٨.٤°F.This represents the average body temperature. It was usually placed in the mouth under the tongue for at a least ٥ minute, and the mouth firmly closed so that breathing has only taken placed through the nose and provided that no hot or cold has been placed in the mouth. (Guyton, ١٩٨٦).

٦ – Chemical studies

a)Determination of alkaline phosphatase (ALP)

Principle of method :-

The method used was that ALP acts upon the AMP–buffered sodium thymolphthalein mono-phosphate .

Procedure

١- For each sample, ٠.٥ µL of alkaline phosphatase substrate was added into labeled test tubes and equilibrate to ٣٧°C for ٣ minutes.

٢- At timed intervals, ٥٠µl of each standard, control, and sample were added to its respective test tube and mixed gently. Deionized water was used as sample for reagent blank.

٣- The mixture was incubated for exactly ١٠ minutes at ٣٧°C.

٤- At timed intervals, ٢.٥ ml of alkaline phosphatase color developer was added to standard, control, and sample with mixing gently.

٥- The absorbance of standard, control, and sample was read by using spectronic ٢١ at ٥٩٠ nm wave length. (according to ALP kit from Techodiagnostics, Anahein).

b)Measurement of total serum protein:

Principle of method:-

Proteins are given an intensive violet–blue complex with copper salts in alkaline medium. Iodide is included as an antioxidant. The intensity of the color formed is proportional to the total protein concentration in sample(Koller,١٩٨٤).The intensity of color was measured photometricaly at ٤٥٠ nm wave length, by using spectronic ٢١.

$$\text{Calculation} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{standard concentration}$$

= gm/dL of total protein in the sample.

Procedure:-

Reagent	Blank	Standard	Sample
Biuret	١.٠ml	١.٠ ml	١.٠ml
Standard	-	٢٥µL	-

Sample	-	-	٢٥ µL

The solutions were mixed and incubated for ٥ minutes at ٣٧°C

c)Determination of serum albumin

Principle of the method:-

Albumin in the presence of bormocresol green at slightly acid pH, produces a color change of the indicator from yellow– green to green blue. The intensity of the color is proportional to the albumin concentration in the sample. The intensity of color was measured by using spectronic ٢١ at ٦٣٠ nm wave length. (according by albumin kit from spinreact).

$$\text{Calculation} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

= gm/dL concentration of albumin in the sample.

Procedure:-

Reagent	Blank	Standard	Sample
R	١.٠ ml	١.٠ ml	١.٠ ml
Standard	-	٥ µL	-
Sample	-	-	٥ µL

The solution was mixed and incubated for ١٠ minutes at a room temperature.

d) Determination of serum globulin:-

Serum globulin was determined by substracting albumin from total serum protein and the results represented the values of serum globulin.(Bishop *et al.,* ٢٠٠٠)

e) Determination of total serum cholesterol

Principle of the method:-

The cholesterol is determined after enzymatic hydrolysis and oxidation.The indicator quinoneimine is formed from hydrogen peroxide and ٤-amino-antipyrine in the presence of phenol and peroxidase. The quantity of this red dye quinoneimine formed is

proportional to the cholesterol concentration, and was measured photometrically by using spectronic ٢١ at ٥٠٥ nm wave length.

$$\text{calculation} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times ٢٠٠ = \text{mg/dL Concentration}$$

of total cholesterol in serum sample.

Procedure:-

Reagent	Blank	Standard	Sample
Standard	-	١٠ µL	-
Sample	-	-	١٠ µL
Working reagent	١ ml	١ ml	١ ml

The solution were mixed and incubated for ٥ minutes at ٣٧°C. (according by cholesterol kit from Biomagreb).

f) Determination of triglycerides

Principle of method:-

The triglycerides are enzymetically hydrolyzed to glycerol and fatty acids according to the following reaction :-

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{glycerol + fatty acids}$$

$$\text{calculation} = \frac{\text{Optical density of sample}}{\text{Optical density of standard}} \times ٢٠٠ = \text{mg/dL Concentration of serum triglycerides.}$$

Procedure:-

Reagent	Blank	Standard	Sample
Standard	-	١٠ µL	-
Sample	-	-	١٠ µL
working reagent	١.٠ml	١.٠ ml	١.٠ml

The reagents were mixed and incubated for ٥ minutes at ٣٧C°, and then measured photometrically by using spectronic ٢١at ٥٠٥ nm wave length. (according by triglycerides kit from Biomagreb).

g) Determination of total calcium in serum and urine samples

principle of method :-

Calcium in the sample, reacts with O–cresolphtaleine at alkaline pH. The colored complex formed is proportional to the amount of calcium present in the sample. The intensity of the color was measured photometrically by using spectronic ٢١ at ٥٧٠ nm wave length.

$$\text{Calculation} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

= mg/ dL concentration of calcium.

Procedure :-

Reagents	Blank	Standard	Sample

R١	١ ml	١ ml	١ ml
R٢	١ ml	١ ml	١ ml
Sample	-	-	٢٠ µL
Standard	-	٢٠ µL	-

The reagents were mixed and incubated for ٥ minutes at room temperature. (according by calcium kit from spinreact).

h) Determination of phosphorus in serum and urine samples

principle of method :-

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. Its subsequent reduction in alkaline medium originates a blue molybdenum. The intensity of color formed is proportional to the inorganic phosphorus concentration in sample. The intensity of color was measured photometrically by using spectronic ٢١ at ٧١٠ nm wave length.

$$\text{Calculation} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

= mg\dL concentration of phosphorus in sample.

Procedure : -

Reagents	Blank	standard	sample
Working reagent	١.٥ml	١.٥ ml	١.٥ml
Standard	-	٥٠ µL	-
Sample	-	-	٥٠ µL

The reagents were mixed and incubated for ١٠ minutes at ٣٧°C. (according by phosphorus kit from spinreact)

I) Determination of serum reduced glutathione(GSH)

Principle of method :-

٥,٥´-dithio bis (٢- nitro benzoic acid) (DTNB) is a disulfide chromogen that is readily reduced by sulfhydryl group of GSH to an intensely yellow compound.The absorbance of the reduced chromogen is measured at ٤١٢ nm and is directly proportional to the GSH concentration (Burtis and Ashwood, ١٩٩٩).

Preparation of reagents :-

١- Precipitating solution: Trichloroacetic acid (TCA) ٥٠٪:-٥٠gm of TCA were dissolved in a final volume of ١٠٠ ml of double distilled water (DDW).

٢- Ethylene diamine tetraacetic acid–disodium (EDTA Na_2). (٠.٢M):- ٣٧.٢٢٤ gm of EDTA Na_2 were dissolved in a final volume of ٥٠٠mL of DDW.

٣- Tris–EDTA buffer(٠.٢M)pH ٨.٩:- ٤٨.٤٥٨ gm of Tris were dissolved in ٨٠٠ ml of DDW. ١٠٠ml of (٠.٢M) EDTA solution were added and brought a final volume of ١ liter with DDW. The pH was adjusted to ٨.٩ by the addition of ١M of HCl.

٤- DTNB reagent(٠.٠١M):-٠.٠١٩٨gm of DTNB was dissolved in ٥ml of absolute methanol, and brought to a final volume of ٢٥ml (This reagent was stable for at a least ١٣ weeks at ٤°C).

٥- GSH standards:- Stock standard solution (٠.٠٠١M) was prepared by dissolving ٠.٠٣٠٧ gm of GSH in a final volume of ١٠٠ml of(٠.٢M) EDTA solution. Dilutions were made in EDTA solution to ٢, ٥, ١٠, ١٥, ٢٠, ٣٠,٤ ٠,and ٥٠µM. (This working standard solution should be prepared daily).

Procedure:-

Serum GSH was determined by using a modified procedure utilizing Ellman's reagent(DTNB),which is summarized as follows:-

Duplicates of each standard and sample test tubes were prepared then pipetted into test tubes.

Reagents	Blank	Standard	Sample
Serum	-	-	١٠٠ µL
Standard	-	١٠٠ µL	-
DDW	٩٠٠ µL	٨٠٠ µL	٨٠٠ µL
TCA	١٠٠ µL	١٠٠ µL	١٠٠ µL

Contents were mixed in vortex mixture intermittently for ١٠–١٥ minutes, and centrifuged for ١٥ minutes at ٣٠٠٠ rpm, then pipetted into test tubes.

Reagents	Blank	Standard	Sample
Supernatant	٤٠٠ µL	٤٠٠ µL	٤٠٠ µL
Tris–EDTA buffer	٨٠٠ µL	٨٠٠ µL	٨٠٠ µL
DTNB reagent	٢٠ µL	٢٠ µL	٢٠ µL

Tubes were mixed in vortex mixture. The spectrophotometer ٢١ was adjusted with reagent blank to read zero absorbance at ٤١٢ nm,

and the absorbance of standards and sample was read within ٥ minutes of the addition of DTNB.

Calculation of serum GSH was obtained from the calibration curve in µM.

j) Determination of serum copper and zinc :-

Atomic absorption spectrophotometer method was used to determine the trace elements(copper and zinc) in serum samples (Burits and Ashwood, ١٩٩٩) .

Procedure

The serum samples were diluted (١:٤)by adding distilled water and then digested with addition of concentrated nitric acid (HNO_3).The samples were injected into auto sample cup of atomic absorption spectrophotometer to read emitted lights. standard curves were done for both copper and zinc. Concentrations of copper standard curve were(٠.٣, ٠.٦, ٠.٩, ١.٢,١.٥,١.٨ mg/L. The concentrations of zinc standard curve were (٠.٣, ٠.٦, ٠.٩, ١.٢, ١.٥ mg/L).

k) Determination of potassium and sodium in both serum and urine samples

Flame emission photometer was used to determine sodium and potassium in both serum and urine samples(Bishop *et al*., ٢٠٠٠).Serum samples were diluted(١:٤),where as urine samples diluted(١:٥);and then samples injected into sample cup of the system to measure emitted lights., Standard curve were made for both sodium and potassium. Since, concentrations of sodium standard curve were (٢٠, ٤٠, ٥٠, ٨٠, ١٠٠, ١٢٠, ١٤٠, ١٦٠ mmol/ L),where as, concentration of potassium standard curve were (٢, ٤, ٦, ٨, ١٠ mmol/L).

L) Statistical analysis :-

All values were expressed as means ±SE. The data were analyzed by using of computer SPSS program. Student's t–test was used to examine the differences between different groups (Daniel, ١٩٩٩).

Results

١- Hormonal measurements :-

Results of TSH,T٣, and T٤ are summarized in table (١) of both hyperthyroid groups ,males and females.

a)TSH :-

Results of TSH of hyperthyroid in males and females (٠.٣٢٦ ± ٠.٢٥٤; ٠.٣٨٦ ±٠.٢٤٥ µU/ml respectively) were significantly (P< ٠.٠١) lower than control subjects, males and females (٢.٢٥٣ ± ٠.٢٥٤; ٢.٥١٤ ± ٠.٢٥٣µU/ml, respectively).

b)T٣ :-

Values of T٣ of hyperthyroid in males and females (٢٤٦ ± ٩.٤٥; ٢٤٩ ± ١٢.٢٩ ng/dL , respectively) were significantly (P<٠.٠١) higher than control subjects, males and females (١٣٨ ± ٦.٩٨٣;١٣٢ ± ٧.٦٣٤ ng /dL respectively).

c)T٤ :-

Result of T٤ of hyperthyroid in males and females (١٣.٥١ ± ٠.٣٢١;١٣.٧٢±٠.٣٣٧µg/dL, respectively) were significantly higher than healthy control subjects, males and females (٧.٨٠٦± ٠.١٥٠; ٨.٢٢± ٠.١٦٦µg /dL, respectively).

٢-Relationship between hyperthyroidism and sex :-

The results which are shown in figure (١) show that the number of affected females was ١٠٠(٧٦.٩٢ %)out of ١٣٠ and the number of affected males was ٣٠(٢٣.٠٧٪).

٣- Relationship between hyperthyroidism and age :-

The results which are illustrated in figure(٢)showed that ≥ ٢٠–٣٠years appears the most affected age group and comprises ٣٣.٠٧٪, where as, the other age groups >٣٠–٤٠ years; >٤٠–٥٠ years; and >٥٠–٦٠years comprised ٢٥.٣٨٪; ٢٢.٣٠٪; ١٩.٢٣٪, respectively.

٤- Hematological studies

a)RBCs count, Hb concentration , and PCV :-

Results of RBCs count ,Hb concentration , and PCV of both males and females affected with hyperthyroidism are illustrated in table (٢).

i) RBCs count :-

Results of RBCs count of hyperthyroid in males and females groups (٥٥٢٠٠٠٠ ±٠.١٦٤,٥ ١٠٠٠٠٠± ٠.١٦٩ cell/mm^3, respectively)

which was a significant increase (P<٠.٠١) higher than control subjects, of males and females(٤٦١٠٠٠٠ ±٠.١٥٩;٤٠٠٠ ٠٠٠±٠.١١٣ cell/mm³,respectively)

ii) Hb concentration :-

Results of Hb concentration of both affected males and females groups (١٥.١٠٦ ± ٠.٣٨٤, ١٤.٠٦٦ ± ٠.٣١٨ gm/dL, respectively) were significantly(P<٠.٠١) higher than control males and females (١٣.٣٨٠±٠.٢٤٣; ١٢.١٦٤ ± ٠.٢٤٦ gm/dL, respectively).

iii) PCV :-

Results of PCV in hyperthyroid patients, males and females groups (٠.٤٥٨±٠.٠٢٥,٠.٤٢±٠.٠١١%, respectively) which were significantly (P<٠.٠١)higher than healthy males and females (٠.٤٢١ ±٠.٠٣٢ ; ٠.٤٠ ±٠.٠٢١٪ , respectively).

b) RBCs indices :-

Values of red blood corpuscles indices (MCV, MCH, and MCHC) of both hyperthyroid, males and females are shown in table (٣).

i)MCV :-

Results of MCV of hyperthyroid, males and females (٨٤.٢٣٠ ± ١.٠٨٠, ٨٣.٨٨ ±١.٠٥١ Fl , respectively) recorded non significant decrease in a comparison with control subjects, (٨٧.١٧٠ ± ١.٨٤١; ٨٦.٨٦ ± ١.١٢٢ Fl, respectively).

ii) MCH :-

Results of MCH of hyperthyroid in males and females groups (٣١.١٦٠ ± ٠.٦٠ ; ٢٩.٩٢٠ ± ٠.٨٠٠ pg, respectively) which were non significantly different compared with healthy males and females(٣٠.٣٧٠±٠.٤٩;٣٠.٢١١±٠.٩٠٢ pg, respectively).

iii) MCHC :-

Values of MCHC of hyperthyroid in males and females groups (٣٢.١٨٠ ± ٠.٨٦١,٣٣.٧٧ ± ٠.٩٢٩ gm/dL, respectively) showed non significant different in a comparison with healthy, males and females(٣٣.٥٨٠ ± ٠.٨٠٤;٣٣.١١±٠.٨٨٥ gm/dL respectively).

c) Hb electrophoresis :-

Results of HbA, HbA٢ , and HbF of males and females groups affected with hyperthyroidism are explained in table (٤) .

i) HbA :-

Values of HbA for hyperthyroid in males and females groups (٩٨.٠٨ ± ٠.٢١٦;٩٨.٢١١ ± ٠.٢٢٨ %, respectively) which non significantly different in a comparison with those control male and female, (٩٨.٠٠ ±٠.٢٠٧;٩٨.٢٦٠± ٠.٣٤٨ %, respectively).

ii) HbA٢ :-

Results of HbA٢ of hyperthyroid, males and females were ١.٤٨ ±٠.١١٢; ١.٥٦± ٠.١٧٤٪, respectively,these values were non significantly different in a comparison with control, males and females (١.٥٤ ± ٠.١٦٨; ١.٧٢ ± ٠.١٢٣٪, respectively).

iii) HbF :-

Values of HbF of hyperthyroid groups, males and females(٠. ٧٤٢ ± ٠ . ١١٣, ٠. ٨٨١ ±٠. ١٠٣ % ,respectively) which were non significantly different when compared with those for healthy males and females (٠.٧٥٢±٠.١٢٣; ٠.٧٨٠±٠.٠٧٤٪, respectively) .

d) Total WBCs count and ESR :-

Results of WBCs count and ESR of both hyperthyroid patient groups, males and females are depicted in table(٥).

i)Total WBCs count :-

Results of WBCs count of hyperthyroid, males and females (٥١٦٠±٠.٤٥١ ;٤٨١٠±٠.٢٢٦ cell/mm^{٣}, respectively) were non significantly different when compared with control, males and females (٥٠٩٠±٠.٣٥١ ; ٤٦٨٠±٠.١٨٤ cell/mm^{٣}, respectively).

ii)ESR

Values of ESR of both hyperthyroid males and females (٤.٢٢±٠.٦٦٦; ٦.٠٠±١.٠٧ mm/h, respectively) were non- significantly different in a comparison with those healthy control groups (٤.٠٢±٠.٨٩٤; ٧.٦١٠±١.٠٧٧ mm/h, respectively).

٥-Measurement of body temperature:-

Values of body temperature which are shown in figure (٣)of both hyperthyroid males and females (٣٨.٢± ٠.٣٩٧, ٣٨.٠٦ ±٠.٤١٨°C, respectively) and were significantly (P<٠.٠٥)higher than healthy control, males and females (٣٧.٤±٠.٣٣١; ٣٧.٣± ٠.٢٧٠°C, respectively) .

٦–Biochemical studies

a)Reduced glutathione(GSH) :-

Results of GSH are shown in figure (٤) for both affected males and females (١١.٠٨٦±١.٠٧٨,١٠.١٨٤±٠.٧٧µM, respectively) were significantly (P<٠.٠١)lower than healthy control, males and females (٢٠.٩٣٣ ± ٠.٨٦٢; ١٩.٤٨٣ ± ٠.٦٨٢µM. respectively).

b)Total serum protein, albumin, and globulin:-

Results of total serum protein, albumin ,and globulin of both hyperthyroid groups males and females are shown in table (٦).

i)Total serum protein :-

Values of total serum protein of both hyperthyroid groups, males and females (٤.٩٠±٠.٣٣٥; ٥.٦٢٣±٠.٢٤١g/dL, respectively), these results were significantly (P<٠.٠١) lower than control males and females (٦.٧٥±٠.٣٣٨; ٧.٠١٠±٠.٢٠٤g/dL, respectively).

ii)Serum albumin :-

Results of serum albumin value of both hyperthyroid males and females were (٣.٥٠ ± ٠.١٧١, ٣.٦٨٥ ± ٠.١٥٨ g/dL, respectively). These results pointed with significant decrease (P<٠.٠١) when compared with those healthy control males and females (٤.٤٤ ± ٠.٢٥٧ ; ٤.٤٨٠ ± ٠.١٧٥g/dL, respectively).

iii) serum globulin :-

Values of serum globulin of both hyperthyroid males and females were(١.٧٠٨ ± ٠.١٦٣, ١.٩٨٥ ± ٠.١٣٩ g/dL, respectively). These results were in significant decrease in a comparison with control, males and females (٢.٠٥٠ ±٠.١٦٠; ٢.٢٧٠ ±٠.١٤٥ g/dL , respectively) .

c) Alkaline phosphatase:-

Results of alkaline phosphates which are expressed in figure (٥) hyperthyroid males and females (٣٦.٨٧٠ ± ١.٤٠٧; ٣٤.٥١١ ± ٠.٥٤٢ IU/L, respectively) were significantly (P<٠.٠١) higher than those for control males and females (٢٢.٨٢٤±١.٨٨٥; ١٩.٥٨٥ ±١.٧٩٦ IU/L, respectively) .

d) Calcium and phosphorus:-

Results of calcium and phosphorus in both serum and urine samples of hyperthyroid males and females are shown in table (٧) .

i) Calcium :-

Values of serum calcium in affected males and females were (١٣.٤٨٣ ± ٠.٣٦٠; ١٣.٠٢٠± ٠.٤١٦ mg/dL, respectively) and were significantly (P<٠.٠١) higher than control males and females (٩.٨٠٢ ±٠.٤٠٠ ; ١٠.٠٧ ± ٠.٥١٠ mg/dL, respectively). Values of urine calcium were (٧٨.٣٧٥±٨.٢٥١;٨٥.٥٠٠ ± ١٠.٧٠٩ mg /dL, respectively)were significantly (P<٠.٠١) higher than healthy males and females (٢٠.٧٦١±٩.٣٨; ٣٠.١٢١ ±١١.٨٠٤ mg/dL, respectively) .

ii) Phosphorus:-

Results of serum phosphorus in affected, males and females groups were ٤.٩٥٠ ± ٠.٢٦٨; ٤.٧٩١ ± ٠.٢٨٥ mg/dL, respectively. These results showed significant increase (P<٠.٠٥) in a comparison with healthy subjects (٣.٩٦٠ ±٠.٢٤٠; ٤.٣٨٣ ±٠.١٩٨ mg/dL, respectively). Values of urine phosphorus were (١٣٥.٠٠٠ ± ١٥.٧٩٠; ١٤٢.١٣٧ ± ١٣.٠٩١ mg/dL, respectively) were significantly (P<٠.٠٥) higher than control subjects of both sex (١١٢.١٣ ±١٣.٣٩٤; ١١٠.٧٥ ±١٢.٢٣٣ mg/dL, respectively).

e) Sodium and potassium:-

Results of sodium and potassium in serum and urine samples of hyperthyroid, males and females were illustrated in table (٨) .

i) Sodium:-

Values of serum sodium of affected, males and females were ١٥٠.٥٠٠±٣.٥٥; ١٥١.٧٠٠±١.٩٨٩ mmol/L, respectively .These results were significantly increase (P<٠.٠١)when compared with healthy subjects of both sex (١٣٧.٤٠٠±١.٥٨; ١٣٩.٩٠٠±١.٨٧٦mmol/L, respectively). Values of urine sodium were (٦٥.٣٠٠±٤.٨٣٩; ٥٨.٥١٠٠± ٣.٦٨٥ mmol/L, respectively) showed significant increase (P<٠.٠١) in a comparison with control males and females (٣٣.٥٠١±٤.٣٤٩; ٢٧.٢٧٢±٢.٩٦٦ mmol/L, respectively.

ii)Potassium :-

Results of serum potassium of hyperthyroid groups ,males and females were (٣.٦٧٠±٠.١٣٤; ٣.٨٧٠±٠.٤١٣ mmol/L, respectively) which showed non significant decrease in comparison with control males and females (٤.١٤±٠.١٦٣; ٤.١٩٠±٠.١٧٩ mmol/L, respectively). Moreover, values of urine potassium of affected males and females were (٥.٨١٠ ± ٠.٢٥٦; ٥.٦٣١± ٠.٤٢٥ mmol/L, respectively). These results were non significant decrease when compared with healthy control males and females (٦.٤٧٠±٠.٣٩٧; ٦.٢٣١± ٠.٤٥٥ mmol/L, respectively).

f) Cholesterol and triglycerides :-

Values of serum cholesterol and triglycerides of hyperthyroid males and females are illustrated in table (٩) .

i) Cholesterol :-

Results of cholesterol of affected males and females were ١٥٥.٨٧١±٨.٧٣٠; ١٤٨.٢٣±٥.١٠٣ mg/dL, respectively. These results pointed out significant decrease (P<٠.٠١) in a comparison with control males and females (١٨٣.٩٨± ٣.٦٠٠; ١٨١.٢٩٤±٣.٤٣٧ mg/dL, respectively).

ii) Triglycerides:-

Values of triglycerides of hyperthyroid males and females (٨٦.٢٠٠±٦.٧١٩; ٨٠.٠٠٠±٧.٧٦٦ mg/dL respectively) were significantly (P<٠.٠١) lower than control males and females (١١٢.٥٠٠±٥.٣٢٥; ١٠١.٦٠٠±٦.١٧٢ mg/dL, respectively).

g) Serum zinc :-

Results of serum zinc of hyperthyroid groups, males and females are shown in figure(٦). These results were ١.٢٤١±٠.٠٧٩; ١.٣٠٠±٠.٠٩٦ mg/L, respectively were significantly (P<٠.٠٥) higher than healthy males and females (٠.٩٠٨±٠.٠٤٩; ٠.٩١٦±٠.٠٧٤ mg/L, respectively).

h) Serum copper :-

Values of serum copper of affected males and females are shown in figure(٧) . These values were (٠.٧٥١±٠.١٠٢, ٠.٨٩١±٠.٠٧٨ mg/L, respectively) and were significantly (P<٠.٠١) lower than healthy males and females (١.١٩٤±٠.١٣٣; ١.٢٣٣±٠.٠٦٧ mg/L, respectively).

Table(١):-The means of thyroid stimulating hormone (TSH), triiodothyronine (T٣), and tetraiodothyronin concentrations in hyperthyroidism of both sex, males and females.

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
TSH µU/ml	٢.٢٥٣± ٠.٢٥٤	** ٠.٣٢٦ ±٠.٢٥٤	٢.٥١٤± ٠.٢٥٣	** ٠.٣٨٦ ±٠.٢٤٥
T٣ ng/dL	١٣٨±٦.٩٨٣	** ٢٤٦±٩.٥٤	١٣٢± ٧.٦٣٤	** ٢٤٩±١٢.٢٩٧
T٤µg/dL	٧.٨٠٦±٠.١٥٠	** ١٣.٥١±٠.٣٢١	٨.٢٢±٠.١٦٦	** ١٣.٧٢±٠.٣٣٧

-Values are means ± SE .

- Means with two asterisks are significantly different at P<٠.٠١ .

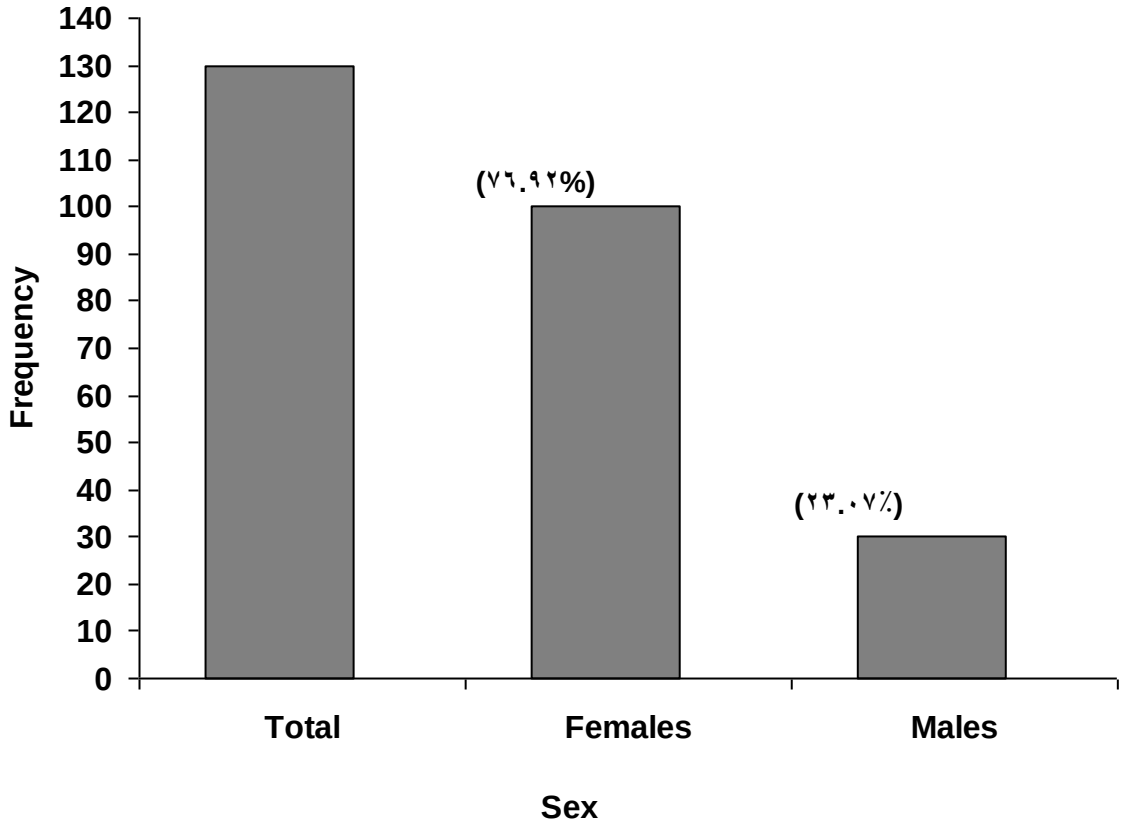


Figure (١):- The relationship between sex and the frequency of hyperthyroidism.

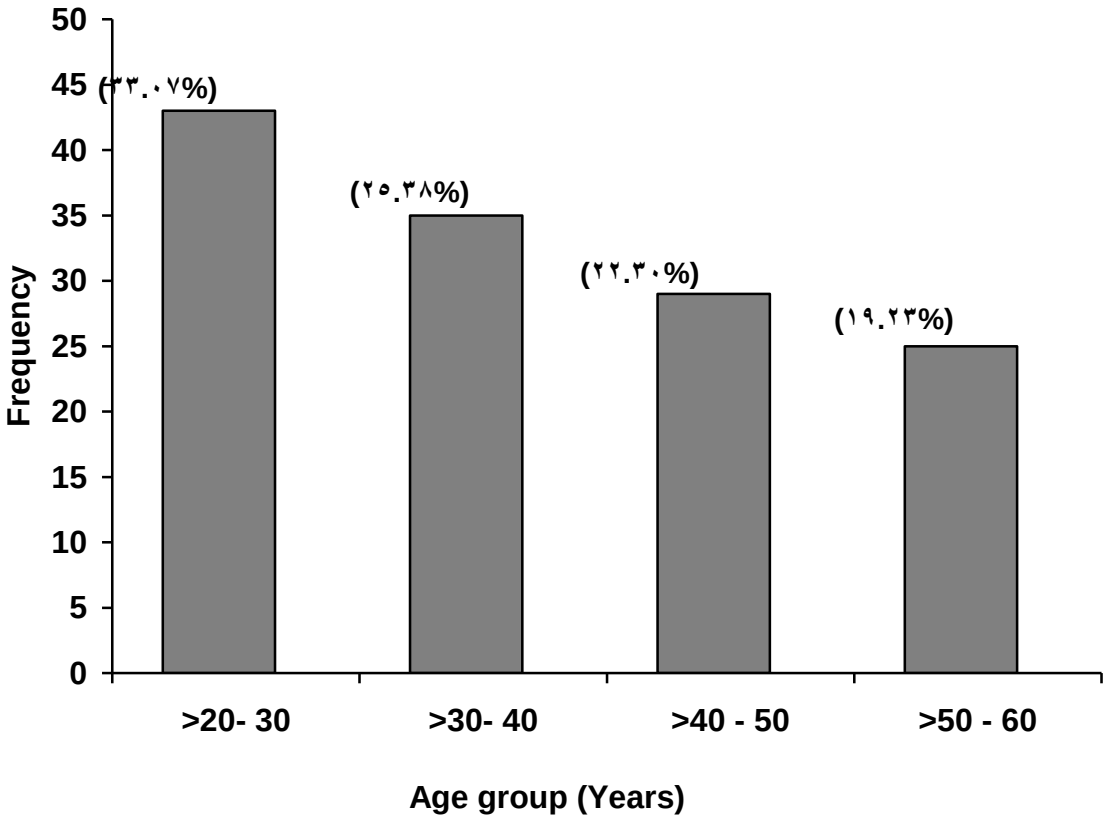


Figure (٢) :- The relationship between age and the frequency of hyperthyroidism .

Table (٢):- The means of red blood corpuscles count (RBCs), hemoglobin concentration (Hb), and packed cell volume (PCV) in hyperthyroid males and females .

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
RBCs million/mm³	٤.٦١٠±٠.١٥٩	** ٥.٥٢٠±٠.١٦٤	٤.٠٠٠± ٠.١١٣	** ٥.١٠٠ ± ٠.١٦٩
Hb gm/dL	١٣.٣٨٠±٠.٢٤٣	** ١٥.١٠٦±٠.٣٨٤	١٢.١٦٤± ٠.٢٤٦	** ١٤.٠٦٦±٠.٣١٨

PCV %	٠.٤٢١±٠.٠٣٢	* ٠.٤٥٨±٠.٠٢٥	٠.٤٠±٠.٠٢١	* ٠.٤٢±٠.٠١١

- Values are means ± SE.

- Means with two asterisk are significantly different at P<٠.٠١.

Table(٣):- The means of red blood corpuscles indices (mean corpuscular volume–MCV, mean corpuscular hemoglobin –MCH, and mean corpuscular hemoglobin concentration–MCHC in hyperth-yroidism males and females.

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
MCV fL	٨٧.١٧٠±١.٨٤١	٨٤.٢٣٠±١.٠٨٠	٨٦.٨٦± ١.١٢٢	٨٣.٨٨ ±١.٠٥١
MCH pg	٣٠.٣٧٠±٠.٤٩	٣١.١٦٠±٠.٦٠	٣٠.٢١١± ٠.٩٠٢	٢٩.٩٢٠±٠.٨٠٠
MCHC gm/dL	٣٣.٥٨٠±٠.٨٠٤	٣٢.١٨٠±٠.٨٦١	٣٣.١١±٠.٨٨٥	٣٣.٧٧±٠.٩٢٩

-Values are means ± SE.

Table (٤):- The means of values of adult hemoglobin (HbA), adult hemoglobin٢(HbA٢),and fetal hemoglobin (HbF)in hyperthyroid males and females.

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
HbA %	٩٨.٠٠±٠.٢٠٧	٩٨.٠٨± ٠.٢١٦	٩٨.٢٦٠±٠.٣٤٨	٩٨.٢١١±٠.٢٢٨
HbA٢ %	١.٥٤±٠.١٦٨	١.٤٨± ٠.١١٢	١.٧٢±٠.١٢٣	١.٥٦±٠.١٧٤
HbF %	٠.٧٥٢±٠.١٢٣	٠.٧٤٢±٠.١١٣	٠.٧٨٠±٠.٠٧٤	٠.٨٨١±٠.١٠٣

-Values are means ± SE .

Table (٥):- The means of erythrocytes sedimentation rate (ESR)and total white blood cells count (WBCs) in hyperthyroid males and females .

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
ESR mm/h	٤.٠±٠.٨٩٤	٤.٢٢± ٠.٦٦٦	٧.٦١٠±١.٠٧٧	٦.٠٠±١.٠٧
WBCs cell/mm³	٥٠٩٠±٠.٣٥١	٥١٦٠± ٠.٤٥١	٤٦٨٠±٠.١٨٤	٤٨١٠±٠.٢٢٦

-Values are means ± SE .

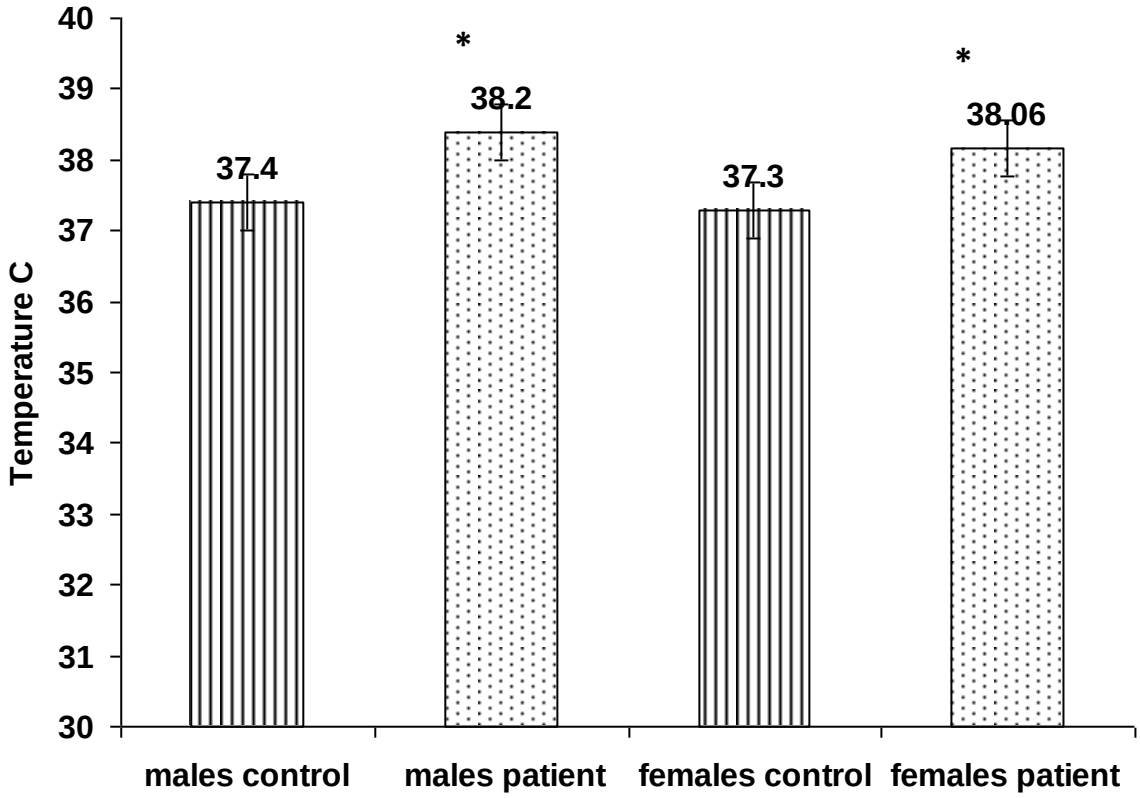


Figure (٣): The means of body temperature values in males and females affected with hyperthyroidism.

- Values are means ±SE.
- Means with one asterisk are significantly different at (P<٠.٠٥).

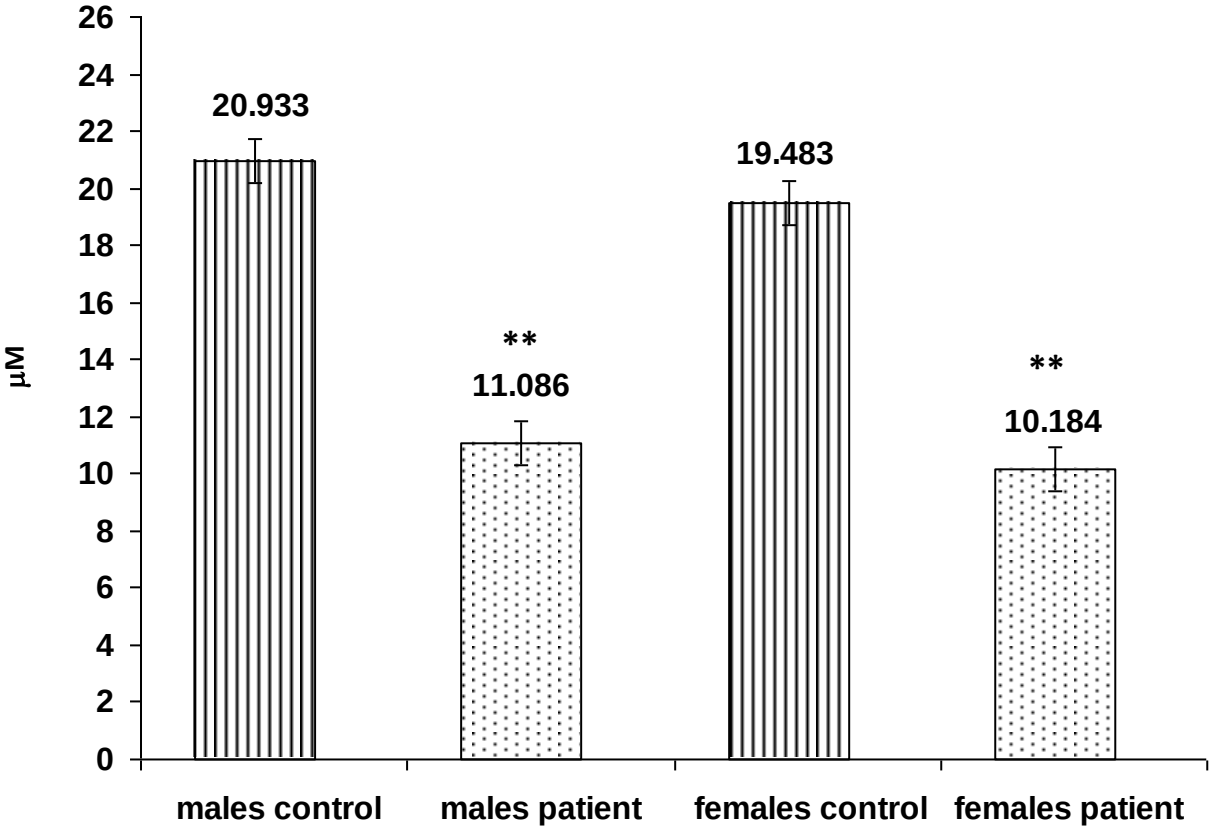


Figure (٤): The means of reduced glutathione (GSH) values in males and females affected with hyperthyroidism.

-Values are means ±SE.

- Means two asterisk are significantly (P<٠.٠١).

Table(٦):-The means of total serum protein, albumin, and globulin values in hyperthyroid males and females .

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
Total serum protein g/dL	٦.٧٥±٠.٣٣٨	** ٤.٩٠± ٠.٣٣٥	٧.٠١٠±٠.٢٠٤	** ٥.٦٢٣±٠.٢٤١
Albumin g/dL	٤.٤٤٠±٠.٢٥٧	** ٣.٥٠٠± ٠.١٧٠	٤.٤٨٠±٠.١٧٥	** ٣.٦٨٥±٠.١٥٨
Globulin g/dL	٢.٣١٠±٠.١٦٠	١.٤٠٨± ٠.١٦٣	٢.٥٣٠± ٠.١٤٥	١.٩٣٨± ٠.١٣٩

- Values are means ± SE .

- Means with the two asterisks are significantly different at p<٠.٠١

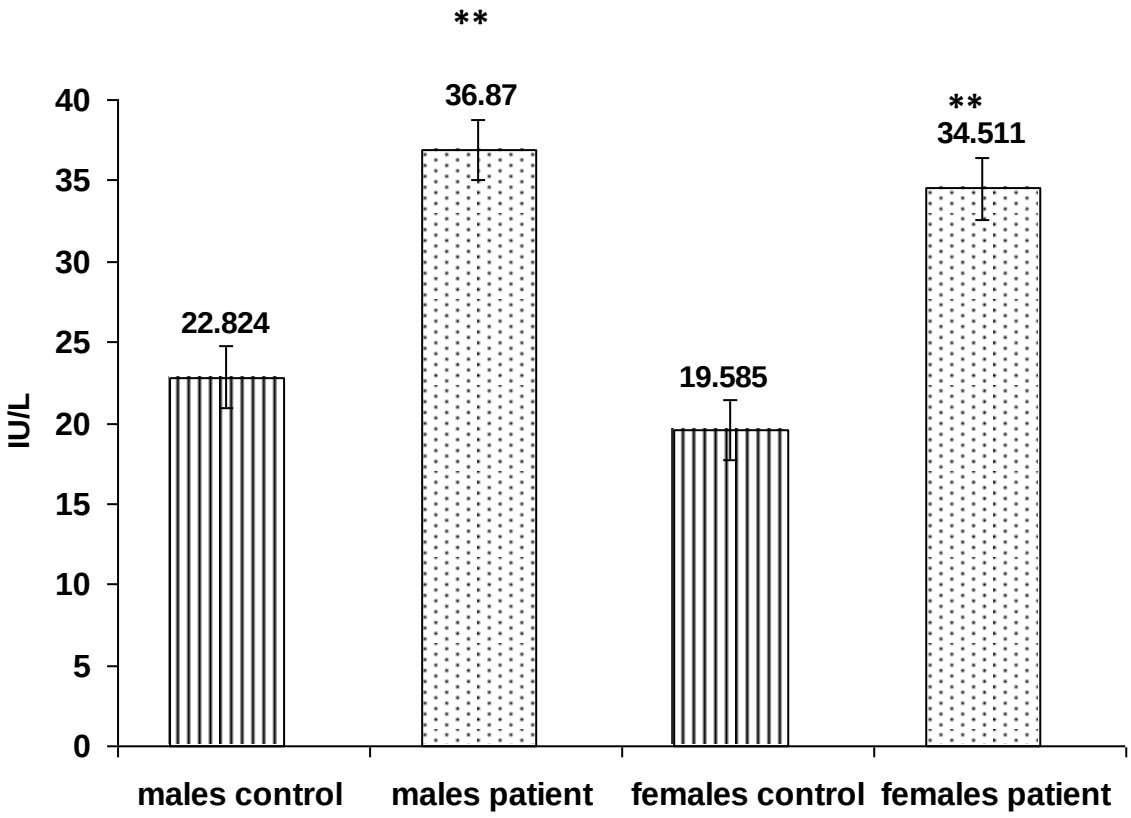


Figure (٥): The means of alkaline phosphatase values in males and females affected with hyperthyroidism.

- Values are means ±SE.

- Values with two asterisks are significantly different at (P<٠.٠١).

Table(٧):- The means of calcium and phosphorus levels in serum and urine samples of hyperthyroid males and females.

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
Serum calcium mg/dL	٩.٨٠٢±٠.٤٠٠	** ١٣.٤٨٣± ٠.٣٦٠	١٠.٠٧±٠.٥١٠	** ١٣.٠٢٠±٠.٤١٦
Urine calcium mg/dL	٢٠.٧٦١±٩.٣٨	** ٧٨.٣٧٥± ٨.٢٥١	٣٠.١٢١±١١.٨٠٤	** ٨٥.٥٠٠±١٠٨.٧٠٩
Serum phosphorus mg/dL	٣.٩٦٠±٠.٢٤٠	* ٤.٩٥٠± ٠.٢٦٨	٤.٣٨٣± ٠.١٩٨	* ٤.٧٩١± ٠.٢٨٥
Urine phosphorus mg/dL	١١٢.١٣±١٣.٣٩٤	* ١٣٥.٠٠٠±١٥.٧٩٠	١١٠.٧٥±١٢.٢٣٣	* ١٤٢.١٣٧±١٣.٠٩١

- Values are means ± SE

- Means with two asterisks are significantly different at P<٠.٠١.
- Means with one asterisks are significantly different at P<٠.٠٥ .

Table (٨):-The means of sodium and potassium levels in both serum and urine samples of hyperthyroid patients, males and females .

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
Serum sodium mmol/L	١٣٧.٤٠٠±١.٥٨	** ١٥٠.٥٠٠±٣.٥٥	١٣٩.٩٠٠±١.٨٧٦	** ١٥١.٧٠٠±١.٩٨٩
Urine sodium mmol/L	٣٣.٥٠١±٤.٣٤٩	** ٦٥.٣٠٠±٤.٨٣٩	٢٧.٢٧٢±٢.٩٦٦	** ٥٨.٥٠٠±٣.٦٨٥
Serum potassium mmo١/L	٤.١٤±٠.١٦٣	٣.٦٧٠± ٠.١٣٤	٤.١٩٠± ٠.١٧٩	٣.٨٧٠± ٠.٤١٣
Urine potassium mmo١/L	٦.٤٧٠± ٠.٣٩٧	٥.٨١٠±٠.٢٥٦	٦.٢٣١ ± ٠.٤٥٥	٥.٦٣١± ٠.٤٢٥

- Values are means ± SE .

- Values with two asterisks are significantly different at P<٠.٠١ .

Table (٩):- The means of cholesterol and triglycerides levels in hyperthyroid males and females .

Parameter	Males		Females	
	Control groups	Patient groups	Control groups	Patient groups
Cholesterol mg/dL	١٨٣.٩٨±٣.٦٠٠	** ١٥٥.٨٧±١٠.٧٣٠	١٨١.٢٩٤±٣.٤٣٧	** ١٤٨.٢٣±٤.١٠٣
Triglycerides mg/dL	١١٢.٥٠٠±٥.٣٢٥	** ٨٦.٢٠٠± ٦.٧١٩	١٠١.٦٠٠±٦.١٧٢	** ٨٠.٠٠±٧.٧٦٦

- Values are means ± SE .

- Values with two asterisks are significantly different at P<٠.٠١ .

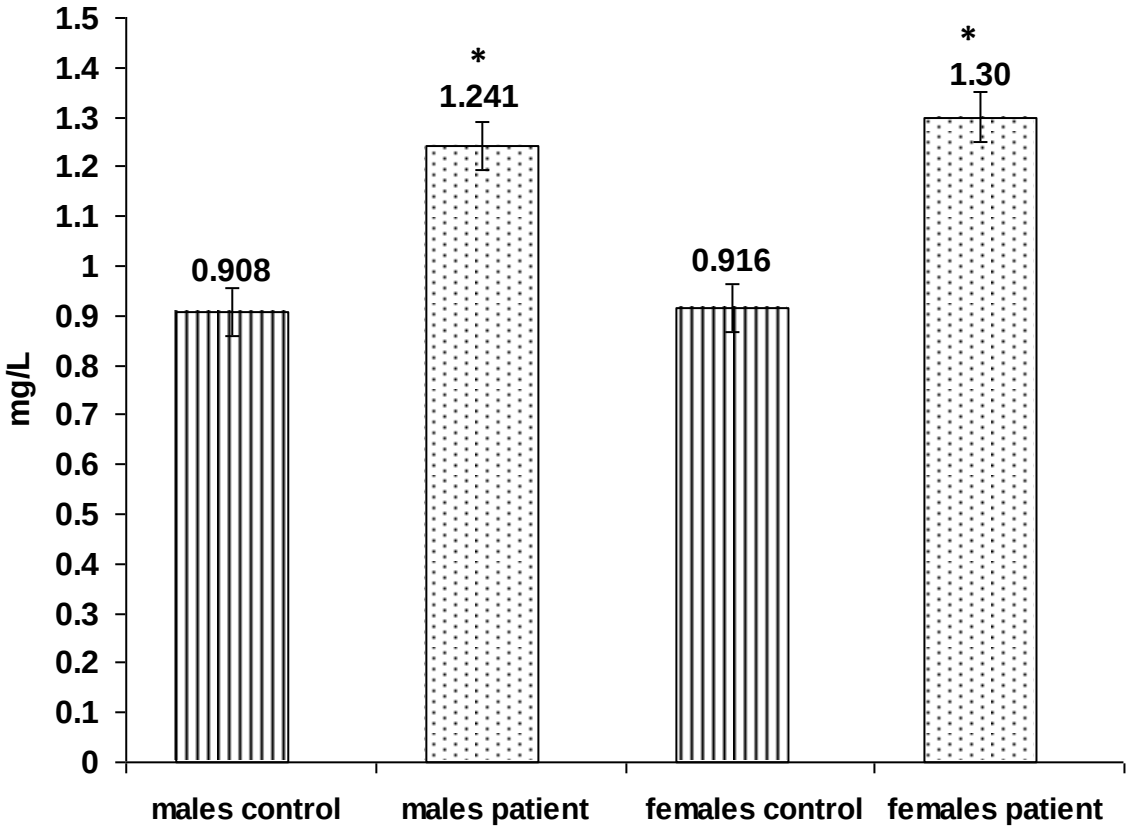


Figure (٦):- The means of serum zinc levels in males and females affected with hyperthyroidism.

- Values are means ±SE.
- Means with one asterisk are significantly different at (P<٠.٠٥).

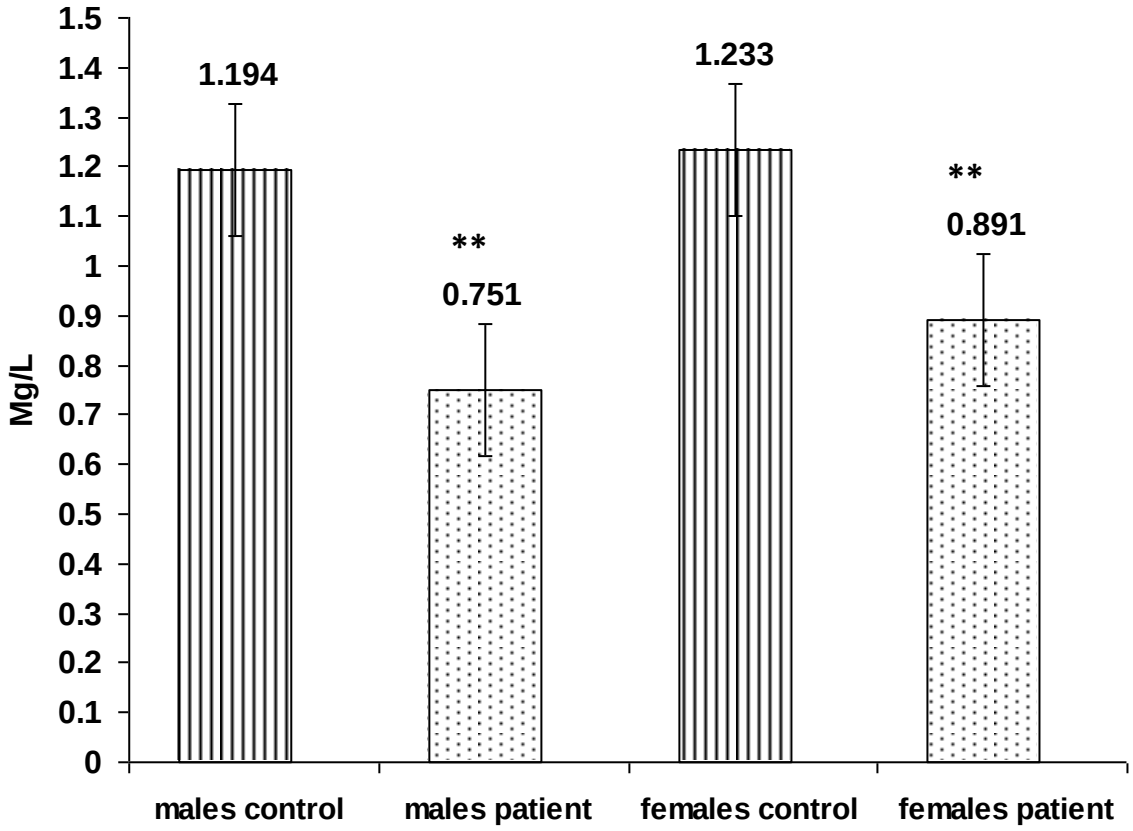


Figure (٧):- The means of copper values in males and females affected with hyperthyroidism.

- Values are means ±SE
- Values with two asterisks are significantly different at (P<٠.٠١).

Discussion

The present study of this thesis concentrated on hyperthyroidism to analysis some of the hematological and biochemical parameters in both males and females which are associated with such disorder.

a) Hormonal Study

The patients group for this study showed an elevated level of T٣ and T٤, while TSH level was reduced when compared with normal control subjects table (١). Looking back to the fact that the physiological mechanism by which such hormones are produced and the relationship between them we can find that thyroxine production begins on the binding of the TSH to their specific receptors on the surface membrane of follicular cells. Thus, the final step is the production of T٣and T٤. In data obtained from this work involved significant increase (p<٠.٠l) levels of T٣ and T٤ associated with significant decrease (P<٠.٠١)of TSH levels of both males and females in comparison with control subjects. From such interesting results, attention should be directed for the fact that excess production of T٣ and T٤ with low level of TSH. There are several explanations and reasons as we believe for such phenomenon. The first acceptable explanation for such results is the presence of anti-

thyroid auto- antibodies in the circulation. In this, autoimmune condition, there is production antibodies against normal thyroid antigens. The source of these antibodies is immune competent plasma cells. The antibodies bind with TSH receptors to initiate and increase T٣ and T٤ synthesis and production regardless of decrease level of TSH by negative feed back mechanism which exerted by T٣ and T٤ on pituitary and hypothalamic axis (Hollowell *et al*.,٢٠٠٢). However, the antibodies that replace TSH and compete to bind with TSH receptors, activate several biochemical mechanisms which are stimulated by TSH such as activate iodide transport mechanism, thyroperoxidase enzyme, thyroglobulin synthesis, reuptake of stored thyroglobulin, and then secretion of T٣ and T٤ (Ali *et al*., ٢٠٠٢). The second reason which may be attributed to increase over production of T٣ and T٤,is solitary or multiple thyroid nodules, which resulted because of hyperplasia for some follicular cells and to increase iodide uptake more than surrounding tissues. Therefore elevated of T٣ and T٤ production, while TSH secretion is depressed and the adjacent tissues in thyroid become inactive (Kosugi and Shinji, ٢٠٠٢). Third reason, thyroiditis as an inflammation of thyroid gland with infectious microorganism, which in turn lead to liberate stored T٣ and T٤ and suppressed of TSH (Burtis and Ashood,١٩٩٩). Finally, attention must be directed at the iodine effects on thyroid gland physiology. There is evidence to suggest that elevated levels of iodine in the diet in endemic area of iodine deficiency are associated with autoimmune thyroid disease in susceptible individuals (Markou *et al.,* ٢٠٠٣). It had also been found that obligatory model of iodine prophylaxis in order to correct the existing status of mild and moderate iodine deficiency lead to hyperthyroidism (Lewinski *et al.,* ٢٠٠٣).

Moreover, study of Lauberg *et al.,*(٢٠٠٠) confirmed that a mild to moderate iodine deficiency for health of the population is an extraordinarily high occurrence of hyperthyroidism in elderly subjects, especially women. Previous studies of Leger *et al.,*(١٩٨٤) and Foley(١٩٩٢) who reported that an increase in iodination of thyroglobulin enhances its immunogenicity. As well as, a high concentration of iodide after oxidation to iodine causes epithelial necrosis and lipid peroxidation from excessive amounts of free radicals which initiate inflammatory and immune disease.

b) Sex and Age

Hyperthyroidism and its relationship with sex in this study is shown in figure (١).The results showed that the number of affected females was ١٠٠ (٧٦.٩٢ %) out of ١٣٠ patients, while the number of affected males was ٣٠ (٢٣.٠٧%). From the above results, it was appeared clearly that females were more susceptible to such disease than males and that agrees with several studies (Bjoro *et al.*, ٢٠٠٠; Ali *et al.,* ٢٠٠٢; Ahmed *et al*., ٢٠٠٤). The reasons which may assist or exacerbate the female to acquire this disease could be due to sex hormones imbalance such as estrogen hormone which are normally elevated in females during puberty and pregnancy. This suggestion was supported by Woeber and Ingher (١٩٧٤)who suggested that high levels of estrogens in euthyroid females increase the total T٣ and T٤ concentration to about ١.٥–٢ time above their normal levels. Lahita *et al*.,(١٩٨٢)who reported that the capacity of sex hormones to influence the functions of immune system and the development of auto immune disease has been firmly established, in general, estrogen hormones were found to stimulate immunity,

while testosterone has the opposite effect. Also Smyth,(١٩٩٤); and Ganong, (١٩٩٧)reported that elevation of total serum T٤ and T٣ without alteration in thyroid status are a consequence of estrogen induced increase in thyroxin binding globulin. In addition, elevation of human chorionic gonadotropin (HCG) during pregnancy which mimic TSH in its structure act as the other cause to render the female more susceptible to this disease (Lazarus and Kokandi, ٢٠٠٠). On the other hand, Gregerman and Davis (١٩٧٨) that reported the effect of androgen hormone has a reversible effect than estrogen, but the male's hormone (androgens) play important role in exhibiting hyperthyroidism but the frequency in females remained higher than that observed in males. Also study of Ueshiba *et al.,* (١٩٩٧) explained that there is a positive relationship between levels of androgens and thyroid hormones this could be due to the fact that the chance of hormonal changes in females is more than males. For example there will be abnormal changes in females at different stages of life such as changes in hormonal balance in maturity and during pregnancy or over change at the postpartum period. Moreover, on the basis of the above mentioned factors, the present study suggests that there are genetically controlled factors such as the exhibition of different gene that are present on X chromosome in females may have direct relation with such disease, such factors may not be found on Y chromosome in males that is why such differences in genes may give the proper explanation of having females more susceptible to these disease than males (AL-Jubori, ١٩٩٨).

The present study also involved the relationship between age and hyperthyroidism which is presented in figure(٢).It appears that the most susceptible age group to this disorder was that ranging from ≥٢٠–٣٠ years and this result agrees with other studies

(Masinkiewicz and Burrow, ١٩٩٩; Bonar *et al*., ٢٠٠٠). Such findings are not surprising because they represent a maturity period of both males and females, pregnancy period, and post partum period of females, this will give a simple explanation for the susceptible to this disease which was discussed in hormonal changes terms that mentioned in relation to sex. Previous studies of Bonar *et al*., (٢٠٠٠)and Guan *et al*.,(٢٠٠١) pointed out that the peak incidence of such disease is in the third to fourth decad of the age. Where as the study of Allahabadia *et al*., (٢٠٠٠) recorded that five decad is more affected. With age, thyroid gland undergoes moderate atrophy and develops non–specific histopathological abnormalities such as fibrosis, increasing numbers of colloid nodules,and some lymphocytic infiltrations (Yassin *et al*.,٢٠٠٠). Mariotti *et al*. (١٩٩٥)reported that with increase age, there is increase nodularity and spread differentiated tumor in thyroid gland, also with age there is a decrease in the metabolic clearance rate of T_4 and a decrease use of T_4 correlates within age related decline in body mass, suggesting that the metabolically active protein rich tissues such as muscles, skin, bones, and viscera decrease, which may lead to reduce use and catabolism of thyroid hormones. Furthermore, Noth and Mazzaferri (١٩٨٥) showed that both hormone levels and target organ responsivity are altered in aging, therefore, aging decrease the mild metabolism of T_4, including its conversion to T_3.

c) Hematological Studies:-

Concerning red blood corpuscles count in hyperthyroidism which are illustrated in table(٢) they showed significant increase (P<٠.٠١) in hyperthyroid males and females when compared with control groups. Oxygen is of vital importance for the metabolism and function of all cells in the human body. Thyroid hormones have been found to augmented oxygen capacity of blood by increasing the production of erythropoietin. Erythropoietin levels are induced by ٥٠ to ١٠٠ fold *in vitro* by physiologically relevant level of hypoxia (Fandrey and Bunn, ١٩٩٣;Justo–Firvida *et al*.,١٩٩٥). Thyroid hormones are required in nearly all tissues, with major effects on oxygen consumption and metabolic rate. Adaptation to this increased metabolic demand is partly achieved by potent effects of thyroid hormones on erythropoiesis and thus blood oxygen capacity increased (Ma *et al*.,٢٠٠٤). At the same time, thyroid hormones directly increase the proliferation of erythroid progenitor (Dainiak *et al*., ١٩٧٨) and thyroid hormones receptors were identified on nucleated erythroid cells isolated from hypoxic animals (Boussions *et al*., ١٩٨٢; Brenner *et al*., ١٩٩٤). A part from the direct effect on erythroid precursors, thyroid hormones, directly enhanced hypoxia inducible erythropoietin formation (Fandrey *et al*.,١٩٩٤). In addition, thyroid hormones directly stimulate erythroid progenitor cells and this effect may be mediated via-beta ٢ receptors**,** since, the increase in erythropoiesis in hyperthyroidism is reflected in the bone marrow which undergo erythroid hyperplasia and these effects are markedly increased in such disease (Peterson *et al*.,١٩٨٣; Ford and Carter, ١٩٨٨). Data of the present study may be attributed to high level of

thyroid hormones which in turn stimulate bone marrow and lead to increase number of red blood corpuscles and this suggestion is agree with other previous studies (Kubota *et al*.,١٩٩٣; Demiroglu, ١٩٩٥). On the other hand, these results did not agree with other studies which reported decrease number of RBCs in such disorder (Franzcse *et al*., ١٩٩٦; Jyo- Oshiro *et al*., ١٩٩٧)

Results of hemoglobin concentration which are presented in table(٢)showed a significant increase(P<٠.٠١)in hyperthyroid patients, males and females in comparison with control group. Over production of thyroid hormones maintains a higher level of hypoxia, and stimulates respiratory center. Also, it increases cellular demands for oxygen because of hyper metabolic rate. Hemoglobin is a major oxygen transporter in blood to meet body requirements of oxygen (Frenandez *et al*., ١٩٩١). As explained in RBCs count, thyroid hormones stimulate erythropoietin production which in turn stimulates erythropoiesis in bone marrow. In addition, thyroid hormone itself stimulate erythrocyte precursors by beta–adrenergic receptors to enhance RBCs production (Ma *et al*., ٢٠٠٤). One of thyroid hormones actions is increased synthesis of multiple enzymes which are responsible for manufacturing of several proteins. Hemoglobin is considered as an intracellular protein, therefore, increasing erythrocytes production in hyperthyroidism is essentially associated with an increase in hemoglobin synthesis (Samules, ١٩٨٨; Nehal and Baquer, ١٩٨٩). These results may be attributed to direct effects of thyroid hormones excess on bone marrow to increase hemoglobin synthesis within red blood corpuscles (Kubota *et al*.,١٩٩٣; Brenner *et al*.,١٩٩٤).On the other hand, the present results do not agree with other studies which reported a decrease of

hemoglobin in hyperthyroidism (Justo–Firvida *et al*., ١٩٩٥; Lawrence *et al*., ٢٠٠٠).

Results of packed cell volume in table(٢)recorded a significant increase(P<٠.٠٥)in subjects with hyperthyroidism of both sexes (males and females) when compared with control subjects. Data of the present study agree with previous studies of Ford and Carter, (١٩٨٨), Kubota *et al*.,(١٩٩٣) but do not agree with studies (Franzcse *et al*.,١٩٩٦; Lawrence *et al*., ٢٠٠٠). As mentioned previously that, PCV test represents the ratio between cellular part and plasma volume. It is not surprising, that an increase in red blood cells resulted in an increase of packed cell volume. Increase in RBCs count because of thyroid hormones excess is the main reason in elevation of PCV in hyperthyroid patients (Motomura and Brent, ١٩٩٨).

The present study also involved the effects of hyperthyroidism on red blood cell indices. Results of mean corpuscular volume(MCV)which are illustrated in table(٣) showed non significant decrease in hyperthyroid males and females in comparison with control group. For explanation of these results, attention must be based on the facts that thyroid hormones excess enhance erythropoiesis, and red blood cells have been found to spend about four to five days in bone marrow to become completely mature (Hoffbrand *et al*., ١٩٩٩). The increase stimulation and enhancement of erythropoiesis may lead to decrease required time to complete maturation of red blood cells and production of immature erythrocytes to enter blood circulation. At the same time, a hypermetabolic rate which resulted from of thyroid hormones excess may also lead to exhaust major nutrient biomolecules which represent essential factors to ensure adequate maturation of red blood cells (Demiroglu, ١٩٩٥). Thus, erythrocytes enter into circulation in a high number with decrease their volume(Ford and

Carter, ١٩٨٨; Kubota *et al.*,١٩٩٣). Concerning the results of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) which are elucidated in table(٣)reported non significant difference in both affected group males and females when compared with those of control subjects. These results may be attributed to that increase of RBCs count, PCV, and Hb concentration which in turn render values of MCH and MCHC with normal values. These results agree with other studies such as Brenner (١٩٩٤)and Ma *et al*. (٢٠٠٤). Concerning the relationship between hyperthyroidism and hemoglobin electrophoresis which are shown in table(٤). It appears that, results of HbA, HbA٢ and HbF reported non significantly differente in patients with hyperthyroidism of both males and females when compared with those of control subjects. Results of the present study do not agree with previous studies (Kendall and Bastomsky, ١٩٨١; Saito *et al*., ١٩٨٢; Krishnamoorthy *et al*., ١٩٨٢; Ford and Carter, ١٩٨٨). These studies indicated that there was slight elevation in HbA٢ and HbF level in hyperthyroidism, and these studies based on the fact that thyroid hormones modulate globin chains synthesis by affecting genes of these chains. In addition, these results were obtained at advanced stages of hyperthyroidism in which the effects of excess thyroid hormones on hemoglobin types become more prominent. In contrast, the present study was obtained at newly diagnosed hyperthyroid patients, thus, suggested that there was not enough time for excess thyroid hormones to exert its effects on hemoglobin types (Saito *et al.,* ١٩٨٢).

Results of erythrocytes sedimentation rate in table(٥) showed non significant difference in hyperthyroid patients males and females when compared with control subjects. Present results agree with previous studies (Skare and Frey, ١٩٨٠; Marc *et al*., ١٩٩٩) which

indicated that ESR level remains within normal values in thyrotoxic patients. Also a study by Parmar and Sturge (٢٠٠٣) indicated that increase in RBCs count in hyperthyroid patients reduces ESR level, at the same time, there was not any inflammatory complication within the body.

Determination of total WBCs count in hyperthyroid patients of both sexes which are shown in table(٥)pointed out non significant differences when compared with control groups. Previous studies had recorded different results. Such as Hrycek (١٩٩٥)who reported that in patients with hyperthyroidism, the number of leukocytes especially neutrophils was decreased. Tsidale and Kemp(١٩٩٤)who recorded increased granulocytes in hyperthyroid patients. Furthermore previous study by Ford and Carter(١٩٨٨)indicated that decrease neutrophils and increased lymphocytes, while monocytes and eosinophils ratios remained within normal values, and they attributed these findings that the pathogenetic immunological events in autoimmune disease take place within thyroid gland. In the present study, it appears that the effects of thyroid hormones on bone marrow are restricted on erythropoiesis process to increase RBCs which in turn will increase oxygen availability for tissue cells (Demiroglu,١٩٩٥).

d) Body Temperature:-

For explanation metabolic and thermogenic effects of excess thyroid hormones, the present study measured the body temperature as a marker of these effects. Results which are shown in figure(٣) shown a significant increase of body temperature ($p<٠.٠٥$)in hyperthyroid groups, (males and females) in comparison with normal groups. Elevation in body temperature in hyperthyroidism belongs to multiple biochemical reactions which were described by several studies. De- Meis *et al*.,(٢٠٠٢) mentioned that in hyperthyroidism an increase in heat production by $Ca^{+٢}$– ATPase which increases four fold in sarcoplasmic reticulum of white muscles and fourteen fold in sarcoplasmic reticulum of red muscles which in turn lead to elevate amount of heat production during ATP hydrolysis. Thyroid status is crucial in energy homeostasis because, thyroid hormones correlated with cellular and mitochondrial oxygen consumption rate. It has also been found that mitochondrial content of energy substances and enzymes such as cytochrome oxidase, ATP synthase, phosphate nucleotides increased when associated with the decrease of ATP/O ratio in hyperthyroidism (Nogueira *et al*. ٢٠٠٢). As a result of increased resting metabolic rate in hyperthyroidism lead to increased chemical energy because of increase oxygen consumption in all body cells especially liver cells and increase hepatic metabolism (Sestoft, ١٩٨٠). Moreover, previous study of Harper and Brand (١٩٩٤)indicated that excess thyroid hormones exert an effects on proton leak across mitochondrial membrane and phosphorylating system since, excess thyroid hormones enhances proton permeability across lipid bilayer of mitochondrial membrane, and also changes in the lipid bilayer due to changes in fatty acids composition of mitochondrial phospholipids, at the same time,

increased the capacity for the transport of cystolic ADP and phosphate because of increased area of mitochondrial membrane(Brand *et al*., ١٩٩٢; Issa *et al*., ١٩٩٢).It must be explained here that, sodium and potassium ATPase which are stimulated by thyroid hormones excess, considered the additional source to increase energy cell expenditure (Monti *et al*., ١٩٩٧). Also, these effects are associated with increased stimulation of aerobic and anaerobic processes especially in muscles lead to increase energy production(Sigurdson and Himm–hagen,١٩٨٨;Valdemararrsson *et al*., ١٩٩٢; Chenzion *et al*., ١٩٩٥). Thyrotoxicosis had been found to affect brown adipose tissues through reduction of the expression of beta ٣ adrenergic receptors, and increase the obligatory of thermogenesis associated with reducing hypothalamic stimulation of brown fat, providing additional mechanism to brown fat thermogenesis in hyperthyroidism (Abelenda and Puerta, ١٩٩٢; Silva, ١٩٩٥).In fact, uncoupling proteins (UCP١)were initially described in brown adipose tissue as a molecules that allowed protons to return to the mitochondrial matrix, by passing ATP synthase therefore, uncoupling oxidative phosphorylation occurs (Klingenberg *et al*., ١٩٩٥). Over production of thyroid hormones stimulates UCP١, thus fatty acids can be oxidized with generation of heat but not ATP. Uncoupling proteins such as, UCP٢ and UCP٣ had been identified. UCP٢ are widely distributed in skeletal muscles, macrophages, spleen, and thymus, while UCP٣ are present in high amount only in skeletal muscles and become stimulated by excess thyroid hormones (Jekabsons *et al.,*١٩٩٩;Griffin and Ojeda, ٢٠٠٠).

e)

Biochemical Studies :-

Regarding the anti-oxidant status in hyperthyroid patients, the present study dealt with reduced glutathione as a marker to explain levels of serum anti-oxidants in hyperthyroid patients which are illustrated in figure(٤).These results showed a significant decrease (P<٠.٠١)in hyperthyroid patients, (males and females) in comparison with control subjects. Present results agree with other previous studies (Adali *et al*., ١٩٩٩; Komosinka *et al.,* ٢٠٠٠). As we know, that, hyperthyroidism is characterized by accelerated mitochondrial respiration and increased oxygen consumption rate by target tissues especially liver (Seven *et al*., ١٩٩٦; Gruyter, ٢٠٠٤). Hence, elevation of oxygen consumption is accompanied with greater electron flow through microsomal, mitochondrial, and peroxisomal electron transport systems of tissues which in turn leads to production of reactive oxygen species such as superoxide (O^{2-}),hydrogen peroxide (H_2O_2), and hydroxyl free radical (OH·) (Seven *et al*.,١٩٩٦). In addition, a higher level of thyroid hormones induces increased respiratory burst activity in human polymorphonuclear cells leukocytes (PMNL) and Kupffer cells in liver. Thus, additional amounts of reactive oxygen species are produced (Videla and Fernandez, ١٩٩٤). The other source that produces oxygen reactive species is NADPH cytochrome P-٤٥٠ reductase, an enzyme localized in endoplasmic reticulum and affected by high levels of thyroid hormones (Landriscina *et al*.,١٩٨٨). Cells of mammals that live in aerobic environments have developed multiple ways (such as glutathione) to remove the reactive oxygen species and protect themselves against deterious effects of these radicals (Lopez *et al*.,

٢٠٠٠). Gredilla *et al.,* (٢٠٠١)who reported that excess thyroid hormones induces oxidative stress and also resulted in higher levels of GSSG/GSH ratio, but this ratio had been found to be lower in hypothyroidism. Previous study of Fernandez and Videla (١٩٩٦) showed that enhanced GSH utilization in the liver of hyperthyroid rats is accompanied by an increment in GSH synthesis which is insufficient to sustain the basal level of GSH.

The present study also involved study of the relationship between hyperthyroidism and total serum protein, albumin, and globulin which are shown in table (٦). Results of total serum protein showed significant decrease (P<٠.٠١) in hyperthyroid subjects of both males and females in comparison with control subjects. The present results were consistent with previous studies (Dolev *et al*., ١٩٨٨ ; Gold and Nejad,٢٠٠٢). As explained previously, albumin and globulin are the major component of serum proteins, thus, decrease in one of them resulted in a decrease of total serum protein. Protein synthesis and degradation are stimulated by thyroid hormones, thus, excessive production of thyroid hormones accelerated protein catabolism, leading increased nitrogen excretion(Griffin and Ojeda, ٢٠٠٠). Moreover, over production of T٣ and T٤ resulted in elevation of body basal metabolic rate which in turn increase catabolism of nutrient molecules such as lipids, carbohydrates, and proteins to produce energy (Deluise and Flier, ١٩٨٣ ; Gold and Nejad,٢٠٠٢). At the same time, excess thyroid hormones stimulates intracellular protein synthesis such as enzymes for energy production and increase synthesis of contractile proteins in cardiac and skeletal muscles may lead to consume some of serum proteins (Danizi and Klein, ٢٠٠٤). Hyperthyroidism is also accompanied with increased oxygen consumption rate which in turn leads to produce reactive oxygen species (Asayama and Kato, ١٩٩٠).So, albumin is one of

important antioxidants, primarily binding free radicals to prevent its toxicity (Goswami *et al*.,٢٠٠٣).The oxidative stress in turn increases protein turnover through change its antigenicity resulted in the elevation its degradation level (Pamplona *et al*.,١٩٩٩).

Results of serum albumin showed a significant decrease (P<٠.٠١)in hyperthyroid patients (males and females) in comparison with control groups. Albumin represents the major component of serum proteins and perform several important physiological functions within the body. Among these functions, its ability to scavenge reactive oxygen species, where it is found to be an important anti–oxidant in plasma, by scavenging free radicals and then prevent their effects (Al-Taee, ٢٠٠٢). As explained previously, such disorder is associated with the liberation of high amounts of reactive oxygen species. Thus, albumin acts with other plasma antioxidants to remove these free radials. This suggestion was supported by several previous studies, which indicated that in hyperthyroidism, serum proteins undergo increased level of oxidative changes especially high molecular weight protein leading to modify their structure and increase its turnover rate (Joe *et al*., ١٩٩٩; Goswami *et al*.,٢٠٠٣). Moreover, in hyperthyroidism, increase energy production and body wasting may attribute to decrease proteins level (Valdemarsson, ١٩٩٢).

Concerning results of serum globulin which showed non significant decrease in both affected males and female when compared with control subjects. As explained previously, globulin, the second important component of plasma proteins (Bishop *et al*.,٢٠٠٥). The present data may suggest that serum globulin also undergo catabolism and oxidative stress but in lesser extent than albumin (Graninger *et al*., ١٩٨٦).

The present study involved the relationship between hyperthyroidism and alkaline phosphatase levels shown in fig(٥). Results showed significant increase (P<٠.٠١) of alkaline phosphatase in hyperthyroid groups (males and females) when compared with those for normal subjects. It must be explained here that,alkaline phosphatase is the main marker of bone formation and secreted mainly by bone forming cells (osteoblasts) to increase precipitation of phosphate with calcium in bone matrix (Langdahl *et al*., ١٩٩٦). In fact, thyroid hormones are considered important hormones to normal growth of bone (Ongphiphadhanakul *et al*., ١٩٩٦). Hence, in hyperthyroidism, there is stimulation to increase bone metabolism through osteoblasts and osteoclasts (bone resorption cells)(Kasono *et al*.,١٩٨٨). Previous studies indicated that excess thyroid hormone induces osteoblasts activity which inturn lead to increase bone formation markers especially alkaline phosphates(Lalan *et al*., ١٩٨٥;Archer and Taylor, ١٩٩٦).Furthermore, liver function is increased in hyperthyroidism because of hypermetabolic rate and oxidative stress, and lead to liberate additional liver alkaline phosphatase iso–enzyme (Gurlek *et al*., ١٩٩٦;Biscoveanu and Hasinki, ٢٠٠٠).

The results of calcium and phosphorus in both serum and urine samples of hyperthyroid patients which are illustrated in table (٧). Results of calcium showed a significant increase (P< ٠.٠١)in both serum and urine samples of both males and females affected with hyperthyroidism. Whereas, results of phosphorus pointed out a significant increase(P<٠.٠٥) in both serum and urine samples of hyperthyroid subjects, males and females when compared with control subjects. Calcium and phosphorus represent the main minerals of bone matrix. Thus, bones consider as store house of both these metals which are responsible for the strength and stability of

the bones. Furthermore, skeleton is metabolically active organ which undergoes continuous remodeling during life and is the more affected organ in hyperthyroid patients (Lowell and Weicker, ١٩٩٩; Shilbayeh, ٢٠٠٣). It had been found that excess thyroid hormones stimulates osteoclast cells and increase their activities to mobilize calcium and phosphorus from bone matrix into blood (Engler *et al*.,١٩٩٩; Kelepouris *et al*., ١٩٩٥).So, excessive mobilization of calcium and phosphorus from bone matrix result in decrease bone mass (osteoporosis) (Glaser and Kapalan, ١٩٩٧; Orwoll *et al*., ٢٠٠٠). Bone resorption, however, is quantitatively more stimulated than bone formation, therefore, bone loss is accelerated in hyperthyroid patients which leads to increase of calcium and phosphorus in circulation (Lalan *et al*.,١٩٨٥; Alikhan and Singh, ١٩٩٦). However, calcium and phosphorus levels are regulated through multiple hormonal mechanisms which include: parathyroid hormone, calcitonin, and ١,٢٥di-hydroxy cholecalceferol (vitamin D٣) (Barber and Elliott, ١٩٩٦). From physiological point view, it appears that parathyroid hormone is inhibited at a higher levels of calcium and phosphorus, but on the other hand, calcitonin, calcium - lowering hormone, must be elevated(Szabo and Ritzl, ١٩٨١; Reverter *et al*., ٢٠٠٥).We conclude that the effects of excess thyroid hormone on osteoclasts activities may exceed the effects of calcitonin hormone (Ganong, ١٩٩٧). Concerning ١,٢٥–dihydroxy cholecalceferol hormone, the biological active form of vitamin D, increases calcium absorption from intestine via Ca^{+2}–H^{+} ATP ase and increase proliferation and differentiation of osteoblasts with increase their function (Fretz *et al*., ٢٠٠٦). Previous study of Macfarlane *et al.*,(١٩٨٢) indicated that excess thyroid hormones is associated with decrease in circulating١,٢٥-dihydroxycholecalceferol and then lead to osteoporosis. In addition, hyperthyroidism is associated with the increase of fatty acids oxidation, ketosis, and decreased oxygen level

may lead to increase acidosis which in turn inhibits synthesis of ١,٢٥–dihydroxy cholecalceferol (Sugden *et al*.,١٩٩٩). As a result of elevation of serum calcium and phosphorus levels, body homeostasis regulation mechanisms act to adjust the amount of these minerals in extracellular fluids. The kidneys are the main way to excrete abnormal high amounts of calcium and phosphorus. Previous studies an indicated increase level of calcium and phosphorus in random urine samples of hyperthyroid patients (De–Menis *et al*.,١٩٩٢; Legovini *et al*., ١٩٩٤). As well as, previous studies indicated that glomerular filtration increase in hyperthyroidism(Ford *et al*., ١٩٨٩; Legovini *et al*., ١٩٩٤ ;Glenn, ١٩٩٥ ; Sabunucu *et al*., ٢٠٠٠).

The present study also included the relationship between excess thyroid hormones and levels of sodium and potassium in both serum and urine samples which are shown in table (٨). Results of sodium reported a significant increase (P<٠.٠١) in both serum and urine samples of hyperthyroid groups, males and females. Whereas, potassium results recorded non significant decrease in both serum and urine samples of hyperthyroid patients, males and females in a comparison with control subjects. As explained previously sodium is the more cation presented in extracellular fluids, while potassium is the main intracellular cation. This distribution of both two ions is dependent essentially on cell membrane bound Na^+-K^+ATPase, which actively transports three sodium ions to the outside of cells simultaneously with the transport two potassium ions into inside of cells (De- Riva *et al*., ١٩٩٥). Na^+-K^+ ATPase had been found to be stimulated by several hormones. One of them, thyroid hormones, since, the metabolic and thermogenic effects of excess thyroid hormones are mediated by increasing Na^+-K^+ ATPase activity (Silva, ١٩٩٥; Chan *et al*., ٢٠٠١). The effects of thyroid hormones on Na^+-K^+ ATPase appear by increasing the content of Na^+-K^+ ATPase subunit

genes and increased mRNA of this enzyme (Awais *et al*., ٢٠٠٠). Furthermore, excess thyroid hormones increase the number of beta adrenergic receptors in the cells of tissues(Haluzik *et al*., ٢٠٠٣),which in turn increase potassium uptake(Claud Bennet and Plum, ١٩٩٦).Insulin hormone had also been found to promote potassium transfer from extra cellular fluids to intra cellular fluids. Thus, insulin hormone promotes uptake of potassium independently of cellular glucose uptake by increasing ATPase activity. Moreover, insulin also reduces sodium permeability; the resultant cellular hyperpolarization of cells produces a passive driving force of potassium accumulation within cells (Glausen and Everts, ١٩٨٩; Harry, ٢٠٠٥). In hyperthyroidism, increased insulin secretion which in turn may affects Na^{+}-K^{+} concentration (Cachefo *et al*.,٢٠٠١).Sodium and potassium are also present in considerable amount in matrix of bones, there fore, over production of thyroid hormones on bones by increasing bones resorption may mobilize some amounts of sodium and potassium into circulation (Shilbayeh, ٢٠٠٣).Results of the present study are consistent with other studies which indicated increase in serum sodium and a decrease serum potassium in hyperthyroidism (Khan and Baron, ١٩٨٧; Nellen *et al*., ١٩٩٩; Jourdian *et al*., ٢٠٠٠).It must be explained here, hormones which regulate sodium and potassium levels in fluids of body. Atrial natriuretic peptide is one of the important hormones which regulate sodium levels. Previous studies showed relationship between excess thyroid hormones and ateriopeptin levels. Thus, thyroid hormones had been found to effect heart muscle through induced production of other physiologically relevant proteins such as alpha-myosin heavy chain,beta-adrenergic receptors, and aterial natriuretic peptid (Ladenson *et al*.,١٩٨٧). Therefore, in hyperthyroidism, increased atriopeptin levels which inturn increase sodium excretion in urine of hyperthyroid patients (Yamaji *et al*., ١٩٨٨). In kidneys, the

reabsorption of sodium ions is mediated by aldosteron hormone. Secretion of aldosteron is controlled by sodium concentration in extracellular fluids and its action involve reabsorption of sodium ions from renal tubules by exchange with potassium and hydrogen ions (Kurtzman *et al.*, ١٩٩٠). It appears that a high levels of sodium ions with low level of potassium in extracellular fluids may inhibit aldosteron secretion which in turn lead to increase sodium excretion in urine (Ganong, ١٩٩٧).Previous studies indicated increased sodium excretion in urine with low level of potassium in hyperthyroid subjects(McCaffrey and Quamme, ١٩٨٤; Disashi *et al.*, ١٩٩٦). In addition, Ford *et al.*,(١٩٨٩)showed that hyperthyroidism exerts direct effect on renal tubules by increasing glomerular filtration rate. As well as, Dolev *et al.*, (١٩٨٨) reported that excess thyroid hormone increase sodium and calcium excretion in urine of hyperthyroid subjects

Regarding levels of cholesterol, the results obtained in the present study pointed out a significant decrease ($P<٠.٠١$) in the cholesterol levels of hyperthyroid groups, males and females when compared with control subjects. These results are shown in table(٩).Present data are consistent with previous studies (Dimitriadis and Rapits, ٢٠٠١; Berghout *et al.*, ٢٠٠٣).For explanation these results, thyroid hormones play an essential role in lipid metabolism through energy production (Erem *et al.*, ١٩٩٩).Parle *et al.*,(١٩٩٢) who observed that excess thyroid hormones exert direct metabolic effects on lipid metabolism which in turn lead to decrease cholesterol level. One of important reasons to decrease cholesterol level, that, hepatic low density lipoprotein (LDL) receptors are sensitive to thyroid hormones, and these receptors decrease in number in hypothyriodism and elevated in hyperthyroidism hence,$T^{٣}$ and $T^{٤}$ increase hepatic LDL receptors which in turn elevate

cholesterol excretion from body via liver (Kovanen, ١٩٨٧; Hayashi *et al.*, ١٩٩٦). Sundaram *et al.,*(١٩٩٧) they explained that total low density lipoprotein cholesterol level were higher in hypothyroidism and lower in hyperthyroidism, and they concluded that LDL has more susceptibility to oxidation in hyperthyroidism. However, fractional synthetic rate of cholesterol had been found to be moderately higher in hyperthyroidism through mRNA level of beta – hydroxyl – beta – methyl–glutaryl –coenzyme A reductase in circulating mononuclear cells increased, where as LDL receptors were unchanged. The decrease in plasma cholesterol level inspite of an enhanced synthetic rate and thus related to increase clearance rate, and the lack of increased expression of LDL receptors suggest that other receptors are implicated (Varas *et al.*, ٢٠٠١). Other reason which explained a decrease of cholesterol level in hyperthyroidism involved that lysosomal acid lipase, the enzyme which hydrolyzes lipoprotein cholesterol esters under thyroid hormones regulation and becomes significantly higher in hyperthyroidism which leads to decrease cholesterol (Coates, ١٩٨٢).It must be explained here that, the possible mechanism could be that thyroid hormones enhance conversion of cholesterol into bile acids. Thyroid hormones influence LDL–independent of alteration synthesis, catabolism, absorption or excretion and also stimulate synthesis of cholesterol and affecting biliary lipid metabolism in large part by influencing energy balance and cholesterol (Abrams and Gudy, ١٩٨١; Eckel, ١٩٨٩).

Results of triglycerides in table(٩)showed a significant decrease (P<٠.٠١)in both hyperthyroid groups, males and females in comparison with control healthy subjects. As explained previously in chapter one, that elevated thyroid hormones are accompanied with hypermetabolic rate which in turn increase consumption of oxygen to oxidiz nutrient molecules to produce energy (Gruyter, ٢٠٠٤).

Triglycerides consider a major source of energy fuel in the body. So, it is not surprising, that triglycerides compounds are consumed to produce high amount of energy in such disease (Boda, ١٩٩٧). Results of the present study were not consistent with Cachefo *et al.,*(٢٠٠١) reported that large stimulation of hepatic lipogenesis in hyperthyroidism is probably explained by both a direct effect of thyroid hormones and increase in insulin level lead to moderate rise in triglycerides levels. On the other hand, our results agree with studies by (Scottolini, ١٩٨٠ ; Tsimihodimos *et al*., ١٩٩٩).One mechanism by which thyroid hormones might decrease plasma triglycerides could be to increase lipoprotein lipase activity, and hyperthyroid patients had remarkable facility in clearing VLDL–TG. Thus, thyroid hormones apparently can promote catabolism of VLDL and lead to decrease triglycerides (Abrams. *et al*.,١٩٨٠).In thyrotoxicosis, fatty acids oxidation and ketogenesis are stimulated, and at the same time, estrification of fatty acids to triglycerides is reduced, as is the secretion of the very low density lipoprotein. As well as, clearance of VLDL and its metabolic products, the LDL, is increased. In addition, increased adipose tissue lipolysis and elevate concentration of plasma free fatty acids also reduce level of triglycerides (Heimberg *et al*.,١٩٨٥; Sugden *et al*.,١٩٩٩). It had also been found that thyroid hormones increase the number of beta–adrenergic receptors in heart muscle, skeletal muscles, and adipose tissues. Also, they may amplify catecholamine action in the post–receptors site. So, sensitivity to catecholamine is markedly increased in hyperthyroidism which increase lipolysis (Greenspan and Gardner, ٢٠٠١).It must be explained here that, adipose tissue norepinephrine decreased in hypothyroidism and increased in hyperthyroidism which lead to increase basal lipolysis.Thus, by affecting local norepinephrine levels and adrenergic post receptors signaling,

thyroid hormones may also influence to increase of lipolysis rate(Haluzik *et al*., ٢٠٠٣).

To clarify the effect of excess thyroid hormones on the level of some trace elements, the present study included determination the levels of serum zinc and copper. Results of serum zinc which are illustrated in fig (٦) showed a significant increase (P<٠.٠٥) in hyperthyroid, males and female groups in comparison with control subjects.As explained in chapter two, zinc is a cofactor for multiple enzymes and found in many tissues such as muscles, RBCs, bones, and liver. Previous studies indicated that increased serum zinc is associated with decreased zinc contents of RBCs, liver, and muscles in hyperthyroid subjects (Buchinger *et al*., ١٩٨٨, Dursun *et al*., ١٩٩٥; Simek *et al*., ١٩٩٧). In addition, other studies showed increase urinary zinc excretion in hyperthyroid subjects (Dolev *et al*.,١٩٨٨;Tsou *et al*.,١٩٩٣). To clarify the reasons which may be implicated in the disturbance of zinc homeostasis, we based on several studies. One of them, study of Chen *et al*.,(٢٠٠٠) who suggested that there may be an interaction between plasma leptin level and thyroid hormones induced abnormality for selected minerals. There is correlation between leptin a concentration and Zn/Cu ratio. Moreover, the previous study of Nakamura *et al.,* (٢٠٠٠) showed increase leptin concentration in hyperthyroidism. On the basis of the mentioned above, we conclude that high level of leptin concentration may lead to increase serum zinc concentration (Mantzoros *et al*., ١٩٩٨; Chen *et al.,* ٢٠٠٠).As well as, catabolic effects of excess thyroid hormones also may be implicated in the liberation of zinc of tissues into blood circulation (Nishi *et al*., ١٩٨٠).

Results of serum copper which are shown in fig (٧) pointed out there was a significant decrease (P<٠.٠١)in hyperthyroid patients

(males and females) in comparison with healthy subjects. Copper deficiency appears to be the most important factor in development of hyperthyroidism. Copper deficiency, the principal nutritional deficiency involved in autoimmune disease especially in the women because women need more copper than men. It seems to be primarily because copper is required for the production of enzymes which convert progesterone into estrogen, whereas men require more zinc which seems to be the mineral necessary to form the enzymes which convert progesterone into testosterone (Klevay and Moore, ١٩٩٠ ; Lu *et al*.,١٩٩٠). Further more, copper seems to be the main mineral for hyperthyroid to take place. Since, copper deficiency lead to elevate level of thyroid hormones. However, copper deficiency may increase progesterone level, which in turn may stimulate thyroid gland. Also, it is essential for monoamino oxidase enzyme which degrades hormones after they have been fulfilled (Saari and Schuschke, ١٩٩٩). Previous studies indicated that some certain minerals like cadmium, aluminum, and mercury are implicated in thyroid disease because they deplate copper. In addition, smokers get hyper thyroidism at a higher rate than non a smokers and the reason is probably because of the high levels of cadmium in tobacco which depletes copper (Bjork *et al*., ٢٠٠٠). Moreover, these minerals such as cadmium and mercury had been found abundantly in fish, plants, and water of Hilla river (Al-Taee, ١٩٩٨).

Conclusions

From data of the present study, we conclude the following points:-

١- The incidence of hyperthyroidism is related with sex and age. Females were more affected than males, and age group of third to fourth decade appears more susceptible group.

٢- The effects of excess thyroid hormones on hematological parameters appear confined with red blood corpuscles and lead to increase these corpuscles in addition to the increase Hb concentration and PCV.

٣- Increase production of reactive oxygen species (ROS) may lead to exhaust serum antioxidants.

٤- Mobilization of calcium and phosphorus from bone may lead to decrease its strength and density and becomes more susceptible to fractures.

٥-Measurment of some minerals(sodium, potassium, calcium, and phosphorus) in both serum and urine samples reflects that excess thyroid hormone has effects on renal clearance.

٦-Serum trace elements (zinc and copper) were affected in over production of thyroid hormones and has been found that copper may be implicated in the incidence of hyperthyroidism.

٧-Increase calcium and phosphorus excretion in urine may be predisposing factor for incidence of renal calculi.

٨-Increase lipolysis and catabolism of proteins resulted in the exhaustion of nutrient molecules and leads to body wasting.

Recommendations

١- Investigation about the iodination procedures of this salts, and determination of the iodide level in serum and urine to explain the effect of iodine level on thyroid gland physiology.
٢- Application of ecological studies of trace elements in drinking water and food stuff to explain the levels of these trace elements and their relationships with incidence of hyperthyroidism.
٣- Determination of extracellular and intracellular antioxidants; and effects of reactive oxygen species on red blood corpuscles and other cells of tissues.
٤- Investigation about stress factors and social status to explain their effects on thyroid gland functions.

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Table (١):- Changes in thyroid stimulating hormone (TSH), triiodothyronine (T٣), and tetraiodothyronin in hyperthyroidism of both sex, males and females

Parameter	Males		Females	
	Patient Subjects	**Control Subjects**	**Patient Subjects**	**Control Subjects**
TSH µU/ml	A ٠.٣٢٦ ±٠.٢٥٤	B ٢.٢٥٣± ٠.٢٥٤	A ٠.٣٨٦ ±٠.٢٤٥	B ٢.٥١٤± ٠.٢٥٣
T٣ ng/dL	A ٢٤٦±٩.٥٤	B ١٣٨±٦.٩٨٣	A ٢٤٩±١٢.٢٩٧	B ١٣٢± ٧.٦٣٤
T٤µg/dL	A ١٣.٥١±٠.٣٢١	B ٧.٨٠٦±٠.١٥٠	A ١٣.٧٢±٠.٣٣٧	B ٨.٢٢±٠.١٦٦

-Values are means ± SE .

- Means with the different letters are significantly different at P< ٠.٠١ .

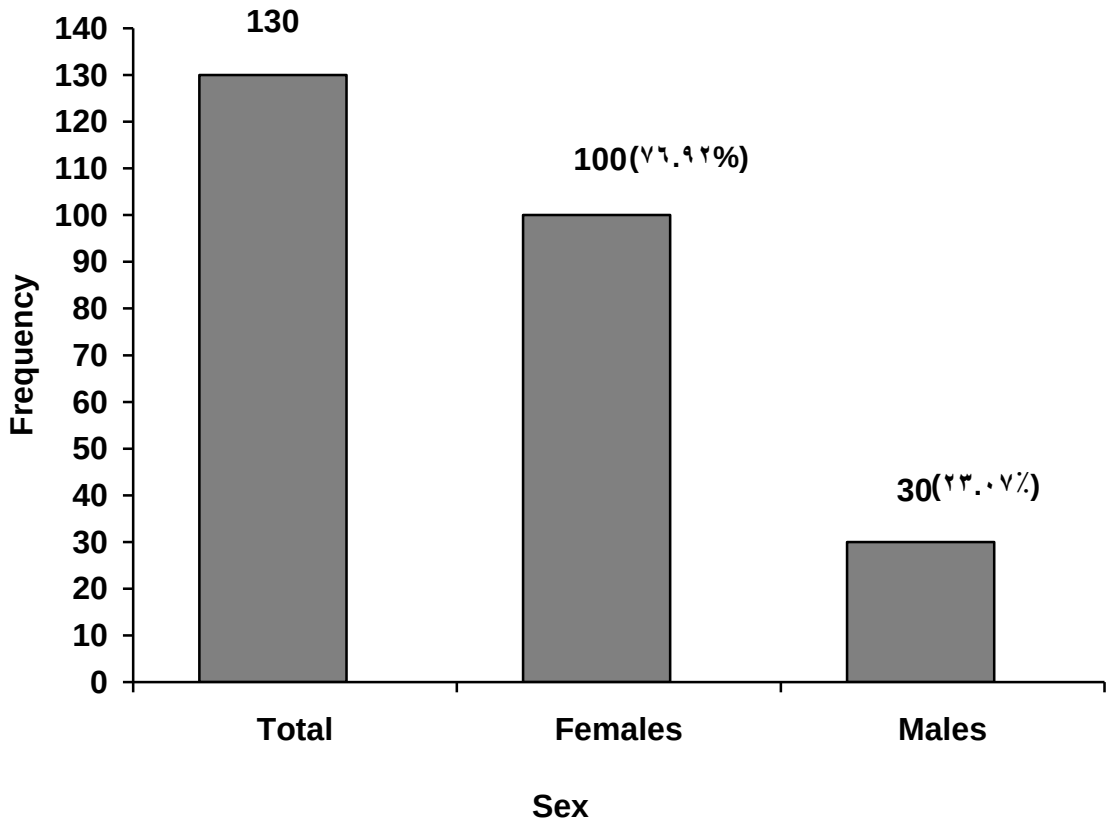

Figure (١):- The relationship between sex and the frequency of hyperthyroidism.

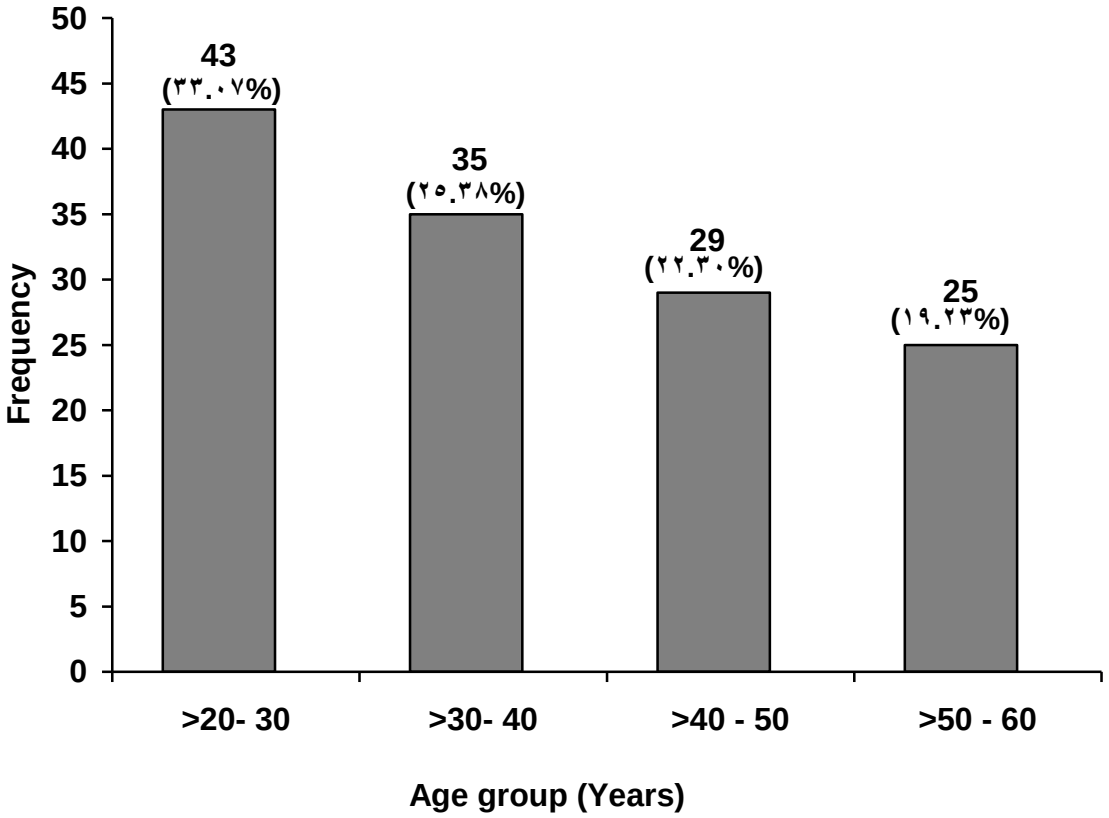

Figure (٢) :- The relationship between age and the frequency of hyperthyroidism.

Table (٢):- Changes in red blood cells (RBCs), hemoglobin concentration (Hb), and packed cell volume (PCV) in hyperthyroid patients, males and females .

Parameter	Males		Females	
	Patient Subjects	**Control Subjects**	**Patient Subjects**	**Control Subjects**
RBCs million/mm³	A ٥.٥٢٠٠±٠.١٦٤	B ٤.٦١٠٠± ١٥٩	A ٥.١٠٠ ± ٠.١٦٩	B ٤.٠٠٠± ٠.١١٣
Hb gm/dL	A ١٥.١٠٦±٠.٣٨٤	B ١٣.٣٨٠±٠.٢٤٣	A ١٤.٠٦٦±٠.٣١٨	B ١٢.١٦٤± ٠.٢٤٦
PCV %	٠.٤٥٨±٠.٠٢٥	٠.٤٢١±٠.٠٣٢	٠.٤٢±٠.٠١١	٠.٤٠±٠.٠٢١

-Values are means±SE.

- Means with different letters are significantly different at P < ٠.٠١ .

Table(٣):- Changes of red blood cell indices (mean corpuscular volume–MCV, mean corpuscular hemoglobin –MCH, and mean corpuscular hemoglobin concentration–MCHC in hyperthyroidism of both sex, males and females.

Parameter	Males		Females	
	Patient Subjects	Control subjects	Patient Subjects	Control Subjects
MCV F١	A ٨٤.٢٣٠±١.٠٨٠	A ٨٧.١٧٠±١.٨٤١	A ٨٣.٨٨ ±١.٠٥١	A ٨٦.٨٦± ١.١٢٢
MCH pg	A ٣١.١٦٠±٠.٦٠	A ٣٠.٣٧٠±٠.٤٩	A ٢٩.٩٢٠±٠.٨٠٠	A ٣٠.٢١١± ٠.٩٠٢
MCHC gm/dL	A ٣٢.١٨٠±٠.٨٦١	A ٣٣.٥٨٠±٠.٨٠٤	A ٣٣.٧٧±٠.٩٢٩	A ٣٣.١١±٠.٨٨٥

-Values are means±SE.

- Means with the same letters are non significantly different .

Table (٤):- Changes in values of adult hemoglobin (HbA), adult hemoglobin٢(HbA٢),and fetal hemoglobin(HbF)in hyperthyroidism in both males and females.

Parameter	Males		Females	
	Patient Subjects	Control Subjects	Patient Subjects	Control Subjects
HbA %	A ٩٨.٠٨± ٠.٢١٦	A ٩٨.٠٠±٠.٢٠٧	A ٩٨.٢١١±٠.٢٢٨	A ٩٨.٢٦٠±٠.٣٤٨
HbA٢ %	A ١.٤٨± ٠.١١٢	A ١.٥٤±٠.١٦٨	A ١.٥٦±٠.١٧٤	A ١.٧٢±٠.١٢٣
HbF %	A ٠.٧٤٢±٠.١١٣	A ٠.٧٥٢±٠.١٢٣	A ٠.٨٨١±٠.١٠٣	A ٠.٧٨٠±٠.٠٧٤

-Values are means±SE .

- Means with the same letters are non significantly different .

Table (٥):- Changes in values of erythrocyte sedimentation rate (ESR)and total white blood cells (WBCs) in hyperthyroidism of both sex, males and females .

Parameter	Males		Females	
	Patient Subjects	**Control Subjects**	**Patient subjects**	**Control Subjects**
ESR mm/h	A ٤.٢٢± ٠.٦٦٦	A ٤.٠±٠.٨٩٤	A ٦.٠٠±١.٠٧	A ٧.٦١٠±١.٠٧٧
WBCs cell/mm³	A ٥١٦٠± ٠.٤٥١	A ٥٠٩٠±٠.٣٥١	A ٤٨١٠±٠.٢٢٦	A ٤٦٨٠±٠.١٨٤

-Values are means ± SE .

- Means with the same letters are non significantly different .

Table(٦):-Changes in total serum protein, albumin, and globulin in hyperthyroidism of both males and females .

Parameter	Males		Females	
	Patient Subjects	Control Subjects	Patient Subjects	Control Subjects
Total serum protein g/dL	A ٤.٩٠± ٠.٣٣٥	B ٦.٧٥±٠.٣٣٨	A ٥.٦٢٣±٠.٢٤١	B ٧.٠١٠±٠.٢٠٤
Albumin g/dL	A ٣.٥٠٠± ٠.١٧٠	B ٤.٤٤٠±٠.٢٥٧	A ٣.٦٨٥±٠.١٥٨	B ٤.٤٨٠±٠.١٧٥
Globulin g/dL	A ١.٧٠٨± ٠.١٦٣	A ٢.٠٥٠±٠.١٦٠	A ١.٩٨٥± ٠.١٣٩	A ٢.٢٧٠± ٠.١٤٥

-Values are means±SE .

- Means with the different letters are significantly different at P<٠.٠١ .

- Means with the same letters are non significantly different .

Table(٧):- Changes in levels of calcium and phosphorus in both serum and urine samples of hyperthyroid patients, males and females.

Parameter	Males		Females	
	Patient Subjects	Control Subjects	Patient Subjects	Control Subjects
Serum calcium mg/dL	A ١٣.٤٨٣± ٠.٣٦٠	B ٩.٨٠٢±٠.٤٠٠	A ١٣.٠٢٠±٠.٤١٦	B ١٠.٠٧±٠.٥١٠
Urine calcium mg/dL	A ٦٤.٣٧٥± ١٤.٢٥١	B ٢٠.٧٦١±٩.٣٨	A ٥٦.٥٠٠±١٨.٧٠٩	B ٣٠.١٢١±١١.٨٠٤
Serum phosphorus mg/dL	a ٤.٩٥٠± ٠.٢٦٨	b ٣.٩٦٠±٠.٢٤٠	a ٤.٧٩١± ٠.٢٨٥	b ٤.٣٨٣± ٠.١٩٨
Urine phosphorus mg/dL	a ١٣٥.٠٠٠±١٥.٧٩٠	b ١١٢.١٣±١٣.٣٩٤	a ١٤٢.١٣٧±١٣.٠٩١	b ١١٠.٧٥±١٢.٢٣٣

-Values are means ± SE .

- Means with different capital letters are significantly at P<٠.- Means with different small letters are significantly different at P<٠.٠٥ .

Table (٨):-Changes in values of sodium and potassium in both serum and urine sample of hyperthyroid patients,

males and females .

Parameter	Males		Females	
	Patient Subjects	Control Subjects	Patient Subjects	Control Subjects
Serum sodium mmol/L	A ١٥٠.٥٠٠±٣.٥٥	B ١٣٧.٤٠٠±١.٥٨	A ٥١.٧٠٠±١.٩٨٩	B ١٣٩.٩٠٠±١.٨٧٦
Urine sodium mmol/L	A ٦٥.٣٠٠±٤.٨٣٩	B ٣٣.٥٠١±٤.٣٤٩	A ٥٨.٥٠٠±٣.٦٨٥	B ٢٧.٢٧٢±٢.٩٦٦
Serum potassium mmo١/L	A ٣.٦٧٠± ٠.١٣٤	A ٤.١٤±٠.١٦٣	A ٣.٨٧٠± ٠.٤١٣	A ٤.١٩٠± ٠.١٧٩
Urine potassium mmo١/L	A ٥.٨١٠±٠.٢٥٦	A ٦.٤٧٠± ٠.٣٩٧	A ٦.٢٣١± ٠.٤٥٥	A ٥.٦٣١ ± ٠.٤٢٥

-Values are means ± SE .

- Values with different letters are significantly at P<٠.٠١ .

- Values with the same letters are non significantly different.

Table (٩) :- Changes in levels of cholesterol and triglycerides in hyperthyroidism of both sex, males and females .

Parameter	Males		Females	
	Patient Subjects	Control Subjects	Patient Subjects	Control Subjects
Cholesterol mg/dL	A ١٥٥.٨٧±١٠.٧٣٠	B ١٨٣.٩٨±٣.٦٠٠	A ١٤٨.٢٣±٤.١٠٣	B ١٨١.٢٩٤±٣.٤٣٧
Triglycerides	A ٨٦.٢٠٠± ٦.٧١٩	B ١١٢.٥٠٠±٥.٣٢٥	A ٨٠.٠٠±٧.٧٦٦	B ١٠١.٦٠٠±٦.١٧٢

-Values are means ± SE .

- Values with different letters are significantly different at P<٠.٠١ .