Biochemical changes in patients suffering from hyperthyroidism

Muhammad O.K. AL- Muhammadi; Dakhel G.O. AL-Watify
University Of Babylon

الخلاصــة

تضمنت هذه الدراسة معرفة تأثير فرط إفراز هرمونات الغدة الدرقية على المتغيرات الكيميوحيوية.

وشملت هذه الدراسة فحص (١٧٠) شخص من المصابين بفرط افراز هرمونات الغدة الدرقية وغير المصابين (الأصحاء) ومن كلا الجنسين الذكور والإناث . حيث كان عدد الإناث المصابات (١٠٠) أنثى وعدد الذكور المصابين (٣٠) ذكراً أما أشخاص السيطرة (الأصحاء) فقد كان عدد الإناث الأصحاء (٢٠) أنثى وعدد الذكور الأصحاء (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 (T3) والثايرونين رباعي اليود (T4) بصورة معنوية بمستوى ($P<0.01$) وانخفاض مستوى الهرمون المحفز للدرقية (TSH) بصورة معنوية بمستوى ($P<0.01$) عند مقارنتها بالأشخاص الأصحاء.أما بخصوص التغييرات الكيموحيوية فقد لوحظ انخفاض الكلوتاثيون المختزل (GSH) بصورة معنوية بمستوى ($P<0.01$) بالمقارنة مع الأشخاص الأصحاء .لوحظ بان قيم خميرة الفوسفتاز القاعدي (ALP) قد سجلت ارتفاعاً معنوياً بمستوى ($P<0.01$) عند مقارنتها بالأشخاص غير المصابين .

أما بخصوص قيم الكالسيوم (Ca^{+2}) والفسفور (PO_4^{-3}) في عينات المصل والإدرار للأشخاص المصابين فقد لوحظ ارتفاعاً معنوياً بمستوى ($P<0.01$) في قيم الكالسيوم وارتفاع معنوي بمستوى ($P<0.05$) في قيم الفسفور عند مقارنتها بالأشخاص الأصحاء .

أما بخصوص العناصر النادرة (Trace elements) فقد لوحظ ارتفاعاً معنوياً بمستوى ($P<0.05$) في قيم الزنك (zinc) وانخفاضاً معنوياً بمستوى ($P<0.05$) في قيم النحاس عند مقارنتها بالأشخاص الأصحاء .أن التغييرات الحاصلة في المعايير الدموية والكيموحيوية يمكن أعزاؤها بصورة رئيسة إلى ارتفاع معدل الايض.

Abstract

The present study was designed to investigate changes occurring in some hormonal and biochemical changes in subjects suffering from hyperthyroidism. A total number used was 170 patients and healthy subjects of both sexes, males and females. The total number of patients was 130; 100 females and 30 males, while the number of control subjects was 40. The ages of all subjects ranged between 20 years to 55 years. It was found that hyperthyroid subjects have a significant increase ($P<0.01$)of triiodthyronine (T3)and tetraiodothyronine (T4) levels and a significant decrease ($P<0.01$) of thyroid stimulating hormone (TSH) levels when compared with those of control groups.

Biochemical changes in serum, showed a significant decrease ($P<0.01$) in the levels of serum reduced glutathione (GSH) in hyperthyroid patients in comparison with healthy subjects. Levels of serum alkaline phosphates (ALP) showed a significant increase($P<0.01$) in hyperthyroid subjects when compared with those control subjects. Levels of calcium in both serum and urine samples showed significant increase($P<0.01$)in hyperthyroid subjects. Values of phosphorus in serum and urine samples showed significant increase($P<0.05$) of hyperthyroid subjects when compared with those healthy subjects. Finally, trace elements results (serum copper and zinc) showed significant decrease($P<0.01$) of copper levels and a significant increase ($P<0.05$) 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 biochemical

parameters may be attributed to hyper metabolic state which arise due to higher production of thyroid hormones which, in turn, affect most of body tissues.

Introduction

Hyperthyroidism is the most endocrine disorder in human and animals, manifest itself in exposure of body tissues to excessive levels of thyroid hormones, tri-iodothyronine (T3) and tetra-iodothyronine or thyroxin (T4),the two main hormones of thyroid gland[1].

This disorder had also been found to affect women five times more than men[2]. Hyperthyroidism is also called thyrotoxicosis, a general term, refers to hyper metabolic state that results because of excess thyroid hormones[3]. 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 T3 and T4[4].

Moreover, high level of thyroid hormones can also result from autonomous production by solitary or multiple thyroid nodules, which secret thyroid hormones autonomously[5].Also, excessive TSH secretion by pituitary tumors resulted in higher stimulation to thyroid gland [6]. The over production of human chorionic-gonadotropin hormone during pregnancy competes with TSH in binding with TSH receptors[7]. Thyroiditis, an inflammatory state of thyroid gland also resulted in liberation of stored hormones (T3 and T4) into blood circulation[8].Finally, the other miscellaneous causes represent a little incidence in hyperthyroidism such as metastatic thyroid carcinoma, and struma ovarri[9].

In general, thyroid hormones play essential roles in regulating vast physiological states in the body[10]. Thyroid hormones play essential roles in regulation the metabolism of major nutrient biomolecules such as carbohydrates, lipids, and proteins[11]. Protein synthesis and breakdown is stimulated by thyroid hormones. The influence of thyroxin on normal body growth is derived largely from the stimulation of protein synthesis[12].Thyroid hormones stimulate the rate of carbohydrates absorption, gluconeogenesis, and insulin secretion[13]. Synthesis, mobilization, and degradation of lipids are controlled by thyroid hormones, because lipids are the major calirogenic molecules[14]. Moreover, thyroid hormones are important factors for the other physiological phenomena such as growth, puberty, and mental development[15]. 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[16;17].However, when thyroid gland enables to produce adequate amount of T3 and T4, consequently lead to hypothyroidism, a disorder which has the opposite features of hyperthyroidism[18].

The aim of the present work was designed to clarify the following points :-

1- Determination of the levels of T3, T4, and TSH.
2- Investigation the effects of hyperthyroidism on the serum anti-oxidants levels(serum reduced glutathione).
3- Investigation the effects of hyperthyroidism on the available minerals (calcium, phosphorus,)in serum and urine samples.
4- Determination of some trace elements such as copper and zinc and their relation with hyperthyroidism.

Materials and Methods

(A):- Materials

Subjects of study:-

This study was carried out over a thirteen months period, from April 2005 to May 2006 in Hilla teaching hospital. The subjects of the study were 170 patients and healthy subjects of both sexes, males and females. The total number of patients were (130) 100 females and 30 males. Forty subjects were used as control group. Their ages ranged between 20 years to 55 years. This study was carried out before starting treatment for all patients. Women of this study were neither pregnant nor receiving contraceptive drugs. All patients were attending to hospital for laboratory diagnosis and treatment, while the 40 control subjects were attending to the public health laboratory in the hospital**.**

(B):- Methods

(1):- Blood collection:-

The collection of blood was performed in Hilla teaching hospital. The tubes were anti-coagulant free as plain tubes, for blood to be used for preparing sera for subsequent biochemical tests. The blood is allowed to clot for 30 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's name, the serum samples were frozen at -20°C for biochemical analysis[19].

(2):- Hormonal studies :

(a):-Determination of thyroid stimulating hormone (TSH)

The ultra sensitive TSH enzyme linked immuno-sorbent assay (ELISA) is based on the principle of solid phase enzyme linked immuno-sorbent assay. The concentration of TSH is directly proportional to the color intensity of the sample test. The absorbance was read at 450 nanometer(nm) (according by TSH kit from Biocheck, Inc.).

(b):-Measurement of total tetraiodothyronine (T4) or thyroxin

The enzyme immunoassay (EIA) is used to determine T4.In the T4 EIA, a certain amount of anti–T4 antibody is coated on microtiter wells. The absorbance was read at 450 nm (according by T4, kit from Biocheck, Inc.).

(c):- Measurement of total triiodothyronine (T3)

Enzyme immunoassay EIA is used to determine T3. In the T3 EIA a second antibody(goat anti-mouse IgG) is coated on micro- titer wells. The optical density was read at 450 nm (according by T3 kit from Biocheck, Inc.)

(3):-Biochemical studies :-

(a):-Determination of serum alkaline phosphatase (ALP)

The method used was that ALP acts upon the AMP–buffered sodium thymolphthalein mono-phosphate .The absorbance of standard, control, and sample was read by using spectronic 21 at 590 nm wave length. (according to ALP kit from Techodiagnostics, Anahein).

(b):- Determination of total calcium in serum and urine samples

Calcium in the sample, reacts with O–cresolphtaleine at alkaline pH. The intensity of the color was measured photometrically by using spectronic 21 at 570 nm wave length (according by calcium kit from spinreact).

(c):-Determination of phosphorus in serum and urine samples

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex. The intensity of color was measured photometrically by using spectronic 21 at 710 nm wave length (according by phosphorus kit from spinreact)

(d):-Determination of serum reduced glutathione(GSH)

5,5´-dithio bis (2- 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 412 nm and is directly proportional to the GSH concentration. Calculation of serum GSH was obtained from the calibration curve in µM[20].

(e):-Determination of serum copper and zinc :-

Atomic absorption spectrophotometer method was used to determine the trace elements(copper and zinc) in serum samples[20] .

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 and taking ($P < 0.05$) as the lowest limit of significant [21].

Results

(1):- Hormonal measurements :-

Results of TSH,T3, and T4 are summarized in table (1) of both hyperthyroid groups ,males and females.

(a):-TSH :-

Results of TSH of hyperthyroid in males and females (0.326 ± 0.254; 0.386 ±0.245 µU/ml respectively) were significantly ($P< 0.01$) lower than control subjects, males and females (2.253 ± 0.254; 2.514 ± 0.253µU/ml, respectively).

(B):-T3 :-

Values of T3 of hyperthyroid in males and females (246 ± 9.45 ; 249 ± 12.29 ng/dL, respectively) were significantly ($P<0.01$) higher than control subjects, males and females (138 ± 6.983;132 ± 7.634 ng /dL respectively).

(c):-T4 :-

Result of T4 of hyperthyroid in males and females (13.51 ± 0.321;13.72 ± 0.337µg/dL, respectively) were significantly higher than healthy control subjects, males and females (7.806± 0.150; 8.22± 0.166µg /dL, respectively).

(2):-Biochemical studies

(a):-Reduced glutathione(GSH) :-

Results of GSH are shown in figure (1) for both affected males and females (11.086±1.078,10.184±0.77µM, respectively) were significantly ($P<0.01$)lower than healthy control, males and females (20.933 ± 0.862; 19.483 ± 0.682µM. respectively).

(b):-Serum Alkaline phosphatase:-

Results of alkaline phosphates which are expressed in figure (2) hyperthyroid males and females (36.870 ± 1.407; 34.511 ± 0.542 IU/L, respectively) were significantly (P<0.01) higher than those for control males and females (22.824±1.885; 19.585 ±1.796 IU/L, respectively) .

(c):-Serum Calcium and phosphorus:-

Results of calcium and phosphorus in both serum and urine samples of hyperthyroid males and females are shown in table (2).

(i):-Serum Calcium :-

Values of serum calcium in affected males and females were (13.483 ± 0.360; 13.020± 0.416 mg/dL, respectively) and were significantly (P<0.01) higher than control males and females (9.802 ±0.400 ; 10.07 ± 0.510 mg/dL, respectively). Values of urine calcium were (78.375±8.251;85.500 ± 10.709 mg /dL, respectively)were significantly (P<0.01) higher than healthy males and females (20.761±9.38; 30.121 ±11.804 mg/dL, respectively).

(ii):-Serum Phosphorus:-

Results of serum phosphorus in affected, males and females groups were 4.950 ± 0.268; 4.791 ± 0.285 mg/dL, respectively. These results showed significant increase (P<0.05) in a comparison with healthy subjects (3.960 ±0.240; 4.383 ±0.198 mg/dL, respectively). Values of urine phosphorus were (135.000 ± 15.790; 142.137 ± 13.091 mg/dL, respectively) were significantly (P<0.05) higher than control subjects of both sex (112.13 ±13.394; 110.75 ±12.233 mg/dL, respectively).

(d):- Serum zinc :-

Results of serum zinc of hyperthyroid groups, males and females are shown in figure(3). These results of 1.241±0.079 and 1.300±0.096 mg/L, respectively were significantly (P<0.05) higher than healthy males and females (0.908±0.049; 0.916±0.074 mg/L, respectively).

(e):- Serum copper :-

Values of serum copper of affected males and females are shown in figure(4) . These values (0.751±0.102, 0.891±0.078 mg/L, respectively) were significantly (P<0.01) lower than healthy males and females (1.194±0.133; 1.233±0.067 mg/L, respectively).

Table(1):-The means of thyroid stimulating hormone (TSH), triiodothyronine (T3), 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	2.253± 0.254	** 0.326 ±0.254	2.514± 0.253	** 0.386 ±0.245
T3 ng/dL	138±6.983	** 246±9.54	132± 7.634	** 249±12.297
T4µg/dL	7.806±0.150	** 13.51±0.321	8.22±0.166	** 13.72±0.337

-Values are means ± SE .
-Means with two asterisks are significantly different at P<0.01 .

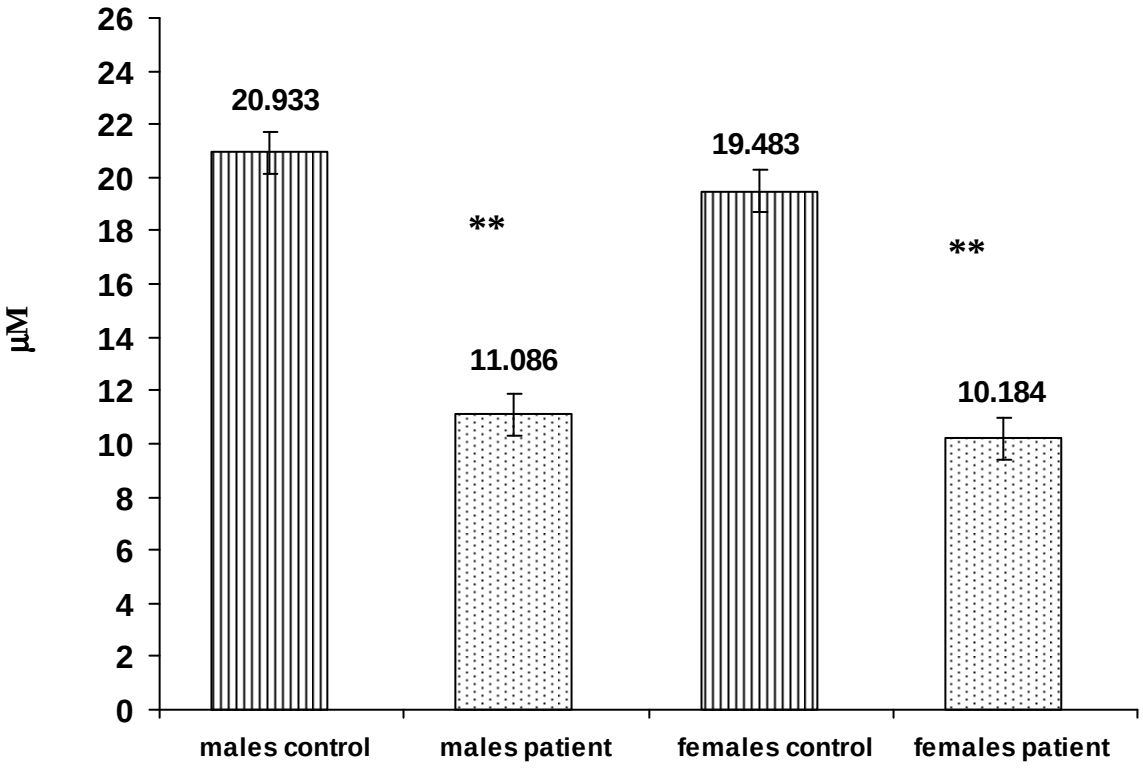

Figure (1): The means of reduced glutathione (GSH) values in males and females affected with hyperthyroidism.

-Values are means ±SE.
-Means with two asterisks are significantly different at (P<0.01).

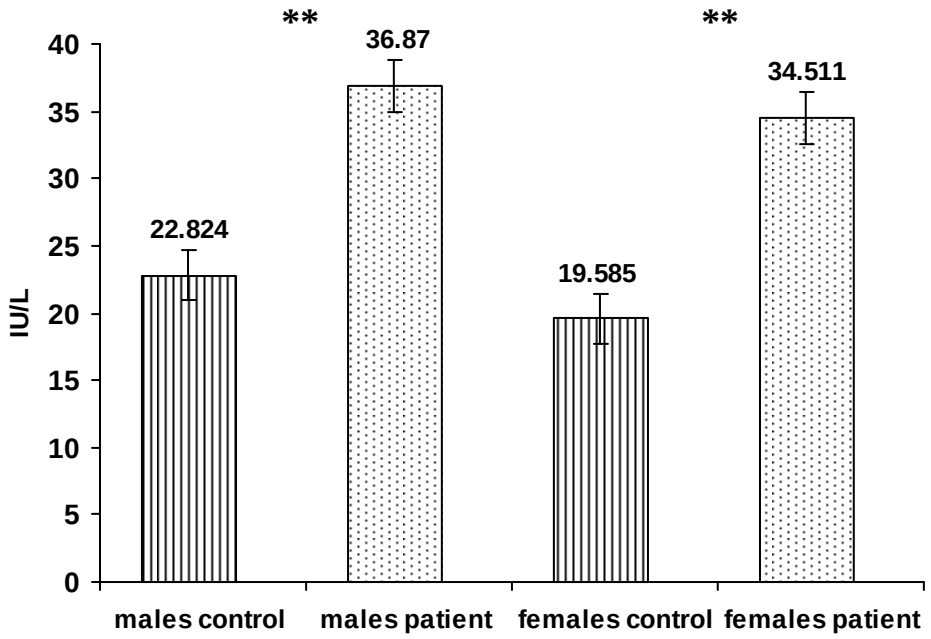

Figure (2): The means of alkaline phosphatase values in males and females affected with hyperthyroidism.

-Values are means ±SE.
-Values with two asterisks are significantly different at (P<0.01).

Table(2):- 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	9.802±0.400	** 13.483± 0.360	10.07±0.510	** 13.020±0.416
Urine calcium mg/dL	20.761±9.38	** 78.375± 8.251	30.121±11.804	** 85.500±108.709
Serum phosphorus mg/dL	3.960±0.240	* 4.950± 0.268	4.383± 0.198	* 4.791± 0.285
Urine phosphorus mg/dL	112.13±13.394	* 135.000±15.790	110.75±12.233	* 142.137±13.091

-Values are means ± SE .
-Means with two asterisks are significantly different at P<0.01.
-Means with one asterisk are significantly different at P<0.05 .

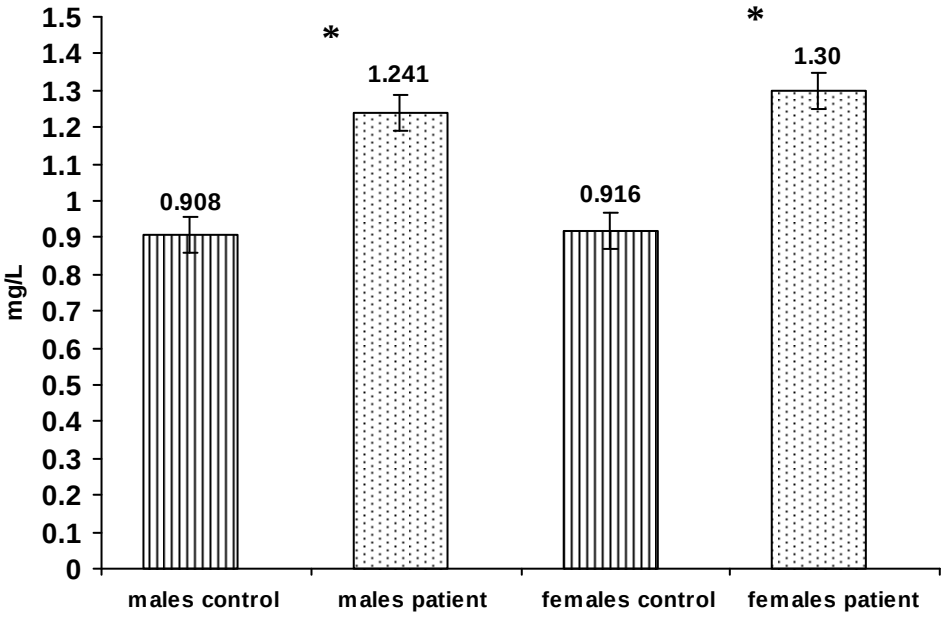

Figure (3):- The means of serum zinc levels in males and females affected with hyperthyroidism.

-Values are means ±SE.
-Means with one asterisk are significantly different at (P<0.05).

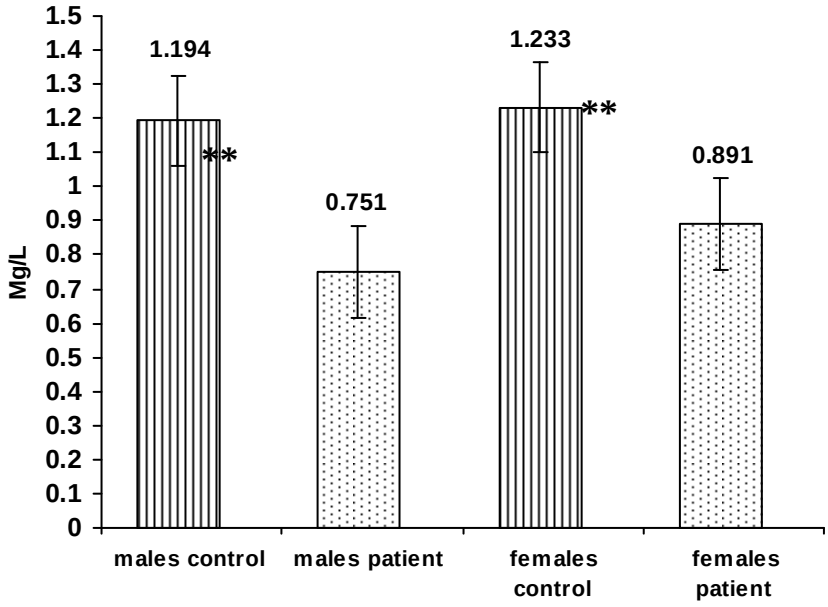

Figure (4):- The means of copper values in males and females affected with hyperthyroidism.

- Values are means ±SE
- Values with two asterisks are significantly different at (P<0.01).

Discussion

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

(a) :-Hormonal Study :-

The patients group of this study showed significant increased levels of T3 and T4 associated with significant decreased of TSH levels of both males and females in comparison with control subjects(table1). The most of the manifestations of patients with hyperthyroidism of this study are related to excess production of T3 and T4 with low level of TSH. There are several explanations and reasons 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 of 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 T3 and T4 synthesis and production regardless of decrease level of TSH by negative feed back mechanism exerted by T3 and T4 on pituitary and hypothalamic axis[22]. 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 T3 and T4[3]. The second reason which may be attributed to increase over one used production of T3 and T4,is solitary or multiple thyroid nodules, which resulted because of hyperplasia of some follicular cells lead to increase iodide uptake more than surrounding tissues. Therefore elevated of T3 and T4 production, while TSH secretion is depressed while the adjacent tissues in thyroid become inactive[5]. Finally, attention was 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 were associated with autoimmune thyroid disease in susceptible individuals[23].

(b):- 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(1).These results showed a significant decrease of as in hyperthyroid patients, (males and females) in comparison with control subjects. Present results agree with other previous studies[24].

The present study showed significant increase of alkaline phosphatase in hyperthyroid groups (males and females) when compared with those of normal subjects (fig.2). 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[25]. It is known that thyroid hormones are considered important hormones to normal growth of bone[26]. Hence, in hyperthyroidism, there is stimulation to increase bone metabolism through osteoblasts and osteoclasts (bone resorption cells)[27]. Previous study indicated that excess thyroid hormone induces osteoblasts activity which in turn lead to increase bone formation markers especially alkaline phosphates[28].Furthermore, liver function is increased in hyperthyroidism because of hyper metabolic rate and oxidative stress, and lead to liberate additional liver alkaline phosphatase iso–enzyme[29].

Results of calcium and phosphorus showed a significant increase in both serum and urine samples of both males and females affected with hyperthyroidism (table 2).

Calcium and phosphorus represent the main minerals of bone matrix. Furthermore, skeleton is metabolically active organ which undergoes continuous remodeling during life and is the more affected organ in hyperthyroid patients[30]. It has been found that excess thyroid hormones stimulates osteoclast cells and increase their activities to mobilize calcium and phosphorus from bone matrix into blood[31]. So, excessive mobilization of calcium and phosphorus from bone matrix result in decrease bone mass (osteoporosis)[32]. 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[33]. However, calcium and phosphorus levels are regulated through multiple hormonal mechanisms which include: parathyroid hormone, calcitonin, and 1,25di-hydroxy cholecalceferol[34]. I can be concluded that the effects of excess thyroid hormone on osteoclasts activities may exceed the effects of calcitonin hormone [35]. Concerning 1,25–dihydroxy cholecalceferol hormone, the biological active form of vitamin D, increases calcium absorption from intestine via Ca^{+2}–H^{+} ATPase and increase proliferation and differentiation of osteoblasts with increase their function[36]. As a result of elevation of serum calcium and phosphorus levels, body homeostasis regulation mechanisms act to adjust the amount of these minerals in extra cellular fluids[37]. 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[38].

Results of serum zinc which are illustrated in fig (3) showed a significant increase in hyperthyroid, males and female groups. Previous studies indicated that increased serum zinc is associated with decreased zinc contents of RBCs, liver, and muscles in hyperthyroid subjects[39;40]. In addition, other studies showed increase urinary zinc excretion in hyperthyroid subjects[41;42]. To clarify the reasons which may be implicated in the disturbance of zinc homeostasis, we based on several studies. One of them, study[43] 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[44] showed increase leptin concentration in hyperthyroidism. On the basis of the facts mentioned above, we conclude that high level of leptin concentration may lead to increase serum zinc concentration[43].As well as, catabolic effects of excess thyroid hormones also may be implicated in the liberation of zinc of tissues into blood circulation[45].

Results of serum copper which are shown in fig (4) pointed out there was a significant decrease in hyperthyroid patients (males and females). 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[46 ; 47]. 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[47]. Previous study indicated that some certain minerals like cadmium, aluminum, and mercury are implicated in thyroid disease because they deplete copper. In addition, smokers get hyperthyroidism 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[48].

Conclusions

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

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

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

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

4:-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.

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

References

(1)-Dahlen, R.(2002). Managing patients with acute thyrotoxicosis. Critical Care Nurse.22(1):20 -26.

(2)-Mayne, P.D.(1998).Clinical Chemistry in Diagnosis and Treatment 6th. ed., Oxford University Press. Inc., P(158- 168).

(3)-Intenzo, C.M.; Depap, A.E; Jabbour, S.; Miller, J.K; and Capuzzi, D.M.(2002). Scintigraphic manifestation of thyrotoxicosis. Radiographics. 23(4):57–69.

(4)-Ali, K.A.; Sharif, M.T.M.; and Alwan, A.F. (2002). Determination of antithyroglobulin and antithyroperoxidase antibodies in patients with hyperthyroidism by application of ELISA technique. Iraqi- J. Comm .Med., 15(1):59-63.

(5)-Kosugi, and Shinji (2002). Non –auto immune hyperthyroidism and hyperfunctioning thyroid adenomas caused by activating mutation of thyrotropin receptor. Nippon. Rinsho., 60(2):91-6.

(6)-Beck, P.P.; Brucker,D. F.; and Persan, L. (1996). Thyrotropin – secreting pituitary tumors. Endocrinol. Rev., 17(3):610- 638.

(7)-AL-Rawi, R.; AL-Duliami, K. S.; and AL- Sammeraie, A.K.(2003). The role of thyroglobulin and iodine in goiter development. J. Basic Med. Scie., 3(4):551-556.

(8)-Summaria, V.; Salvatori, M.; Rufini, V.; Mirk, P.; and Garganese, M.C. (1999). Diagnostic imaging in thyrotoxicosis. Rays. 24 (2):273-300.

(9)-Greenspan, F.S. and Gardner, D.G. (2001). Basic and Clinical Endocrinology. 6th.ed., McGraw- Hill Medical Publishing. Philadelphia, P (201-212).

(10)-Kirsten, D.(2000).The thyroid gland: physiology and pathophysiology. Neonatal Netw., 19(18): 11-26.Abrams, J.J. and Grundy, S. M.(1981).Cholesterol metabolism in hypo and hyperthyroidism in man. J. Lipid Res., 22(2): 23–38.

(11)-Weber, G.; Vigone, M.C.; Stroppa, L.; and Chiumello, C. (2003).Thyroid function and puberty. J. Pediatr. Endocrinol.,2:25-7.

(12)-Karytko, A.I. and Cutter, L.(1997). Thyroid hormones and glucocorticoid regulation of pituitary growth hormone-releasing hormone receptor gene expression. Journal of Endocrinol., 152:R 13-R 17.

(13)-Dimitraidis, G.D. and Raptis, S.A. (2001). Thyroid hormone excess and glucose intolerance. Exp. Clin. Endocrinol. Diabetes. 109(2):39-S 225.

(14)-Nogueira, V.; Walter, L.; Averet, N.; Fontaine, E.; Rigoulet M.; and Leverve, X.M. (2002).Thyroid status is a key regulator of both flux and efficiency of oxidative phosphorylation in rat hepatocytes. J. Biol. Energ. Biomember., 34 (1):55-66.

(15)-Monden, T.; Nakajima, Y.; Hashida, J.; Ishiis, T.; and Shibuja, N.(2006). Expression of thyroid hormone receptors iso form down regulated by thyroid hormone in human medulla blastomal cell. Endocrin. J., 53(2):181-7.
(16)-Krassas, G.E. (2000).Thyroid disease and female reproductive. Fertil –Sterile., 74(6):106-115.
(17)-Kadhim, F.H. (2003). Hyperlipidemia and thyrotropin hormone level among Iraqi women. J. Basic Med. Scien., 3(1):109-110.
(18)-Norigoshi, Y.; Takashi, K.; Toshihiro, T.; Takao, T.; and Toshihiro, M. (2001).Usefulness of thyrotropin (TSH)-releasing hormone test and nocturnal surge of TSH for diagnosis of hypothyroidism. J. Clin. Endocrinol. and Metab., 68(3): 1054-1060.
(19)-Bishop, M.L.; Dube-Engelkirk, J.L.; and Fody, E.P. (2000). Clinical Chemistr: Principles, correlation's, procedures.. 4th. ed., J.B. Lippincott Williams and Wilkins. Philadelphia P(405-416).
(20)-Burtis, C.A. and Ashwood, E.R. (1999). Tietiz Fundimentals of Clinical Chemistry. 4th. ed. W. B. Saunders Comp., Vol.2., USA P(1500-1503).
(21)-Daniel W.W. (1999). Biostatistics :a Foundation for Analysis in the Health Sciences. 7th ed. John Wiley. Philadelphia .P(83).
(22)-Hollwell; Joseph, G.; Staehling ; Norman, W.; Flanders, W ;and Hannon, W. (2002). Serum TSH, T4, and thyroid antibodies in united states population. J. Clin. Endocrinol. Metab.,87 (2): 89-199.
(23)-Markou, K.B.; Georgopoulos, N.A. ; Marki, M.; Vlasopoulou ,B.; Anastasiou, E.; Vaginakis, G.A.; and Kouloubi, K.(2003).Improvement of iodine deficiency after iodine supplementation in school children of Azerbaijan was accompined by hypo and hyperthyrotropenemia and increased title of thyroid autoantibodies. J. Endocrinol. Invest. 26(2):43-8.
(24)-Komosinka, K.; Olczyk, K.; Kucharz, E. J.; Marcisz ,C.; and Winsz–Szczat, K. (2000).Free radical activity and antioxidant defense mechanism in patient with hyperthyroidism due to Grave's disease during therapy. Clin. Chem. Acta.,300 (1-2):7-17.
(25)-Langdahl, B.L.; Loft, A.G.; Moller, N.; Week, J.; and Eriksen, E.F. (1996). Skeletal responsivness to thyroid hormones is not altered at menoponse. Bone. 19(5): 57-64.
(26)-Ongphiphadhanakul, B. ; Puavilai, G.; and Rajatanavin, R. (1996). Effect of TSH-suppressive doses of levothyroxine on bone mineral density in Thai-women. J. Med. Assoc. Thai., 79 (9): 56-7.
(27)-Kasono, K.; Sato, K., Han, D.C.; Fujii, Y. ;and Shizume, K.(1988). Stimuation of alkaline phosphatase activity by thyroid hormone in mouse osteoblast – like cells : a possible mechanism of hyperalkaline phosphatase in hyperthyroidism. Bone. Miner.,4(4):23-63.
(28)-Archer, F.J. and Taylor, S. M. (1996). Alkaline phosphatase bone isoenzyme and osteocalcin in the serum of hyperthyroid cats. Can. Vet. J., 37(12):9-35.
(29)-Biscoveanu, M. and Hasinki, S. (2000). Abnormal results of liver function tests in patients with Grave's disease. Endocrinol. Pract., 6(5):36-97.
(30)-Shilbayeh, S.(2003).Prevalence of osteoporosis and its reproductive risk factors among Jordanian women: a cross sectional study. Osteoporos. Int., 14(11):29-40.
(31)-Engler, H.; Oettli, R.E.; and Riesen, W.F. (1999). Biochemical marker of bone turnover in patients with thyroid dysfunction and euthyroid controls :a cross sectional study : Clin. Chim. Acta.,289(1-2):72-159.

(32)-Orwoll, E.S.; Bevan, L.; and Phipps, K.R. (2000).Determination of bone mineral density in older men. Osteoporos.Int., 11(10):12-81.
(33)-Alikhan, Z. and Singh, A. (1996). Hyperthyroidism–manifested as hypercalcemia. South. Med. J., 89(10) : 27–88.
(34)-Barber, P.J. and Elliott, J. (1996). Study of calcium homeostasis in feline hyper–thyroidism. J. Small. Anim. Pnim. Pract., 34 (12): 575 – 82.
(35)-Ganong, W. F.(1997).Review of Medical Physiology.18th. ed., Appleton and Lange. Philadelphia ,P (496).
(36)-Fretz, J.A.; Zella, L.A.; Kims, N.K.; and Pik, J.W. (2006). 1,25–dihydroxy–vitamin D3 regulates the expression of LDL receptors related protein 5- Via DNA– sequence. Mol. Endocrinol.,13:342-615.
(37)-Macfarlane, I.A.; Mawer, E.B.; Berry, T.; and Hann, J. (1982).Vitamin D-metabolism in hyperthyroidism. Clin. Endocrinol., 17 (1) : 51-9.
(38)-Legovini, P. ;De -Menis, Da–Rin, G.; Roiter, I. ;and Breda, F. (1994). Serum levels of Tetartrate resistant isoenzyme, acid phosphatase, for the evaluation of bone remodeling hyperthyroidism. Minerva. Endocrinol., 19 (3) : 33-8.
(39)-Dursun, N.; Karatoy, M.; Akar, S.; and Biberoglu, G.(1995).The influence of hyperthyroidism on zinc distribution in adult rats. Jpn. J. Physiol.,45(1):197 – 202.
(40)-Simek, G.; Ican, G.; Unal, E.; Hatem, H.;and Yigit, G.(1997).Calcium, magnesium, and zinc status in experimental hyperthyroidism. Biol. Trace. Elem. Res., 57(2):13-71.
(41)-Dolev, E.; Deuster, P.A. ; Solomen B.; Trostamun, U.H.; and Wartofsky, L. (1988).Alteration in magnesium and zinc metabolism in thyroid disease. metabolism. 37(1):7- 61.
(42)-Tsou, C.T.;Chen, M. D.; Lin, W. Hand Ho, L.T.(1993).Alterations of zinc levels in patients with thyroid disorders .hung. Hua. I. H sueh. Tsa. Chih.,51:57 -60.
(43)-Chen, M. D.; Song, Y. M.; Tsou, C. T.; Lin, W. H.; and Sheu, W. H. (2000). Leptin concentrations and the Zn - Cu ratio in plasma in women with thyroid disorder. Biol. Trace. Elem. Res., 75(3) : 99 – 105.
(44)-Nakamura, T.; Nagasaka, S.; Ishikawa, S.; Hayashi, H.; Saito, T.; Kusaka, I ; and Higashiyama, M. (2000). Association of hyperthyroidism with serum leptin levels. Metabolism. 49(10): 285-8.
(45)-Guyton, W.D. (2004). Antioxidants in patients with hyperthyroidism. Clin. Lab. Med., 42(2) :154-158.
(46)-Guyton, W.D. (2004). Antioxidants in patients with hyperthyroidism. Clin. Lab. Med., 42(2) :154-158.
(46)-Nishi, Y.; Kawate, R.; and Usui, T.(1980). Zinc metabolism in thyroid disease. Post grad. Med. J., 56(662): 33-7.
(47)-Saari, J.T.; and Schuschke , D.A. (1999). Cardiovascular effects on dietary copper deficiency. Biofactor. 10(4):59–75.
(48)-Bjork, J.; Alpin, M.; Mauritzson, N.; Stromberg, U.; Johansson,B.; and Hagmar, L. (2000). What other factor may be cause anemia 2.Epidemiology.11(3):91-225.