

Oxidative Stress Induced Histological Changes in Thyroid of Hypothyroidism Patients

Widad Jumaa Hamid

Department of Clinical Laboratory Sciences College of Pharmacy,

AL- Mustansriya University

Email-wedad_jouma @yahoo.com

Abstract:

Hypothyroidism is a condition resulting from insufficient production or diminished action of either T3 and/or T4 thyroid hormones.

The present study is to look for a correlation between oxidative stress and histological changes of thyroid gland in hypothyroidism patients. 30 hypothyroidism patients and 30 healthy individuals as control subjects were included in the study.

We measured their serum tri-iodothyronine, thyroxin and thyroid-stimulating hormone (T3, T4, TSH) respectively. Estimation of MalondyIdehyd (MDA) and Glutathione (GSH) in serum and in homogenized thyroid tissue was carried out by standard methods. TSH was elevated in both serum and in homogenized tissue of patients compared to controls, while T4 and T3 were significantly lower in the patients than in controls. Melanodyldehyd (MDA) as Lipid Peroxidation marker were significantly higher in both serum and in homogenized tissue of patients than in the controls, while (GSH) were significantly lower in both serum and in homogenized tissue of patients than in the control subjects.

Hypothyroidism patients showed histological changes in thyroid gland tissues include, hyaline thickness of the blood vessel walls, necrotic follicles, a strong inflammatory reaction, and peeling of necrotic cells in the follicles.

Keywords: *Glutathione Melanodyldehyd, Oxidative stress, Thyroid gland, Hypothyroidism, Thyroid hormones .*

الخلاصة:

زيادة فعالية الغدة الدرقية ينتج عن عدم او قلة في افراز هرمونات الغدة الدرقية هرمون الثايروكسين و هرمون ثلاثي الثيرودين.

الهدف من البحث هو دراسة العلاقة بين فرط الاكسدة والتغيرات النسيجية في نسيج الغدة الدرقية للمصابين بزيادة فعالية الغدة الدرقية. تمت دراسة 30 مريضا مصاب بزيادة فعالية الغدة الدرقية وتمت مقارنةهم ب 30 من الاصحاء.

تم قياس كل من هرمون الثايروكسين و هرمون ثلاثي الثيرودين و الهرمون المحفز للدرقية في دم المرضى وقورنت النتائج مع الاصحاء. وتم قياس الهرمون المضاد للاكسدة (الكلوتاتيون) ومؤشر فرط الاكسدة (المالانالدهايد) في كل من المصل والسائل النسيجي للغدة الدرقية .

اثبتت نتائج الدراسة الحالية ارتفاعا ملحوظا في الهرمون المحفز للدرقية مع نقصان في معدلات هرمون الثايروكسين و هرمون ثلاثي الثيرودين في دم المرضى مقارنة مع الاصحاء.

كما اثبتت الدراسة الحالية انخفاضاً في مستوى الهرمون المضاد للاكسدة (الكلوتاتيون) في كل من المصل والسائل النسيجي للغدة الدرقية في دم المرضى مقارنة مع الاصحاء. بينما بينت نتائج الدراسة الحالية ارتفاعاً ملحوظاً في مؤشر فرط الاكسدة (المالانالدهايد) في كل من المصل والسائل النسيجي للغدة الدرقية في دم المرضى مقارنة مع الاصحاء.

الدراسة النسيجية وضحت وجود تغيرات نسيجية في نسيج الغدة الدرقية للمصابين بزيادة فعالية الغدة الدرقية (تنخن في جدران الوعية الدموية وتعفن وتحلل والتهابات شديدة في حويصلات الغدة الدرقية).

Introduction:

Hypothyroidism, is a clinical state that results from hypersecretion of thyroid hormones, principally triiodothyronine (T3) and thyroxine (T4). The most common cause of hyperthyroidism is toxic diffuse goiter, or Graves' disease. Less common causes are toxic nodular goiter, or autonomous functioning thyroid nodules, and thyroid cancer^[1].

In basal conditions, thyroid epithelial cells produce moderate amounts of reactive oxygen species (ROS) that are physiologically required for thyroid hormone synthesis.

They are not necessarily toxic because they are continuously detoxified either in the process of hormone synthesis or by endogenous antioxidant systems^[2].

It is noted that oxidative stress arises when highly reactive free radicals produce oxidative damages to the macromolecular structures of the cell.

Thyroid gland plays a central role in generating generalized oxidative stress in diseased condition^[3].

The thyroid gland is located in the lower part of the neck near your Adam's apple. It secretes two essential thyroid hormones: triiodothyronine (T3) and thyroxine (T4) which are responsible for regulating cell metabolism in every cell in the body^[3].

They promote optimal growth, development, function and maintenance of all body tissues.

They are also critical for nervous, skeletal and reproductive tissue as well as regulating body temperature, heart rate, body weight and cholesterol^[4].

In a healthy people a normal thyroid gland secretes all of the circulating T4 (about 90 to 100mg daily) and about 20% of the total circulating T3 (about 30mg daily). The T4 made by the thyroid gland circulates throughout the body and is converted into

roughly equal amounts of T3 and reverse T3^[3].

All of the biological activity of thyroid hormones is due to T3. Because 80% of serum T3 is derived from T4 in tissues such as the liver and kidney^[5].

T4 is considered a pro-hormone^[6]. The synthesis and secretion of the two thyroid hormones is influenced by a hormone released by the pituitary gland called thyroid-stimulating hormone (TSH).

The synthesis and release of TSH from the pituitary gland is influenced by thyroid hormone levels as well as a hormone released from the hypothalamus called thyrotropin-releasing hormone (TRH)^[5].

The activity of the thyroid gland is regulated by a negative feedback loop, in which thyroid hormones interact with receptors in the pituitary gland to inhibit TSH and at the hypothalamus to inhibit TRH secretion^[6].

Hypothyroidism is a condition resulting from insufficient production or diminished action of either T3 and/or T4 thyroid hormones.

Hypothyroidism is characterized by a generalized reduction in metabolic function that most often manifests itself as slowing of physical and mental activity^[7].

The most common signs and symptoms of hypothyroidism are: weight gain, fatigue, lethargy, sleepiness, cold hands and/or feet, low body temperature, depression/anxiety, constipation, headache, menstrual problems, reduced sex drive, hair loss, swollen eye lids and general fluid retention, poor memory and concentration and dry skin, hair and/or nails^[7].

Oxidative stress (OS) has recently been documented in hypothyroidism, a disease more prevalent in women. In general, OS is reported to be more prevalent in males. However, the effect of gender on OS and protein glycation in hypothyroidism has not been addressed.

Glutathione (GSH), malondialdehyde (MDA), levels were analysed. GSH was found to be lower, whereas MDA, levels were higher in patients^[8].

Thyroiditis is an inflammation of the thyroid gland that has several etiologies and can be associated with normal, elevated, or depressed thyroid function, often with evolution from one condition to another.

The differentiation is based primarily on the clinical setting, rapidity of symptom onset, family history, and presence or absence of prodromal symptoms and neck pain^[9].

Materials and Methods

The present study was conducted in Al-Yarmook Teaching Hospital in the period between 2011 to 2012. 30 patients with hypothyroid syndrome and 30 healthy individuals as control subjects were enrolled in the study.

Blood samples were collected from both hypothyroidism and the control healthy groups. From each patient 10 mills of venous blood was collected to use in this study. The blood was left to clot and then centrifuged at 3000 pm for 10 minutes.

The separated plasma samples were stored at 20°C until the biochemical analysis was performed.

Hormonal analysis (T3, T4, and TSH) was performed by radio-immunoassay technique (RIA) using immunotech-Kits^[10].

Serum glutathione is determined by a modified procedure utilizing Elman's reagent (Sedlak and Lindsay 1968)^[11].

In brief; an equal volume (150ml) of serum and 4% sulfosalicylic acid were mixed, centrifuged (2000 rpm at 4°C for 15 minutes to 150 of supernatant add 4.5 ml of 0.1 mm Elman's reagent (5.5 dithiobis, 2-Nitrobenzoic acid DTN) in phosphate buffer of pH 8.0 (prepared by a mixer of 0.6 M KH₂PO₄ and 0.08 M. Na₂ HPO₄), then read at spectrophotometrically at 412 nm.

Malondialdehyde is an end product of lipid per oxidation.

It reacts with thiobarburic acid (TBA) to produce a colored complex. Malondialdehyde was measured according to the method of (Fong *et al.*, 1973)^[12].

The byproduct of lipid per oxidation malondialdehyde (MDA) level was measured in (thyroid glands) tissues homogenates depends on the formation of pink chromophore because of the reaction between (MDA) and thiobarbituric acid TBA, which can be measured spectrophotometrically according to the method of (Buege and Aust, 1978)^[13].

In this method 2 ml of TBA reagent [10.375 g (TBA), 15g trichloroacetic acid (TCA) dissolved in 100 ml of 0.25 N hydrochloric acid at 45°C], was added to 1 ml of the homogenate.

The mixture was incubated in a boiling water bath for 10 minutes, cooled and then centrifuged at 3000 r pm for 10 minutes.

Light absorbance of the clear supernatant was determined spectrophotometrically at 535 nm against Blank. MDA concentration was calculated using a molar absorptivity coefficient $1.5 \times 10^5 \text{ m}^{-1} \text{ cm}^{-1}$ (Sinhner and LU, 1958)^[14].

The results were expressed as n moll MDA/g tissue. Glutathione (GSH) levels were determined according to the method of Ellman (Ellman, 1959)^[15].

In which 0.5 ml of 4% sulphosalicylic acid was added to equal volume of tissue homogenate for precipitation of protein.

After centrifugation 0.5 ml of clear supernatant was mixed with 4.5 ml (DTNB) reagent [0.1Mm DTNB IN 0.1 m phosphate buffer pH8. The light absorbance of the mixture was measured at 412 nm after 2 minutes.

For histological preparation of thyroid gland tissue, formalin fixed thyroid gland tissue was embedded in paraffin,

sectioned at 6 μm thickness and stained with hematoxylin and eosin (H&E).

Statistically Analysis:

The data of this study, were compiled into the computerized data file and the frequency, distribution and statistical description (mean, range, and SD) were derived using SPSS statically software.

We used statistical analysis of variance (ANOVA) test and least significantly difference (LSD) test by

probability of less than 0.05 (P<0.05) according to ^[16].

Results

All the parameters of antioxidant enzymes and lipid peroxidation are summarized in Table 1 and Table 2, the parameters of oxidative stress in Hypothyroidism compared with healthy people Parameters of Thyroid Function Test The estimates of T3, T4, and TSH are summarized in Table3.

Table-1: Parameters of oxidative stress in serum

| Parameters | Hypothyroidism (mean±SD) | Control (mean±SD) |
|------------|--------------------------|-------------------|
| Number | 30 | 30 |
| MDA μmol/g | 3.05±0.121 | 1.32±0.244 |
| GSH μmol/g | 1.92±0.202 | 5.08±0.253 |

Table-2: Parameters of oxidative stress in tissue homogenate

| Parameters | Hypothyroidism (mean±SD) | Control (mean±SD) |
|------------|--------------------------|-------------------|
| Number | 30 | 30 |
| MDA μmol/g | 6.72±0.209 | 1.266±0.178 |
| GSH μmol/g | 0.88±0.181 | 4.95±0.0289 |

Table-3: Parameters of T3, T4, and TSH in Hypothyroidism syndrome.

| Parameters | Hypothyroidism (mean±SD) | Control (mean±SD) |
|------------|--------------------------|-------------------|
| Number | 30 | 30 |
| T4 n mol/L | 38.79±21.78 | 105.09±9.79 |
| T3 n mol/L | 0.48±0.22 | 1.19±0.05 |
| TSH mμ/L | 33.55±18.05 | 2.49±1.04 |
| | | |

Serum MDA was significantly higher in the Hypothyroidism groups in comparison to control group.

The serum GSH levels were significantly lower in the Hypothyroidism groups in comparison to control group.

Histological examples of thyroids of Hypothyroidism showing increase fibrosis,

increase interstitial tissue ,decrease in follicle numbers, decrease in follicle diameter (Fig-3, Fig-4, Fig.-5 and Fig.-6), compare with Histological examples of thyroids of under normal nutrition condition (Fig.-1 and Fig.-2).

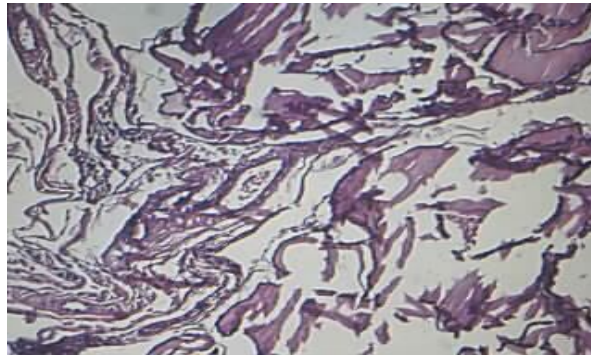


Figure-1: Histological examples of thyroids of under normal nutrition conditions (H&E) stain 200x.

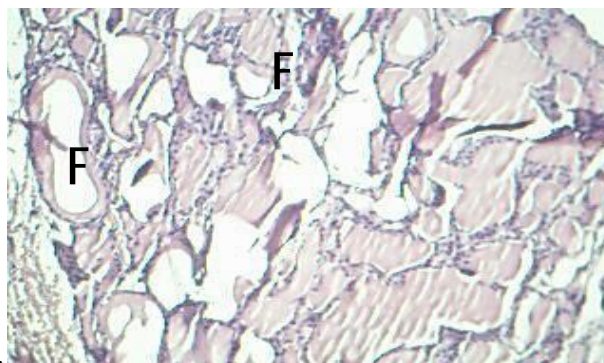


Figure-2: Histological examples of thyroids of under normal nutrition conditions (H&E) stain 400x.

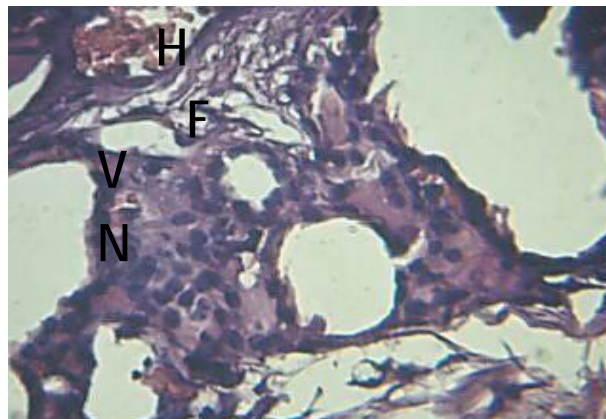


Figure-3: Histological examples of thyroids of Hypothyroidism showing increase fibrosis F, increase interstitial tissue H, decrease in follicle numbers N, decrease in follicle diameter V. (H&E) stain 400x.

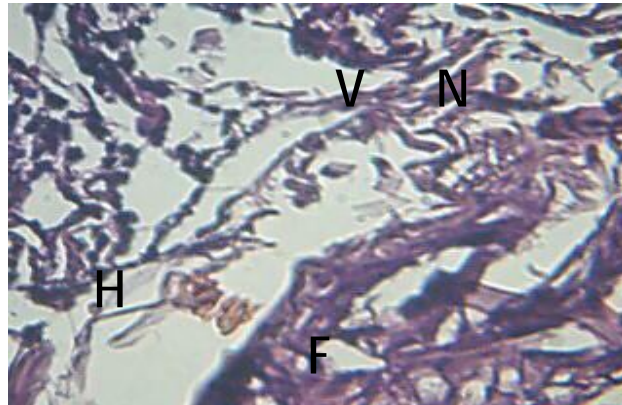


Figure-4: Histological examples of thyroids of Hypothyroidism showing increase fibrosis F, increase interstitial tissue H ,decrease in follicle numbers N,decrease in follicle diameter V. (H&E) stain 400x.

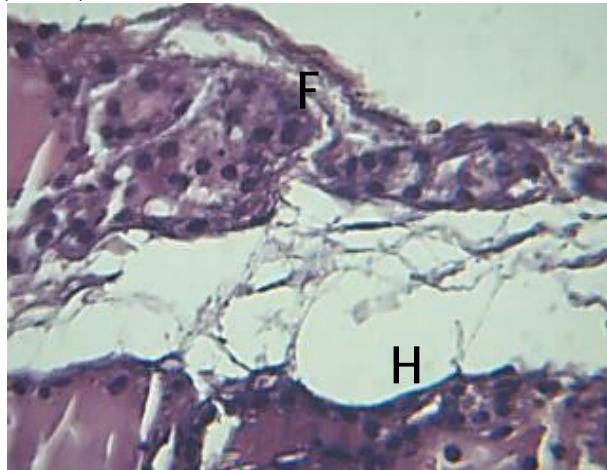


Figure-5: Histological examples of thyroids of Hypothyroidism showing increase fibrosis F, increase interstitial tissue H, (H&E) stain 400x

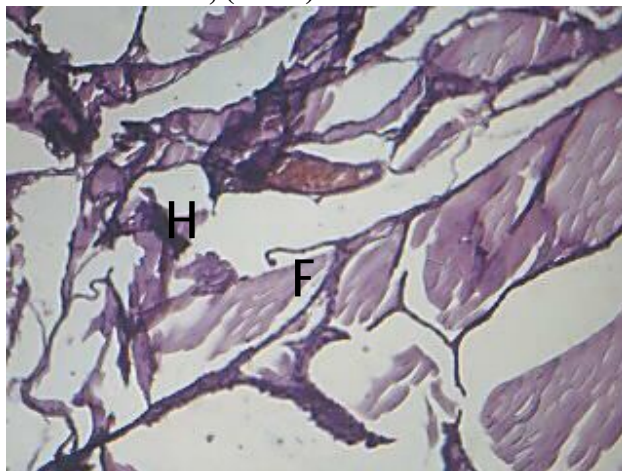


Figure-6: Histological examples of thyroids of Hypothyroidism showing increase fibrosis F, increase interstitial tissue H, (H&E) stain 200x.

Discussion:

The serum levels of thyroid hormones were significantly lower in Hypothyroidism patients compared to control, while the serum TSH levels were significantly higher as expected due to pituitary's response to the decreased hormone levels.

Oxidative stress involves a depletion of the protective antioxidant, substance that prevents or slows the breakdown of another substance by oxygen.

Synthetic and natural antioxidants are used to slow the deterioration of gasoline and rubber, and such antioxidants as vitamin C (ascorbic acid), defenses of the body.

Oxidative stress causes increased production of reactive oxygen species, a molecules and ions of oxygen that have an unpaired electron, thus rendering them extremely reactive.

Many cellular structures are susceptible to attack by ROS contributing to cancer, heart disease, and cerebrovascular disease, enhanced lipid peroxidation, decreased glutathione^[17].

This link between iodine and autoimmune consequences has been recently emphasized in a survey in China that showed that excessive iodine intake is associated with a higher prevalence of Hashimoto's disease and hypothyroidism^[18]. It is therefore crucial for thyrocytes to be efficiently protected against excessive ROS production; otherwise, it would not be possible for these cells to be kept alive and, obviously, to function properly^[18].

Thus, to face the oxidative challenge and survive, thyrocytes have developed protective systems that limit the toxicity of endogenously and naturally produced ROS. They include antioxidant enzymes such as superoxide dismutases, catalase, glutathione peroxidases, and peroxiredoxione^[19].

The balance between thyroid regeneration and fibrosis appears to

determine in part whether hypothyroidism will occur^[20].

Abnormal distribution of hyaline probably disrupts thyroid hormone secretion, as shown by Li et al.^[18].

Therefore, despite an increased TSH concentration during the monitoring period, T3 concentration was low but close to the minimum normal level.

This finding indicates that T3 occurs in non-thyroid tissues by T4 deiodination and this is the reason why its concentration, contrary to the concentration of T4, does not depend on tissue status^[19,20].

One of the major effects of thyroid hormones is to increase mitochondrial respiration^[21], which results in increased generation of ROS, leading to oxidative damage to membrane lipids. There is a good deal of evidence to indicate that metabolic depression brought about by hypothyroidism is associated with a decrease in free radical production and a subsequent protection against lipid peroxidation^[21].

This supports the notion that reduced demand for oxygen in hypothyroidism serves as a protective factor in tissue injury due to ROS. In fact, it has been shown that hypothyroidism is able to prevent the increase in lipid peroxidation and the diminution in GSH as well the tissue damage induced by intracolonic administration of trinitrobenzene sulfonic acid (experimental model of colitis)^[22].

The finding of our results shows Hypothyroidism also cause the increase in MDA which induced damage thyroid gland tissue as same as in the study of^[23].

Effect of hypothyroidism against oxidative stress and tissue damage in several experimental models^[24].

Was observed by histological (necrotic tubules, percentage of tubules with hyaline casts and area of tubular lumen occupied by hyaline casts) and biochemical (creatinine and BUN) analyses.

In addition, the results in increased generation of ROS, leading to oxidative damage to membrane lipids.

There is a good deal of evidence to indicate that metabolic depression brought about by hypothyroidism is associated with a decrease in free radical production and a subsequent protection against lipid peroxidation^[18].

This supports the notion that reduced demand for oxygen in hypothyroidism serves as a protective factor in tissue injury due to ROS. In fact, it has been shown that hypothyroidism is able to prevent the increase in lipid peroxidation and the diminution in GSH^[8].

Hypothyroidism also prevents the increase in MDA and decreases the susceptibility to oxygen radical-induced lung damage in newborn rats exposed to prolonged hyperoxia^[8].

The majority of the studies in hypothyroid animals have found no change or a decrease in tissue markers of oxidative stress (thiobarbituric acid reactive substances, MDA or oxidized glutathione) (Table-1) supporting a decreased production of ROS in this experimental model.

The most studied enzymes in hypothyroid animals are SOD, Cu, ZnSOD, MnSOD, CAT, GPx, and GR. The effect of hypothyroidism on the antioxidant enzymes in several tissues is not consistent. In some cases the change of antioxidant enzyme activity seems to be tissue specific^[23].

On the other hand, within a single tissue, the response of the antioxidant enzymes to hypothyroidism is not always similar^[24].

Conclusion:

According to the present study we could conclude that hypothyroidism cause tissues undergo several biochemical and histological changes that predispose them to oxidative damage.

Therefore we suggest that hypothyroidism patients may benefit from supplements of antioxidants.

References:

- 1- Gilles, R. Cf.; Den Heijer, M.; Ross, A. H.; Sweep, F. C. G. J. and Wetzels, J. F. M. Thyroid function in patients with proteinuria. *Netherlands Journal of Medicine*, 2008. Vol. 66(11). Pp: 483–485.
- 2- Mogulkoc, R.; Baltaci, A. K.; Oztekin, E, Ozturk, A. and Sivrikaya, A. Short-term thyroxine administration leads to lipid peroxidation in renal and testicular tissues of rats with hypothyroidism. *ActaBiologica Hungarica*, 2005. Vol. 56 (3-4). Pp: 225-232.
- 3- Iglesias, P. and Díez, J. J. Thyroid dysfunction and kidney disease. *European Journal of Endocrinology*, 2009. Vol. 160 (4). Pp: 503-515.
- 4- Nadkarni, G. D. and Sawant, B. U. Role of antioxidant defence in renal path physiology: primacy of glutathione peroxides. *Indian Journal of Experimental Biology*. 1998. Vol. 36 (6). Pp: 615-617.
- 5- Fernández, V.; Castillo, I.; Tapia G.; Romanque, P.; Uribe-Echevarría, S. and Uribe, M. Thyroid hormone preconditioning: protection against ischemia-reperfusion liver injury in the rat. *Hepatology*, 2007. Vol. 45 (1). Pp: 170-7.
- 6- Metso, S.; Jaatinen, P.; Huhtala, H.; Luukkaala, T.; Oksala, H. and Salmi, J. Long-term follow-up study of radioiodine treatment of hyperthyroidism. *Clin Endocrinol*. 2004 Vol. 61. Pp: 641-648.
- 7- Poncin, S.; Colin, I. M. and Decline, B. N-acetylcysteine and 15 deoxy- Δ 12, 14-prostaglandin J2 exert a protective effect against autoimmune thyroid destruction in vivo but not against

- interleukin-1 α / interferon γ -induced inhibitory effects in thyrocytes in vitro. *The American Journal of Pathology*. 2010. Vol. 177 (1). Pp: 219-228.
- 8- Grinberg, L.; Fibach, E.; Amer, J. and Atlas, D. N-Acetylcysteine amide, a novel cell-permeating thiol, restores cellular glutathione and protects human red blood cells from oxidative stress. *Free Radic. Biol. Med.* 2005. Vol. 38. Pp: 136-145.
 - 9- Torlak1,V.; Zemunik,T.; Modun4, D.; Capkun1,V.; Pesutic-Pisac,V.; Arkotić, A.; Pavela-Vrancic, M. and Stanicic, A. 131I-induced changes in rat thyroid gland function. *Braz J Med Biol Res*, August. 2007. Vol. 40 (8) Pp: 1087-1094.
 - 10- Evered, D. C.; Ormston, B. J. and Smith, P. A. Grades of Hypothyroid. *Biol. Chem.* 1973. Vol. 1. Pp: 657-662.
 - 11- Sedlak, J. and Lindsay, R. H. *Analytical Biochemistry*. 1968. Vol. 314. Pp: 1311-1317.
 - 12- Fong, K. L.; McKay, P. B. and Poyer, J. L. Evidence that per oxidation of liposomal membranes is initiated by hydroxyl free radicals produced during flavin enzyme activity. *Biol. Chem.* 1973. Vol. 1. Pp: 248-7792.
 - 13- Budge, J. A. and Aust, S. D. Microsomal lipid per oxidation. *Methods. Enzymol.* 1978. Vol. 52. Pp: 302 -310.
 - 14- Sinhuber, R. O. and Lu, I. C. Lipid per oxidation. *Food Technology*. 1958. Vol. Pp: 12-9.
 - 15- Ellman, G. L. Tissue Salfhydryl group. *Arch. Biochem. Biophys.* 82:70-77. Elsevier science publishing co. 1959. Pp: 920.
 - 16- Duncan, R. C.; Knapp, R. G. and Miller, M. C. *Introductory Biostatistics of Health Sciences*. A Wileg Medical Publication, John Wiley and Sons, London 1983. Pp:161-179.
 - 17- Messarah, M.; Boumendjel, A. and Chouabia, A. Influence of thyroid dysfunction on liver lipid peroxidation and antioxidant status in experimental rats. *Experimental and Toxicologic Pathology*. 2010. Vol. 62 (3). Pp: 301-310.
 - 18- LiFLu, S.; Zhu, R.; Zhou and Cai, L. Heme oxygenase-1 is induced by Thyroid hormone and involved in thyroid hormone preconditioning-induced protection against renal warm ischemia in rat. *Mol Cell Endocrinal* 2011.Vol. (2). Pp: 221-229.
 - 19- Veronica, T. V.; Diana, B.; Martha, F.; Edilia,T.; Rogelio, H. P. and Omar, N. Jose Pedraza-ChaverriHypothyroidism attenuates protein tyrosine nitration, oxidative stress and renal damage induced by ischemia and reperfusion: effect unrelated to antioxidant enzymes activity. *J. Biol Chem.* 2013. 2010. Vol. 5. Pp: 9070.
 - 20- Studer, H.; Forster, R.; Conti, A.; Kohler, H.; Haerberli, A. and Engler, H. Transformation of normal follicles into thyrotropin-refractory "cold" follicles in the aging mouse thyroid gland. *Endocrinology*. 1978. Vol. 102. Pp: 1576-1586.
 - 21- Gerber, H.; Peter, H. J. and Studier, H. Age-related failure of endocytosis may be the path genetic mechanism responsible for "cold" follicle formation in the aging mouse thyroid. *Endocrinology*. 1987. Vol. 120. Pp: 1758-1764
 - 22- Tanrikulu, S.; Doğru, S. and Özderya, A. The 8-oxoguanine DNA N-glycosidase 1(hOGG1) Ser 326 Cys variant affects the susceptibility to Graves' disease. *Cell Biochemistry and Function*. 2011. Vol. 29 (3): Pp: 244–248.
 - 23- Pomorski, L. and Bartos, M. Histologic changes in thyroid nodules after percutaneous ethanol injection in patients subsequently operated on due to new focal thyroid lesions. *APMIS*; 2002. Vol. 110. Pp: 172-176.
 - 24- Madhu, M.; Patil, Sharangouda, J. and Saraswati, B. Atropine Sulphate Induced Changes in Uterine, Adrenal, Liver and Thyroid Gland in Female Albino Rats. *Journal of Pharmacology and Toxicology*, 2009. Vol. 4.Pp: 236-245.