Evaluating of some serum biochemical parameters in thyroid dysfunction women in Kalar’s General Hospital

Hero Helal Muhammed Saed 1**, Zeyan Abdullah Ali 2*
1- Department of Chemistry, College of Education, University of Garmian, Kalar, Kurdistan region, Iraq.
2- Department of Chemistry, College of Education, University of Salahaddin-Erbil, Erbil, Kurdistan region, Iraq.

1. INTRODUCTION

Thyroid glands is an endocrine gland, found in the neck, and are one of the most important glands. Thyroid glands produces three hormones: Triiodothyroxine (T3), tetraiodothyroxine (T4, known as thyroxine), and calcitonin. This gland uses tyrosine, and iodine to manufacture the T3 and T4 hormones. The circulating hormone is bound to protein which is called thyroid binding globulin. Only the free hormone (unbound) is active (Moore, 2016). The most important function of thyroid gland is to regulate the physiological actions of the body (Mohamedali, et al. 2014). There are different factors that affect the function of thyroids including gender, age, urinary iodine...
concentration, smoking, age, and race, in addition to physical activity, education level, daily caloric intake, poverty status, and weight (Shon et al., 2008, Flegal, 2010, and Six-Merker, et al. 2016). Diseases of the thyroid are the most common afflictions involving the endocrine systems. The most common types of thyroid diseases are hypothyroidism and hyperthyroidism. Hypothyroidism is a state presenting with low levels of thyroxine, and elevated thyrotropin, which results in the slowing of metabolic processes (Park, 2013). While hyperthyroidism is the condition with induced secretion of thyroxine by the thyroid gland (Burton, 2011). Thyroid hormone induces the generation of free radical and hence the resultant reduces in antioxidant state in hyperthyroidism (Mancini et al., 2013). It is postulated that the thyroid hormones play a role in inducing the production of generalized oxidative stress (OS) and variations in the thyroid hormone levels in vivo have been considered the main physiological modulators of OS in the cells (Babu, et al. 2011). Peroxynitrite, is a toxic product formed from the reaction between superoxide radicals (O$_2^-$) and nitric oxide (NO) which has been implicated in the pathologies of inflammatory diseases, and central nervous system. Peroxynitrite leads to lipid peroxidation, oxidation of sulfhydryl, and the alteration of DNA and proteins (Ahmad et al., 2015). Ceruloplasmin is a glycoprotein contains copper (Cu). The physiological role of ceruloplasmin involves redox reactions in the plasma. CP can function as an antioxidant or oxidant depending on some factors, such as the presence of ferritin binding sites and free ferric ions. CP is important in the control of lipid oxidation in the membrane and is a major defense against the effects of reactive oxygen species in the cells and erythrocytes, and have a high capacity to degrade exogenous hydrogen peroxide H$_2$O$_2$ (Bhattacharya et al., 2014). Vitamin C is non-enzymatic antioxidant and is a class of essential nutrient vitamins that is essentially required for many biochemical and physiological functions in humans body and animals including immune functions, as a cofactor, as a reducing agent, the synthesis of collagen, L-carnitine, and sparing action of other vitamins (like vitamin E, A) from oxidation (Clemens and Tóth, 2013, Grosso et al., 2013, Omidi et al., 2015, and Pacier and Martirosyan, 2015). There are several studies showed that vit. C is important for the central nervous system and a decrease in the concentration of this vitamin causes the damage of the cells (Karim et al., 2011) and helps maintain of DNA, enzymes, lipids, proteins, and other antioxidants in their normal form by scavenging reactive oxygen and nitrogen specie and reducing metal ions (Ge, et al. 2008). Albumin is responsible for several important pharmacological and physiological functions. Impairment in the function of albumin during disease has been documented, which leading to the notion that human serum albumin administration could partially restore these functions. Alb transports endogenous compounds such as metals, fatty acids, cholesterol, hormones, amino acids, bile pigments, drugs, and other substances through the blood and is also important for healing and tissue growth (Alva et al., 2011, and Garcia-Martinez et al., 2013). It has been considered that the Alb not only as an antioxidant, but also, as the major circulating antioxidant in plasma exposed to continuous OS (Ambade et al., 2014). Glutathione is an important tripeptide antioxidant, exists in two forms: reduced glutathione GSH and oxidized glutathione disulfide (GSSG), and is the first line of defense against OS (Petrulea, et al. 2010). GSH is widely distributed in cells and involved in many biological activities which include: detoxification of many xenobiotics, neutralisation of reactive oxygen species, and
maintenance the levels of thiol in proteins in the reduced state, removing toxic peroxides formed in the growth and metabolism under aerobic conditions, and playing a role as a coenzyme with some enzymes including, thiol-transferase, GSH peroxidase, and GSH-S-transferase (Das and Chainy 2001). The concentrations of GSH in the tissue and plasma are inversely related to the OS levels (Mohamed and Elnegris, 2015). The aim of this study is to evaluate some biochemical parameters like peroxynitrite, ceruloplasmin, vitamin C, albumin, and glutathione in hypo, hyperthyroid dysfunction women before and after treatment.

2. MATERIALS AND METHODS

The population of this study consists of (160) patients (women) aged (16-65) years as a thyroid dysfunction (hypo and hyper) disease; in addition to (40) healthy women as control group aged (16-65) years.

About (200) blood samples of women were collected from equivalent number of clinically diagnosed to be thyroid patients in Kalar General Hospital in the period between June (2015) until January (2016). They were divided into four groups:

1- Group (1) included (40) patients as the hypothyroidism before treatment.
2- Group (2) included (40) patients as the hypothyroidism after treatment (L-thyroxin 50 µg).
3- Group (3) included (40) patients as the hyperthyroidism before treatment.
4- Group (4) included (40) patients as the hyperthyroidism after treatment (Carbamizole 5 mg).

In addition, Blood was also collected from (40) healthy women as controls.

About (5 ml) of blood sample were obtained from patients and healthy individual groups, and centrifuged at 1500 rpm for 10 minutes to get the serum, which was divided to small portions and kept frozen at (-86°C) until further analysis.

2.1 Anthropometric Assessment of Subjects

Body mass index (BMI) was calculated from dividing weight in Kilogram by the square of height in meter. In adults BMI is a reliable and valid indicator of overweight and obesity at the population level because calculation requires only height and weight. BMI correlates well with percentage of body fat and is relatively unbiased by height (Figueroa, 2008).

Weight (Kg) of subjects were taken using standardized scales and Height (m) using a standardized measuring tape. BMI correlates well with thyroid problem Clinically Hypothyroidism causes an increase in body weight, while hyperthyroidism reduces it.

\[
\text{BMI} = \frac{\text{Weight (Kg)}}{\text{Height (m)}^2}.
\]

2.2 Estimation of serum peroxynitrite (ONOO\textsuperscript{-}) level

Serum peroxynitrite level was estimated according to the method of Mohammed, et al., (2011).

2.3 Estimation of serum Ceruloplasmin

The serum Ceruloplasmin activity was measured according to Menden method (Menden et al., 1977).
2.4 Estimation of Albumin concentration in serum

The serum Albumin was measured using routine clinical chemistry methods according to the Doumas method (Doumas, 1997).

2.5 Estimation of vitamin C in serum

Vitamin C level is based on the oxidation of ascorbic acid in serum according to the Omaye method (Omaye, et al., 1979).

2.6 Estimation of Glutathione in serum

Glutathione can be estimated according to the modified procedure utilizing Elman’s reagent by Banerjee method (Banerjee, 1999).

2.7 Statistical analysis

Statistical analysis was carried out by Statistical Package for the Social Science SPSS and Excel program. The results were expressed as mean ± standard deviation. The P-value was considered significant if it is < 0.05.

3. RESULTS AND DISCUSSION

Demographic characteristics of the study population are shown in table (1) and figure (1). In table (1), there were no differences in age between all studied groups as compared with control group. While, BMI in Group (1), and Group (2) had a significantly increased compared to control subjects, and there were a significant decreased in Group (3) and Group (4) as compared to control subjects. However, a significant increase between Group (1) and Group (2), and a significant decrease between Group (3) and Group (4) were found.

The relationship between body weight and thyroid function in individuals with euthyroid has been given a great medical concern. Some authors have studied the effect of the thyroid hormones on BMI, and it has been demonstrated that the thyroid dysfunction affects on body weight. Hypothyroidism leads to induce in body weight, whereas hyperthyroidism decrease it (Milionis, and Milionis, 2013). Our results is in agreement with the data of Humerah et al., (2016), who showed that in hypothyroid patients, increased BMI strongly supporting the previously reported correlation between the level of TSH and obesity (Marinou et al., 2010).

Table 1. Demographic characteristics of control, and all studied groups

| Groups       | N  | Age (years) | P     | BMI (kg/m²) | P   |
|--------------|----|-------------|-------|-------------|-----|
| Control      | 40 | 36.07±14.13 | N.S   | 23.361±3.591| P   |
| Group (1)    | 40 | 35.74±11.46 | N.S   | 30.804±4.962| S   |
| Group (2)    | 40 | 36.71±11.64 | N.S   | 26.621±3.324| S   |
| Group (3)    | 40 | 37.71±11.85 | N.S   | 20.768±4.572| S   |
| Group (4)    | 40 | 37.27±11.74 | N.S   | 22.057±5.090| S   |
Table (2) and figure (2) shows the concentration of serum peroxynitrite and ceruloplasmin of control, and all groups.

In table (2), the mean values of serum peroxynitrite in the circulating showed a significant increase in all groups as compare with control group. Also, the mean value of serum peroxynitrite in Group (1) are significantly higher than those of Group (2), and significant increase between Group (3) and Group (4) was found. The data showed a significantly increased peroxynitrite in both hypothyroid and hyperthyroid patients compared to controls. Increased in the formation of peroxynitrite has been related to many pathogenic diseases (Trujillo, et al. 2008). According to the results of this study a significant increase in peroxynitrite was found before the treatment of hypothyroidism. The peroxynitrite levels were found to be significantly lower after the treatment in hypothyroidic patients but still higher than the control after the treatment. In addition, the effect of nitric oxide is destroyed by superoxide radicals and stabilized by superoxide dismutase. The results are the elimination of these two radicals. If SOD is insufficient and/or excess amount of NO is generate, the $O_2^{•-}$ combines with short lived NO to produce a toxic peroxynitrite anion. The excessive amounts of NO can leads to damage of the cells either by toxicity of the molecule itself, or by peroxynitrite which is formed by its reaction with a superoxide radical. Excessive generation of nitric oxide causes several autoimmune disorders (Baskol et al., 2007). The results of the present study was in agreement with authors stated that peroxynitrite increased in patients with hypothyroidism and hyperthyroidism (Erdamar et al., 2008), but disagree with the same authors who reported that the peroxynitrite levels is reduced to about normal levels by treatment (Erdamar et al., 2008).

Also, in table (2), the level of CP non-significantly decreased in the all groups as compared to control. Also a non-significant increase between Group (1) and Group (2), and non-significant decrease between Group (3) and Group (4) were depicted. The thyroid hormones regulate synthesis and the degradation of non-enzymatic antioxidants, such as CP and transferrin. In our study the level of CP showed no variation in all the groups. This finding is in a disagreement with a study done by Sinha et al., (2015) who has been reported that the plasma CP level in patients with hyperthyroidism was increased. The explanation for this rise is that the ceruloplasmin is one of the acute phase reactant which increase in response to inflammation conditions. Also, some authors suggested that the level of CP was decrease in
thyroid dysfunction and this could be due to counter balance of the reactive oxygen species produced in the processes of lipid peroxidation and presence of copper or iron ions (Sirajwala et al., 2007). There are a number of factors which influence on the activity of antioxidant system: the physiological state of the thyroid gland, in addition to the dose and the duration of treatment (Solati et al., 2007). The current study was done only for a short term of six months but ‘long term of treatment with antioxidants (AO) along with L-thyroxin will definitely be beneficial as thyroid hormones have a strong effect on OS and the AO system giving rise to higher levels of CP and simultaneous decreasing of serum malondiadehyde (Bhattacharya et al., 2014). It was concluded that the patients with hypothyroid, even after treatment for six months, a still raised lipid peroxidation levels and its consequences are probably aggravated by the low levels of ceruloplasmin (Bhattacharya et al., 2014).

Table 2. Peroxynitrite and Ceruloplasmin levels for all groups in comparison with control.

| Groups    | N  | Peroxynitrite (µmol/L) | P    | Ceruloplasmin (U/L) | P    |
|-----------|----|------------------------|------|--------------------|------|
| Control   | 40 | 47.55 ± 2.75           |      | 0.0493±0.0047      |      |
| Group (1) | 40 | 56.59±5.41             | S    | 0.0473±0.0026      | N.S  |
| Group (2) | 40 | 52.82±4.93             | S    | 0.0481±0.005       | N.S  |
| Group (3) | 40 | 61.36 ± 5.30           | S    | 0.0483±0.0029      | N.S  |
| Group (4) | 40 | 55.52±5.91             | S    | 0.0478±0.0031      | N.S  |

Figure 2. Peroxynitrite, and Ceruloplasmin values for all groups in comparison with control.

Table (3) and figure (3) depicts the value of albumin, vitamin C, and glutathione in sera of control and all groups respectively. The level of Alb decreased significantly in the Group (1) compared to control, and a non-significant in Group (2), (3), and (4) as compared with control was reported. On the other hand, a significant differences between Group (1) and Group (2), and a non-significant between Group (3) and Group (4) were noticed. In the present study, a significant decrease in the level of albumin was observed between hypo-thyroidism and control group. This result was in agreement with results of pervious study (Kim et al., 2010). Vlassara et al., (2001) reported that albumin levels decreased and this decrease is due to increase synthesis of lipid peroxide and increase in the production of free radicals which leads to increase the permeability of the membranes and leaking the proteins outside the vascular system. In hyperthyroidism, our study was in a disagreement with Maes et al., (1999) who found that Alb was significantly decreased in patients with hyperthyroid, and the probable
explanation of this is that the Alb is the major transporter protein of zinc in plasma (Ali et al. 2007).

In table (3), the levels of vitamin C is significantly decreased in both Group (1) and Group (3) when compared with control, while a non-significantly decreased in both Group (2) and Group (4) when compared with control were observed. Also, a significantly increase of Group (1) with Group (2), and Group (3) with Group (4) were seen. In contrast with our results, Alicigüze et al., (2001) and Mohan et al., (2004) have described increase oxidative stress and low levels of vit C in hyperthyroidism patients at the same time, it also indicate that the vitamin become oxidized and it is consumed in exerting its antioxidant action. On the other hand, report from Moncayo et al., (2008) found decreased of vit. C levels in patients with hypothyroidism, hyperthyroidism, thyroid carcinoma, and thyroiditis. Our results were in disagreement with a study done by Uma et al., (2015) who observed that the level of vit C increase in patients with hypothyroid and hyperthyroidism. The high levels of vit. C is indicating the effect of OS on vit. C. Immediately after exposure to an OS, a decrease in antioxidants capacity is seen using the available antioxidants, but overtime there may be a response in the tissue, so the increase is seen in antioxidants (Zagorodna, 2005).

From the table (3), serum glutathione levels showed a significant decrease in all groups in comparison with the control. Also, a non-significant increase in GSH level of Group (1) with Group (2) and Group (3) with Group (4) were found. In mammals, the ROS levels are partially controlled by endogenous antioxidants; mainly GSH. The concentrations of GSH in the tissue and plasma are inversely related to the oxidative stress levels. A suppression of glutathione tissue signifies that reactive oxygen species and OS are inducing and the tissues became unprotected against the OS (Mohamed and Elnegris, 2015). It has been suggested that the regulation of GSH metabolism in mitochondria is obtained under the control of thyroid hormone (Das et al. 2001). In agreement with the results of the current study, some previous studies demonstrated that the serum level of glutathione decrease in hypothyroid (Pasupathi et al., 2008) and hyperthyroid patients (Ali et al., 2012, and Ayuoba et al., 2016).

Table 3. Albumin, Vitamin C, and Glutathione concentrations for control, and all studied groups

| Groups     | N  | Albumin (g/L)   | P     | Vitamin C (mg/dL) | P     | Glutathione (µmol/L) | P     |
|------------|----|----------------|-------|------------------|-------|----------------------|-------|
| Control    | 40 | 4.555±0.312    | P     | 7.973 ± 0.430    | P     | 4.111±0.116          | P     |
| Group (1)  | 40 | 3.610±0.333    | S     | 3.98 ± 0.72      | S     | 1.61 ± 0.308         | S     |
| Group (2)  | 40 | 4.479±0.346    | N.S   | 7.756±0.479      | N.S   | 1.749±0.270          | S     |
|            |    | 4.657±0.319    | N.S   | 3.703±0.35       | S     | 1.433±0.173          | S     |
| Group (3)  | 40 | 4.485±0.560    | N.S   | 7.91 ± 0.44      | N.S   | 2.867±0.299          | S     |
| Group (4)  | 40 |                | N.S   |                  |       |                      |       |
4. CONCLUSIONS

From the results of the present study there was significantly increased in the levels of peroxynitrite in all patients, before and after treatment compared to healthy control. Whereas, no significant differences in CP in the sera of both patient groups compared to control group were found before as well as after treatment. The albumin levels showed a non-significant differences in all studied groups except for untreated hypothyroid who showed significantly lower levels than those of control group. The concentration of vit. C was significantly lower in both patient groups before treatment compared to control. But on treatment, the vit. C levels showed a non-significant differences when compared with control group. While, serum GSH concentration showed a significant decrease in all groups in comparison with the control.

REFERENCES

AHMAD, R., SAH, A. K., and AHSAN, H. (2015) Peroxynitrite Modified Photoadducts as Possible Pathophysiological Biomarkers: A Short Review. J Mol Biomark Diagn. 7. Issue 1: 1-3

ALI, E. A., TAHSSEN, Y. H., SALEH, B. O. M. (2007) Study of Some Trace Elements in Hyperthyroidism Patients, The Iraqi Postgraduate Medical Journal, 6(2):113-117.

ALI, W. J. H., ALI R. K. H., ALFALLOUJI, S. (2012) The Correlation between Oxidative Stress and Thyroid Hormones in Serum and Tissue Homogenized of Hypothyroidism Patients, Medical Journal of Babylon, 9(4): 843- 849.

ALICIGUZEL, Y. (2001) "Zinc metabolism and thyroid status". Free-Radical-Biol-Med. 30: 665-670.

ALVA, S., PARAMESHA, S., SUCHETHA K. N., and MAHESH, D. G. K. M. (2011) Antioxidant Status And Serum Total Protein Levels In Elderly Women, International Journal of Applied Biology and Pharmaceutical Technology, 2: Issue-3: 521-524.

AMBADE, V., SONTAKKE, A., and BASANNA, D. (2014) Total Antioxidant Capacity: Correlation with Other Antioxidants and Clinical Utility of Their Levels in Chronic Obstructive Pulmonary Disease, International Journal of Biochemistry Research & Review, 4(2): 150-162

AYUOBA, N. N., EL-SHITANYC, N. A, and ALAMA M. N. (2016) Thymo-quinone protects against hypothyroidism-induced cardiac histopathological changes in rats through a nitric oxide/antioxidant mechanism, Biomedical Research, 27 (1):93-102.

BABU, K., JAYARAAJ, I. A., and PRABHAKA, J. (2011) Effect of Abnormal thyroid hormone
changes in lipid peroxidation and Antioxidant imbalance in Hypothyroid and Hyperthyroid patients, Int J Biol Med Res., 2(4): 1122-1126.

BANERJEE, B. D., SETH, V., BHATTACHARYA, A., PASHA, S. T., and CHAKRABORTY, A. K. (1999) Biochemical effects of some pesticides on lipid peroxidation and free-radical scavengers, Toxicology letters, 107(1): 33-47.

BASKOL, G., ATMACA, H., TANRIVERDI, F., BASKOL, M., KOCER, D., and BAYRAM, F. (2007) Oxidative Stress and Enzymatic Antioxidant Status in Patients with Hypothyroidism before and after Treatment. Exp Clin Endocrinol Diabetes. 115: 522-526.

BHATTACHARYA, A., SAHA, R., MONDAL, T., CHOUDHURI, S., and GADOTTI, S. G. G. SILVESTRINI, A., FESTA, R., TIANO, L., PONTECORVI, A., and MEUCCI, E. (2014) Ceruloplasmin and serum MDA levels in hypothyroid patients, IJBAR, 5(8): 369-372.

BURTON, J. (2011) Hyperthyroidism. MEDSURG Nursing. 20(3): 152-153.

CLEMENS, Z., and TÓTH, C. (2013) "Vitamin C and Disease: Insights from the Evolutionary Perspective," Journal of Evolution and Health, 1: Issue 1: 1-22.

DAS, K., and CHAINY, G. B. N. (2001) Modulation of rat liver mitochondrial antioxidant defense system by thyroid hormone. Biochimica et Biophysica Acta. 1537: 1-13.

DOUMAS, B. T., WATSON, W. A., and BIGGS, H. G. (1997) Albumin standards and the measurement of serum albumin with brom cresol green, Clinica chimica acta, 258(1): 21-30.

ERDAMAR, H., DEMIRCI, H., YAMAN, H., ERBIL, M. K., YAKAR, T., SANCAK, B., ELBEG, S., BIBEROĞLU, G., and YETKIN, I. (2008) The effect of hypothyroidism, hyperthyroidism, and their treatment on parameters of oxidative stress and antioxidant status. Clin Chem Lab Med. 46(7): 1004-1010.

FIGUEROA, B., VÉLEZ, H., and IRIZARRY-RAMIREZ, M. (2008) Association of thyroid-stimulating hormone levels and body mass index in overweight Hispanics in Puerto, Rico. Ethn Dis, 18(2Supp2): S2151-S2154.

FLEGAL, K. M., CARROLL, M. D., OGDEN, C. L., & CURTIN, L. R. (2010). Prevalence and trends in obesity among US adults, Journal of the American Medical Associatio,., 303(3): 235-241.

GARCIA-MARTINEZ, R., CARACENI, p., BERNARDI, M., GINES, p., ARROYO, V., and JALAN, R. (2013) Albumin: Pathophysiologic Basis of Its Role in the Treatment of Cirrhosis and Its Complications. Hepatology. 58: 1836-1846.

GE, M., O’REILLY, A., BAILLIE, N., MED D. H., TWENTYMAN, G., D. OBS, STURT, J., H, FITZPATRICK, M., TAYLOR, T., and OBS, D. (2008) Vitamin C: Evidence, application and commentary. NZFP. 35: 312-318.

GROSSO, G., BEI, R., MISTRETTA, A., MARVENTANO, S., CALABRESE, G., MASUELLI, L., GIGANTI, M. G., MODESTI, A., GALVANO, F., and GAZZOLO, D. (2013) Effects of Vitamin C on health: a review of evidence, Front Biosci., 1(18): 1017-29.

HUMERAH, S., SIDDIQUI, A., and KHAN, H. F. (2016) Pattern of Altered Lipid Profile in Patients with Subclinical and Clinical Hypothyroidism and its Correlation with Body Mass Index, Journal of the College of Physicians and Surgeons Pakistan, 26 (6): 463-466.

KARIM, R., NAHAR, Z., ISLAM, M. S., AHMED, M. U., MUSTAFA,A., SHOHAG, M. H., AL MARUF,A., and HASNAT, A. (2011) Serum MDA and Vitamin C level in Conversion Disorder Patients, Dhaka Univ. J. Pharm. Sci, 10(1): 59-64.

KIM, M. K., KWON, H. S., BAEK, K., LEEJ, H., PARK, W. C., SOHN, H. S., LEE, K., and SONG, K. (2010) Effects of Thyroid Hormone on A1C and Glycated Albumin Levels in Nondiabetic Subjects With Overt Hypothyroidism, Diabetes Care, 33: 2546–2548.

MAES, M., DEVOS, N., DEMEDTS, P., WAUTERS, A. and NEELS, H. (1999) Lower serum zinc in major depression in relation to changes in serum acute phase proteins, J Affect Disord, 56(2-3): 189-194.

MANCINI, A., RAIMONDO, S., SEGNI, C. D., and PERSANO, M., (2013) Thyroid Hormones and Antioxidant Systems: Focus on Oxidative Stress
in Cardiovascular and Pulmonary Diseases. Int. J. Mol. Sci. 14. p. 23893-23909.

MARINOU, K., TOUSOULIS, D., ANTONOPOULOS, A. S., STEFANADI, E., and STEFANADIS, C. (2010) Obesity and cardiovascular disease: from pathophysiology to risk stratification, Int J Cardiol, 138: 3-8.

MENDEN, E. E., BOIANO, J. M., MURTHY, L., and PETERING, H. G. (1977). Modification of a p-Phenylenediamine Oxidase Method to Permit Non-Automated Ceruloplasmin Determinations in Batches of Rat Serum or Plasma Microsamples, Analytical Letters, 10(3): 197-204.

MILIONIS, A., and MILIONIS, C. (2013) Correlation between Body Mass Index and Thyroid Function in Euthyroid Individuals in Greece, ISRN Biomarkers, 2013: 1-7.

MOHAMED, D. A, and ELNEGRIS, H. M. (2015) Histological Study of Thyroid Gland after Experimental Exposure to Low Frequency Electromagnetic Fields in Adult Male Albino Rat and Possible Protective Role of Vitamin E, J Cytol Histol, 6: Issue 6: 1-6.

MOHAMEDALI, M., MADDIKA, S. R., VYAS, A., IYER, V., and CHERIYATH, P. (2014) Thyroid Disorders and Chronic Kidney Disease", International Journal of Nephrology, 1-6.

MOHAMMED, S. M., AMIN, I. A., and SABRI, Z. Z. (2012) Nitric Oxide, Peroxynitrite and Malondialdehyde Levels as Markers for Nitrosative/Oxidative Stress in Iraqi Patients with Systemic Lupus Erythematosus. Iraq J. Pharm. Sci. 21: 87-92.

MOHAN, K. K. M., BOBBY, Z., SELVARAJ, N., KUMAR, D. A., CHANDRA, K. B., SEN, S. K., RAMESH, R., and RANGANATHAN, P. (2004) Possible link between glycated hemoglobin and lipid peroxidation in hyperthyroidism, Clin. Chim. Acta, 342: 187-92.

MONCAYO, R., KROISS, A., OBERWINKLER, M. KARAKOLCU, F., STARZINGER, M., KAPELARI, K., TALASZ, H., AND MONCAYO. H. (2008) The role of selenium, vitamin C, and zinc in benign thyroid diseases and of selenium in malignant thyroid diseases: low selenium levels are found in subacute and silent thyroiditis and in papillary and follicular carcinoma, BMC Endocr Disord, 8(2): 1-12.

MOORE, L. (2016) "Thyroid disease in pregnancy: A review of diagnosis, complications and management", World J ObstetGynecol, 5: 66-72.

OMAYE, S. T., TUMBALL, J., and SUBERLICH, H. E. (1979) Selected methods for the determination of ascorbic acid in animal cells, tissues and fluids. Elsevier. 62: 3-11.

OMIDI, A., KHEIRIE, M., SARIR, H. (2015) Impact of vitamin C on concentrations of thyroid stimulating hormone and thyroid hormones in lambs under short-term acute heat stress, Veterinary Science Development, 5(5965): 103-106.

PACIER, C., and MARTIROSYAN, M. (2015) Vitamin C: optimal dosages, supplementation and use in disease prevention, Functional Foods in Health and Disease, 5(3): 89-107.

PARK, W. R., OH, T. K., and JEON, H. J. (2013) Prospective observation of 5-year clinical course of subclinical hypothyroidism in Korean population. Journal of Korean Medical Science. 28(11): 1622-1626.

PASUPATHI, P., and LATHA, R. (2008) Free radical activity and antioxidant defence mechanisms in patients with Hypothyroidism, Thyroid Sci, 3(12): 1-6.

PETRULEA, M. S., DUNCEA, I., HAZI, G., DRAGOTOIU, G., DECEA, N., and MUREŞAN, A. (2010) Oxidative Stress In Experimental Hypothyroidism: Effect Of Vitamin E Supplementation, Clujul Medical, LXXXIII (2): 245-249.

SHON, H. S., JUNG, E. D., KIM, S. H., and LEE, J. H. (2008) "Free T4 is negatively correlated with body mass index in euthyroid women", Korean Journal of Internal Medicine, 23: 53-57.

SINHA, S., KAR, K., DASGUPTA, A., BASU, S., and SEN. (2015) Correlation of Serum zinc with TSH in hyperthyroidism, Asian Journal of Medical Sciences, 7: Issue 1: 66-69.

SIRAJWALA, H. B., DAHHI, A. S. , MALUKAR, N. R. , BHALAMI, R. B. , and PANDYA, T. P. (2007) Serum ceruloplasmin levels as an
extracellular antioxidant in diabetes mellitus patients, JIACM, 8(2): 135-138.

SIX-MERKER, J., MEISINGER, C., JOURDAN, C., HEIER, M., HAUNER, H., PETERS, A., and LINSEISEN, J. (2016) Treatment of Thyroid Dysfunctions Decreases the Risk of Cerebrovascular Events in Men but Not in Women: Results of the MONICA/KORA Cohort Study, PLOS ONE, 11(5): 1-15.

SOLATI, S. M., ATTAEI, L., and AZIZI, F. (2007) Lipid oxidation, antioxidants and paraoxonase enzyme activity in patients with subclinical thyrotoxicosis, Darunriz Va Mitabulim –Iran, 8(4): 317-323.

TRUJILLO, M., FERRER-SUETA, G., and RADI, R. (2008) Peroxynitrite Detoxification and Its Biologic Implications. Antioxidants & Redox Signaling. 10(9): 1607-1620.

UMA, T. SANGEETHA, B., and HARITHA, B. (2015) The Study Of Lipid Profile Levels, Oxidative Stress And Thyroid Status In Thyroid Disorders, RA Journal of Applied Research, 1: Issue 2: 55-61.

VLASSARA, H., M. BROWNLE, CERAMI, A. (2001) Non-enzymetic glycosylation of peripheral nerve protein in diabetes mellitus, 78(8): 5190-5192.

ZAGORODNA, O. S. (2005) Free radicals in biology and medicine, Spring, 77: 222, 1.