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Estimation of Serum Protein Carbonyl and Vitamin D in Overt and Subclinical Iraqi Hypothyroidism Patients

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Abstract

Hypothyroidism is a disease caused by a dysfunction in the thyroid gland. The disease is divided into overt hypothyroidism (OHT) and subclinical hypothyroidism (SHT) according to the clinical signs and thyroid function hormones levels. Oxidative stress is a serious health situation related to many pathological conditions, including hypothyroidism. Oxidative damage can be caused at any cellular level including lipids, proteins, and nucleic acids. Protein carbonyl (PCO) is an indicator of protein damage caused by oxidative stress. Vitamin D perform a significant part in the human health, and its deficiency has become a global issue. We have tested the role of PCO and vitamin D in OHT and SHT types of hypothyroidism and investigated the possibility of using these two parameters in the screening of hypothyroidism. For this purpose 120 individuals, and included in three groups equally, OHT patients, SHT patients, and healthy control. The results were showed a significant (p<0.05) reduction in serum level of vitamin D for OHT patients compared to control and SHT groups, while the differences of vitamin D level were non-significant (p>0.05) between SHT and control groups. The level of PCO was elevated significantly in OHT and SHT groups compared to control, while SHT and OHT groups were showed a non-significant differences in PCO levels. Additionally, no correlation was found between vitamin D and PCO neither in OHT patients, nor in SHT patients. The sensitivity of vitamin D in the screening of OHT was good, but it was poor in SHT, while the sensitivity of PCO was good in both OHT and SHT groups. The results suggest a role of vitamin D deficiency in the pathophysiology of OHT, as well as elevated oxidative stress caused by protein carbonylation in both OHT and SHT groups.

keywords:Hypothyroidism, Hashimoto's disease, Protein carbonyl, vitamin D;

1. Introduction

Hypothyroidism has grown in rates of incidence since the last few decades [1, 2]. The disease is more frequent in women than men [3], nevertheless, hypothyroidism was reported in men with serious rates of incidence [4]. The etiology of the disease can attributed mainly to two major reasons; either iodine deficiency (goiter) [5], or immunological reasons (Hashimoto's disease) [6]. Furthermore, it can be categorized according to the clinical signs and thyroid function test to overt hypothyroidism (OHT) or subclinical hypothyroidism (SHT). The OHT is characterized by an elevated levels of thyroid stimulating hormone (TSH) and reduced levels of thyroxine (T4) [7], while SHT is characterized by elevated levels of TSH and normal levels of T4 hormone [8].

Free radicals contribute to oxidative damage of cells [9], which leads eventually to increase cell apoptosis and various pathological effects [10]. Because of the instability and extreme reactivity of the free radicals, they react with the macromolecules of the cellular compartments initiating a sequence of damaging reactions [11] including lipid peroxidation [12], protein oxidation [13], and nucleic acid modifications [14]. The damaging effects of free radicals are termed as oxidative stress [15]. Protein carbonyl (PCO) has been used as indicator of oxidative stress, in which it represents the content of oxidative protein damage caused by free radicals and reactive oxygen species (ROS) [16]. The literature has shown an elevated oxidative stress status in both OHT and SHT conditions of hypothyroidism [17, 18].

Vitamin D is an important cholesterol-derivative molecule with various benefits to the human health [19]. Vitamin D_3 is the type that produced by human in sequential steps that starts in the skin and ended in the kidneys [20]. Vitamin D would also be viewed as a required substrate for the production of a group of hormones that are well known for regulating calcium

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metabolism [21]. Furthermore, the active form of vitamin D_3 binds to a nuclear receptor of many target cells of most tissues, making vitamin D_3 to act as endocrine signalling agent [22]. The deficiency of vitamin D is considered as the most commonamong other vitamins [23]. A previous study has linked the deficiency of vitamin D with the severity of hypothyroidism [24]. In this study, we have measured the oxidative damage on protein level by evaluating PCO in overt and subclinical hypothyroidism, as well as, we have tested the role of vitamin D in OHT and SHT patients. Additionally, the study was included a sensitivity test of using PCO and vitamin D in the screening of OHT and SHT.

2. Experimental

2.1. Hypothyroidism patients

This work was performed on hypothyroidism patients inConsultant Department at Al-Yarmook Teaching Hospital (Baghdad, Iraq). A total of 80 hypothyroidism patients whom diagnosed by the medical senior doctor, and they were divided equally into two groups, 40 individuals with OHT disease and 40 individuals with SHT. In addition to, 40 healthy individuals with same age were used as control for the study. All the three groups were contained males and females in equivalentpercentage. All the volunteerspatients were collected from September to December 2021.

The separated blood serum was stored in three different tubes at -20 °C until the time of analyses.

2.2. Methods

The stored serum of participants was used to analyze the concentrations of TSH, triiodothyronine (T3), T4, PCO, and vitamin D. The thyroid function tests were performed on cobas e411 device, while the level of PCO was performed by an Enzyme-Linked Immune Sorbent Assay (ELISA) human kit based on technology (Biont, China). Human vitamin D was analyzed by using competitive ELISA kit of human vitamin D (Human, Germany). Both PCO and vitamin D were analyzed by using ELISA microplate reader from Human (Germany). The results were processed statistically through analysis of variances (ANOVA), the highest significant difference (HSD) test, Pearson's correlation, and the receiver operating characteristics (ROC) curve.

3. Results

The anthropometric characteristics of the subjects whichenrolled in this study are listed in Table 1. The differences of age were non-significant (p>0.05) among control (37.85±8.67 year), OHT patients (38.13±11.67 year), and SHT patients (38.73±11.03 year).

The value of BMI was observed to be significantly (p<0.05) higher in the OHT patients (27.85±2.72 $kg.m^{-2}$), and the SHT patients (25.73±2.67 $kg.m^{-2}$), compared to the control subjects (23.02±2.12 $kg.m^{-2}$). Furthermore, the BMI value was remarkablyhigher (p<0.05) in OHT compared to SHT patients.

The value of waist circumference was observed to be significantly higher (p<0.05) in the OHT patients (99.90±8.72 cm), and the SHT patients (94.28±9.61 cm), compared to the control subjects (80.98±5.36 cm). Moreover, WHpR was significantly higher (p<0.05) in the OHT patients (0.87±0.06), and the SHT patients (0.82±0.09), compared to the control subjects (0.78±0.06). Moreover, the waist circumference, and WHpR values were significantly higher (p<0.05) in OHT compared to SHT patients.

The thyroid function was determined by measuring the levels of TSH, T3 and T4 hormones. The results of these hormones are shown in Table 2 in the form of Mean±SD. The level of TSH was significantly elevated (p<0.05) in the serum of OHT patients (15.98±5.04 mIU/L) and SHT patients (14.1±3.06 mIU/L), compared to control (2.37±0.76 mIU/L). Furthermore, OHT patients have shown significant higher levels (p<0.05) of serum TSH compared to SHT patients.

The level of T3 hormone was significantly reduced (p<0.05) in the serum of OHT patients (0.98±0.34 nmol/L) compared to SHT patients (1.25±0.36 nmol/L) and control (1.33±0.31 nmol/L). Nevertheless, non-significant differences (p>0.05) of T3 hormone levels were observed between SHT patients and control.

The level of T4 hormone was significantly reduced (p<0.05) in the serum of OHT patients (2.64±1.12 µg/dL) compared to SHT patients (7.61±1.61 µg/dL) and control (7.38±1.18 µg/dL). Nevertheless, non-significant differences (p>0.05) of T4 hormone levels were observed between SHT patients and control.

The level of vitamin D_3 was reduced significantly (p<0.05) in the serum of OHT patients (14.11±5.19 ng/mL) compared to SHT patients (17.58±7.39 ng/mL) and control (20.12±4.92 ng/mL). Nevertheless, the differences of serum vitamin D_3 level were non-significant (p>0.05) between SHT patients and control, Table 2.

The level of PCO was significantly elevated (p<0.05) in the serum of OHT patients (22.10±7.67

ng/mL), and SHT patients (19.12±5.04 ng/mL), compared to control (13.24±5.05 ng/mL). Nevertheless, PCO level was non-significantly higher (p>0.05) in the serum of OHT patients compared to SHT patients, Table 2.

There were no significant correlation (p>0.05) between vitamin D and PCO neither in OHT patients (r=-0.123, P=0.449), nor in SHT patients (r=-0.030, P=0.855), as shown in Figure 1.

The ROC curve has indicated that PCO is a good sensitive marker in the diagnosis of OHT, in which it has exhibited an AUC of 0.856 with p-value 0.0001, when tested with respect to people with normal thyroid function. Furthermore, the cut-off PCOvalue obtained for OHT was shown to be 16.19 ng/mL with 80% sensitivity and 75% specificity. This means that when the level of PCO is obtained higher than 16.19 ng/mL it may refer to an overt hypothyroidism. The ROC curve of PCO for OHT is shown in Figure 2.

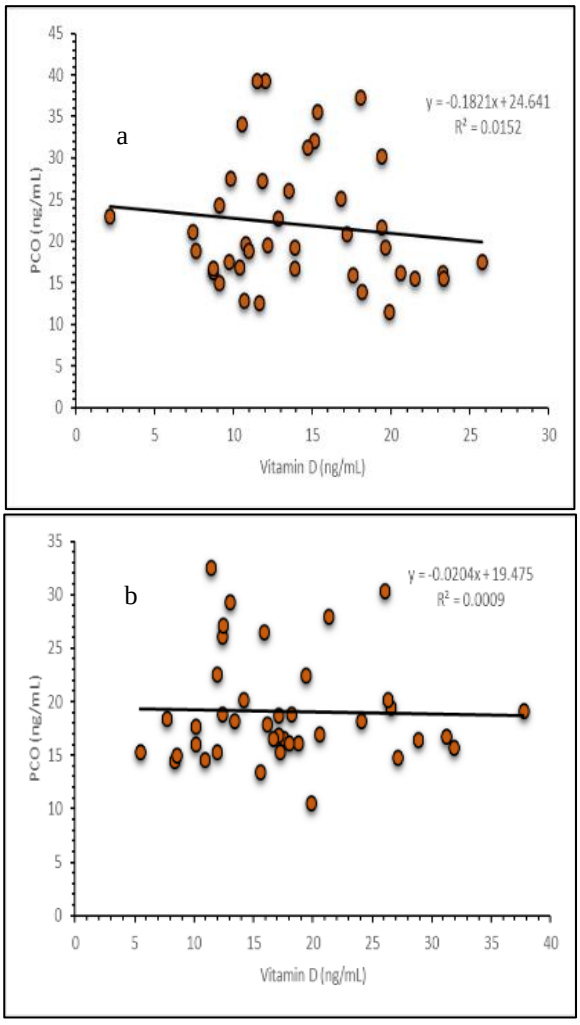

Figure 1: The correlation between PCO and vitamin D in OHT patients (a) and SHT patients (b).

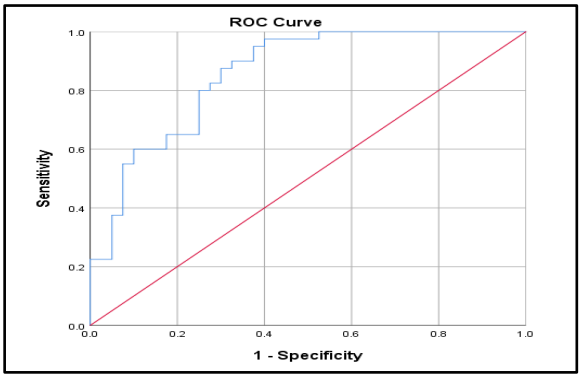

Figure 2: ROC curve of PCO for Overt hypothyroidism compared to healthy control

The ROC curve has indicated that PCO is a good sensitive marker in the diagnosis of SHT, in which it has exhibited an AUC of 0.815 with p-value 0.0001, when tested with respect to people with normal thyroid function. Furthermore, the cut-off PCO value obtained fromPCO for SHT was shown to be 15.29 ng/mL with 85% sensitivity and 70% specificity. This means that when the level of PCO is obtained higher than 15.29 ng/mL it may refer to an overt hypothyroidism. The ROC curve of PCO for SHT is shown in Figure 3.

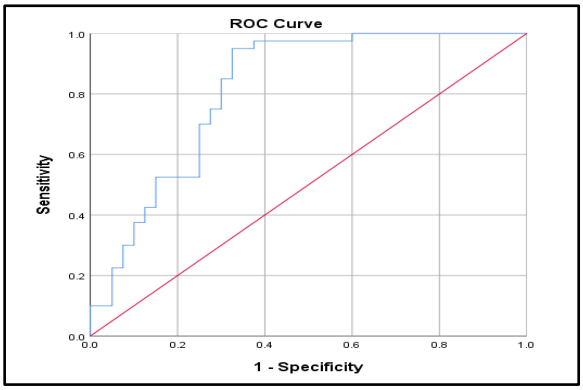

Figure 3: ROC curve of PCO for Subclinical hypothyroidism compared to healthy control

The ROC curve has indicated that vitamin D_3 is a good sensitive marker in the diagnosis of OHT, in which it has exhibited an AUC of 0.800 with p-value 0.0001, when tested with respect to people with normal thyroid function. Furthermore, the cut-off value obtained for vitamin D_3 for OHT was shown to be 15.41 ng/mL with 80% sensitivity and 65% specificity. This means that when the level of vitamin D_3 is obtained below 15.41 ng/mL it may refer to an overt hypothyroidism. The ROC curve of vitamin D_3 for OHT is shown in Figure 4.

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Table 1: Characteristic of the study subjects

Parameter		Control, N=40 Mean±SD	OH, N=40 Mean±SD	SbH, N=40 Mean±SD	*p*-value
Age (year)		37.85±8.67[a]	38.13±11.67[a]	38.73±11.03[a]	0.931
BMI (kg/m2)		23.02±2.12[a]	27.85±2.72[b]	25.73±2.67[c]	0.0001*
Waist circumference (cm)		80.98±5.36[a]	99.90±8.72[b]	94.28±9.61[c]	0.0001*
WHpR		0.78±0.06[a]	0.87±0.06[b]	0.82±0.09[c]	0.0001*
Gender	Male%	50%	50%	50%	0.999
	Female%	50%	50%	50%	

Same letters indicate non-significant differences ($p>0.05$), while different letters indicate significant differences ($p\leq0.05$) according to the post-Hoc Tukey's test.
* Significant at $p\leq0.05$ according to ANOVA test.

Table 2: The levels of biochemical parameters in patients and control

Parameter	Control, N=40 Mean±SD	OH, N=40 Mean±SD	SbH, N=40 Mean±SD	*p*-value
TSH (mIU/L)	2.37±0.76[a]	15.98±5.04[b]	14.1±3.06[c]	0.0001*
T3 (nmol/L)	1.33±0.31[a]	0.98±0.34[b]	1.25±0.36[a]	0.0001*
T4 (µg/dL)	7.38±1.18[a]	2.64±1.12[b]	7.61±1.61[a]	0.0001*
Vitamin D3 (ng/mL)	20.12±4.92a	14.11±5.19b	17.58±7.39a	0.0001*
PCO (ng/mL)	13.24±5.05a	22.10±7.67b	19.12±5.04b	0.0001*

Same letters indicate non-significant differences ($p>0.05$), while different letters indicate significant differences ($p\leq0.05$) according to the post-Hoc Tukey's test.
* Significant at $p\leq0.05$ according to ANOVA test.

The ROC curve has indicated that vitamin D_3 is a poor sensitive marker in the diagnosis of SHT, in which it has exhibited an AUC of 0.642 with *p*-value 0.028, when tested with respect to people with normal thyroid function. Furthermore, the cut-off value obtained for vitamin D_3 for SHT was shown to be 17.67 ng/mL with 70% sensitivity and 60% specificity. This means that when the level of vitamin D_3 is obtained below 17.67 ng/mL it may refer to a subclinical hypothyroidism. The ROC curve of vitamin D_3 for SHT is shown in Figure 5.

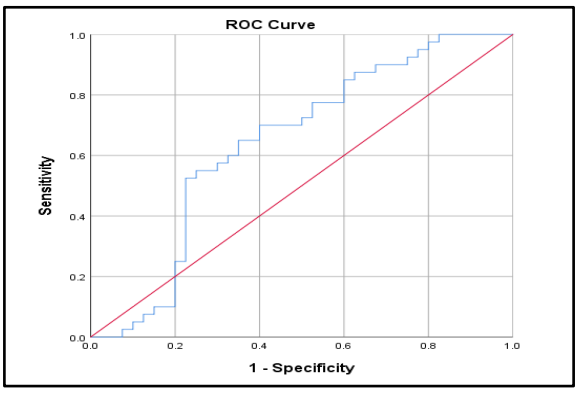

Figure 4: ROC curve of vitamin D_3 for Overt hypothyroidism compared to healthy control.

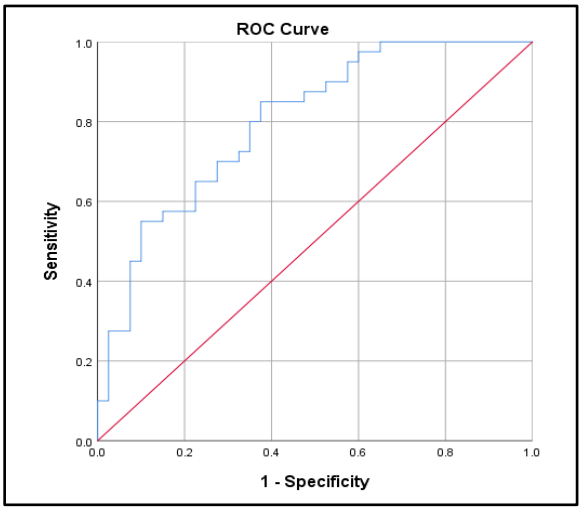

Figure 5: ROC curve of vitamin D_3 for Subclinical hypothyroidism compared to healthy control.

4. Discussion

The study was designed on the bases of standard criteria, in which the age and gender distribution were similar in the three groups to eliminate the effects of these two variables on the clinical results of the study, of particular the oxidative stress (PCO). While the BMI, was obtained as significantly higher in OHT and SHT patients compared to control. Several studies

have reported that patients with OHT or SHT disease were either obese or overweight [25-28].

Choi *et al.*[29] have reported that vitamin D3 insufficient is associated with increasing the risk of autoimmune thyroid diseases in women below menopause age. Furthermore, the previous study have indicated that of 6685 subjects (males and females) the percentages of autoimmune thyroid patients were 21.2% in people with vitamin D_3 deficiency, 15.5% in people with vitamin D_3 insufficiency, and 12.6% in people with normal vitamin D_3 levels. The results of Choi *et al.* enable them from presuming an important role of vitamin D_3 in the health of the ThG. This scientific observations are in agreement with the results of the present study.

Haribabu*et al.*[30] have reported significantly elevated levels of MDA and PCO in patients with OHT and SHT diseases compared to the control of their study. The authors have also found that the levels of MDA and PCO are higher in OHT compared to SHT patients. The resultsof Haribabu*et al.* team are in agreement with the present study, except for the significant increasing ofPCO levels in OHT compared to SHT which was inconsiderableat the present study. Furthermore, the authors have concluded that an oxidative damage caused in lipid peroxidation and protein oxidation in patients with hypothyroidism, and they have suggested a role of elevated TSH levels to the development of oxidative stress. Öztürk*et al.*[31]have tested the levels of PCO and MDA in Hashimoto's patients with OHT and SHT. The workers have reported a significant elevation of PCO and MDA in patients with OHT. Nevertheless, they have reported significant elevated levels of PCO but not MDA in the serum of SHT patients. Additionally, the workers have reported significant higher levels of both PCO and MDA in OHT patients compared to SHT patients. The findings of Öztürk*et al.* are in agreement with the results of MDA and PCO which was significant in the present study, and the significant elevated levels of PCO in OHT patients compared to SHT patients which was non-significant in the present study. Chandra and Iqbal[32] have investigated the oxidative stress in newly diagnosed hypothyroidism patients. The authors have been reported a significant growing in thelevels of MDA and PCO in the serum of newly diagnosed patients compared to the control of their study, this indication matching with the results of oxidative stress in the present study.

Cvek*et al.*[33] have examined the level of vitamin D_3 in OHT and SHT patients. The workers have reported a non-significant role of vitamin D_3 in both SHT and OHT, which is disagreed with the current results. On the other hand, the authors have indicated a significant lower vitamin D_3 levels in OHT compared to SHT patients, which is in agreement with the current study. Makadia*et al.*[34] have reported a non-significant differences of serum vitamin D_3 levels in patients with SHT compared to the control of their study. This outcomes are in agreement with the obtained data from the comparison of vitamin D_3 between control and SHT in the present study. Sudha*et al.*[35] have reported a non-significant differences of vitamin D_3 between newly diagnosed patients with SHT and control, which is in agreement with the current observations. Nevertheless, the authors have indicated a high prevalence (38%) of vitamin D_3 deficient people in SHT patients. Metwalley*et al.*[36] have examined the status of vitamin D_3 in Egyptian hypothyroidism patients. The authors have reported significant lower levels of serum vitamin D_3 in hypothyroidism patients compared to the control of their study. Moreover, they have reported a significant lower levels of serum vitamin D_3 in OHT patients compared to SHT patients. This report was in agreement with the current observations of vitamin D_3.

5. Conclusions

The results of our study revealed the PCO level increased in both OHT and SHT patients. This means that oxidative stress enhances on protein level in hypothyroidism. The oxidative damage of protein was non-significantly differencebetween OHT and SHT, and this may indicate that protein damage starts at very early stage of hypothyroidism. Further investigations are required on PCO in hypothyroidism disease. Furthermore, a significant shortageof vitamin D in the serum of OHT patients, while it was approximate to the control in SHT patients. This may to the role role of vitamin D deficiency in the onset of OHT.

6. Conflicts of interest

There are no conflicts to declare.

7. Acknowledgments

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8. References

[1] Ragusa, F., et al., Hashimotos' thyroiditis: Epidemiology, pathogenesis, clinic and therapy. Best Practice & Research Clinical Endocrinology & Metabolism, 2019. 33(6): p. 101367.

[2] Taylor, P.N., et al., Global epidemiology of hyperthyroidism and hypothyroidism. Nature Reviews Endocrinology, 2018. 14(5): p. 301-316.
[3] Terranova, P., Hypothyroidism☆, in Reference Module in Biomedical Sciences. 2017, Elsevier, pp. 1-669.
[4] Vanderpump, M.P., Epidemiology of thyroid disorders, in The thyroid and its diseases. 2019, Springer. p. 75-85.
[5] Terranova, P., Hypothyroidism, in xPharm: The Comprehensive Pharmacology Reference, S.J. Enna and D.B. Bylund, Editors. 2007, Elsevier: New York. p. 1-3.
[6] Ahmed, R., S. Al-Shaikh, and M. Akhtar, Hashimoto thyroiditis: a century later. Advances in anatomic pathology, 2012. 19(3): p. 181-186.
[7] Imaizumi, M., et al., Risk for progression to overt hypothyroidism in an elderly Japanese population with subclinical hypothyroidism. Thyroid, 2011. 21(11): p. 1177-1182.
[8] Peeters, R.P., Subclinical hypothyroidism. New England Journal of Medicine, 2017. 376(26): p. 2556-2565.
[9] Halliwell, B. and J.M. Gutteridge, Free radicals in biology and medicine. 2015: Oxford university press, USA.
[10] Mohammed, M.T., et al., Free radicals and human health. International Journal of Innovation Sciences and Research, 2015. 4(6): p. 218-223.
[11] Alkadi, H., A review on free radicals and antioxidants. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 2020. 20(1): p. 16-26.
[12] Su, L.-J., et al., Reactive oxygen species-induced lipid peroxidation in apoptosis, autophagy, and ferroptosis. Oxidative medicine and cellular longevity, 2019. 2019.
[13] Unsal, V., M. Cicek, and İ. Sabancilar, Toxicity of carbon tetrachloride, free radicals and role of antioxidants. Reviews on environmental health, 2021. 36(2): p. 279-295.
[14] Adwas, A.A., et al., Oxidative stress and antioxidant mechanisms in human body. J. Appl. Biotechnol. Bioeng, 2019. 6(1): p. 43-47.
[15] Sies, H., Oxidative stress: Concept and some practical aspects. Antioxidants, 2020. 9(9): p. 852.
[16] Chevion, M., E. Berenshtein, and E. Stadtman, Human studies related to protein oxidation: protein carbonyl content as a marker of damage. Free radical research, 2000. 33: p. S99-108.
[17] Santi, A., et al., Association between thyroid hormones, lipids and oxidative stress biomarkers in overt hypothyroidism. Clinical Chemistry and Laboratory Medicine, 2010. 48(11): p. 1635-1639.
[18] Chakrabarti, S.K., et al., Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. Indian journal of endocrinology and metabolism, 2016. 20(5): p. 674.
[19] Holick, M.F., Vitamin D: A millenium perspective. Journal of cellular biochemistry, 2003. 88(2): p. 296-307.
[20] Adams, J.S. and M. Hewison, Update in vitamin D. The Journal of Clinical Endocrinology & Metabolism, 2010. 95(2): p. 471-478.
[21] Reid, I.R., Vitamin D☆, in Encyclopedia of Endocrine Diseases (Second Edition), I. Huhtaniemi and L. Martini, Editors. 2018, Academic Press: Oxford. p. 75-81.
[22] Bikle, D., Nonclassic actions of vitamin D. The Journal of Clinical Endocrinology & Metabolism, 2009. 94(1): p. 26-34.
[23] Prentice, A., Vitamin D deficiency: a global perspective. Nutrition reviews, 2008. 66(suppl_2): p. S153-S164.
[24] Mackawy, A.M.H., B.M. Al-Ayed, and B.M. Al-Rashidi, Vitamin d deficiency and its association with thyroid disease. International journal of health sciences, 2013. 7(3): p. 267-275.
[25] Cikim, A.S., et al., Evaluation of endothelial function in subclinical hypothyroidism and subclinical hyperthyroidism. Thyroid, 2004. 14(8): p. 605-609.
[26] Ashizawa, K., et al., Metabolic cardiovascular disease risk factors and their clustering in subclinical hypothyroidism. Clinical endocrinology, 2010. 72(5): p. 689-695.
[27] Kung, A., R. Pang, and E. Janus, Elevated serum lipoprotein (a) in subclinical hypothyroidism. Clinical endocrinology, 1995. 43(4): p. 445-449.
[28] Yasar, H.Y., et al., Serum irisin and apelin levels and markers of atherosclerosis in patients with subclinical hypothyroidism. Archives of endocrinology and metabolism, 2019. 63: p. 16-21.
[29] Choi, Y.M., et al., Low levels of serum vitamin D3 are associated with autoimmune thyroid disease in pre-menopausal women. Thyroid, 2014. 24(4): p. 655-661.
[30] Haribabu, A., et al., Evaluation of protein oxidation and its association with lipid peroxidation and thyrotropin levels in overt and subclinical hypothyroidism. Endocrine, 2013. 44(1): p. 152-157.
[31] Öztürk, Ü., et al., Oxidative stress parameters in serum and low density lipoproteins of Hashimoto's thyroiditis patients with subclinical and overt hypothyroidism. International Immunopharmacology, 2012. 14(4): p. 349-352.
[32] Chandra, K. and J. Iqbal, Protein carbonylation in freshly diagnosed hypothyroidism is independent of thyrotropin levels. LaboratoriumsMedizin, 2018. 42(5): p. 177-181.
[33] Cvek, M., et al., Vitamin D and Hashimoto's Thyroiditis: Observations from CROHT Biobank. Nutrients, 2021. 13(8): p. 2793.
[34] Makadia, M.G., et al., Study of Glycated Haemoglobin (HbA1c) In Non-Diabetic Subjects with Subclinical Hypothyroidism. Journal of clinical and diagnostic research : JCDR, 2017. 11(4): p. BC01-BC04.
[35] Sudha, K., et al., Prevalence of vitamin D deficiency and its relationship with subclinical hypothyroidism. International Journal of Pharma and Bio Sciences, 2013. 4(4).
[36] Metwalley, K.A., et al., Vitamin D status in children and adolescents with autoimmune thyroiditis. Journal of Endocrinological Investigation, 2016. 39(7): p. 793-797.