The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7818- 7824

Oxidative Stress Biomarkers in Thyroid Dysfunction: From Overt Hypothyroidism to Low Normal Thyroid Hormones

Maha Rakha[1], Nashwa S Ghanem[1], Laila A Rashed[2], Dina Ali Ezzat[3], Heba Habeeb[1], Hend A Elsheimy[1*]
[1]Department of Internal Medicine, [2]Department of Medical Biochemistry and Molecular Biology, [3]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Egypt.
***Corresponding author:** Hend A Elsheimy**, Email: hendaelshemy@gmail.com, Mobile: +201091648680,**
https://orcid.org/0000-0001-8790-9804

ABSTRACT

Background: Increased oxidative stress causes damage to all molecular targets potentially because of discrepancy between antioxidant defensive mechanisms and reactive oxygen species (ROS). Previous studies have reported enhanced oxidative stress in subclinical hypothyroidism (SCH). Dyslipidemia and oxidative stress screening can help the overall management of hypothyroidism and decrease cardiovascular morbidity.

Objective: This study evaluates the degree of oxidative stress in hypothyroid dysfunction, including low-normal thyroid states, by estimating some serum oxidative biomarkers and correlating them to lipid profile as well as defining the cut-off value of thyroid-stimulating hormone, free thyroxin, and free triiodothyronine associated with increased oxidative biomarkers.

Methods: This study included 60 adult patients with hypothyroidism who were subdivided into three equal groups. Group A included overt hypothyroidism, group B consisted of subclinical hypothyroidism (SCH), and group C that contained low-normal thyroid status (*high TSH, low FT4 and FT3 levels within the normal values*). A control group, which included 20 healthy control subjects. Oxidative biomarkers, thyroid hormones, and lipids were estimated.

Results: Urinary allantoin and serum MDA were significantly higher in groups A, B, and C compared with the control group ($P < 0.001$) with a significant positive correlation with TSH, total cholesterol, and low-density lipoprotein cholesterol *(*$P < 0.001$). Significant dyslipidemia was demonstrated in patients with overt hypothyroidism and SCH compared to the control group ($P < 0.001$ and $P = 0.008$ respectively). FT3 was an independent predictor for MDA and urinary allantoin. The receiver operating characteristic curve analysis detected the cut-off values of TSH (≥ 4.2 and 3.6), FT4 (≤ 1 and 1.3), and FT3 (≤ 3.06 and 3.08) and predicted allantoin and MDA elevation, respectively ($P < 0.001$) and hence oxidative stress.

Conclusion: Hypothyroidism, even low-normal thyroid status is a state of increased oxidative stress. The efficacy of levothyroxine treatment and antioxidant supplements in these individuals should be tested.

Keywords: Allantoin, Malondialdehyde, Oxidative stress, Thyroid hormones, Cut-off value.

INTRODUCTION

Increased oxidative stress causes damage to all molecular targets potentially caused by the discrepancy between antioxidant defensive mechanisms and reactive oxygen species (ROS) [(1)]. Previous studies have reported enhanced oxidative stress in subclinical hypothyroidism (SCH). Dyslipidemia and oxidative stress screening can help the overall management of hypothyroidism and decrease cardiovascular morbidity [(2)].

A recent concept that evolved low-normal thyroid status, which is defined as high normal thyroid-stimulating hormone (TSH) levels and/or low-normal free thyroxin (FT4) levels, could accelerate the pathogenesis of the atherosclerotic cardiovascular disease. The low-normal thyroid state is possibly associated with a mild increase in plasma triglycerides (TGs), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-c), as well as insulin resistance. Impaired high-density lipoprotein cholesterol (HDL-c) inhibits oxidative modification of LDL-c and has been related to low-normal thyroid states [(3)].

The occurrence of increased oxidative stress independent dyslipidemia in low-normal thyroid states remained to be elucidated. Overt hypothyroidism and SCH have been linked to an increased risk of coronary events and worsening heart failure; hence, low-level thyroid hormone may be considered a cardiovascular risk factor [(4)].

Malondialdehyde (MDA) was described as a marker for increased lipid peroxidation, which leads to increased free radical production and altered structure and function of proteins [(5)].

The metabolism of purine in humans ends with uric acid production, which is a powerful antioxidant and ROS scavenger. Allantoin is the main product of free radical-induced uric acid oxidation that is excreted in urine and could be used as an oxidative stress biomarker in humans regardless of uric acid level [(6)].

This study aimed to assess the degree of oxidative stress among different grades of hypothyroidism, even the low-normal thyroid status, by estimating serum MDA and urinary allantoin levels as oxidative stress markers. Secondary endpoints were to correlate them to lipid profile and define the cut-off value of TSH, FT4, and FT3 associated with increased oxidative stress biomarkers and in turn oxidative stress.

Received:19/7/2022
Accepted:21/9/2022

MATERIALS AND METHODS

Study design and allocation:

This cross-sectional case-control study included 80 subjects (74 females and 6 males) with ages ranging from 24 years to 50 years. They were divided into three groups with varying degrees of primary hypothyroidism and 20 healthy control subjects according to the institutional values *(TSH of 0.4–4.5 mIU/L and FT4 of 0.8–2.1 ng/dL).*

-Group (A): 20 patients with overt hypothyroidism (TSH > *4.5 mIU/L* and FT4 < *0.8 ng/dL).*
-Group (B): 20 patients with SCH *(TSH > 4.5 mIU/L and normal FT4 levels).*
-Group (C): 20 patients with low-normal thyroid hormone levels (We *selected TSH levels of 4–4.5 mIU/L and FT4 of 0.8–1.5 ng/dL since there is no universal definition for the low-normal thyroid states).*
All patients were selected from Kasr Al-Ainy Hospital Endocrinology Clinic, Cairo University from January 2018 to January 2019.

Exclusion criteria:

Patients who are smokers, with hyperthyroidism, receiving drugs that are known to affect thyroid function, diabetics, with significant clinical cardiovascular, liver, and renal diseases, known active malignancy, pregnancy, and patients receiving antioxidant medications.

All patients underwent the following:

1- Full history and complete physical examination.
2- Laboratory analysis:

Freshly voided urine samples and 10 mL of whole blood were drawn from participants under aseptic conditions. Plain evacuated tubes were used for chemistry. The tubes were inverted 180° (upside down) at least five times, and caution was taken not to place them in direct sunlight or low temperatures. Samples were left to clot and centrifuged for 20 min at 5000 rpm. Samples were stored at −80 °C till assay. FT4, FT3, and TSH were assayed by enzyme-linked immunosorbent assay. MDA was assayed by **NWLSSTM** kits, and the assay is based on the MDA reaction with thiobarbituric acid. Human allantoin was assayed by kits provided by ***MYBIOSOURCE*** which employs the Double Antibody Sandwich Technique.

Data and Statistics

The Statistical Package for the Social Sciences version 12 was used for data coding, entry, and analysis. Data were double-checked. Qualitative variables were expressed in numbers and percentages, whereas quantitative variables were expressed as mean and standard deviations. The Mann-Whitney test was used to compare two different groups, and more than two different groups were compared using the one-way analysis of variance. Spearman's non-parametric correlation was used to test for the association between quantitative variables. *The receiver operating characteristic* (ROC) curves were done to detect the sensitivity and specificity. *P*-values of ≤0.05 were considered statistically significant. Results were demonstrated as tables and graphs.

Ethical approval:

The study protocol conformed to the Helsinki declaration ethical guidelines and was approved by the Institutional Review Board and Ethical Committee of Cairo University. Written informed consents were obtained from the enrolled study participants.

RESULTS

The mean TC and LDL-c were higher in groups A and B compared with the control group, both with high statistical significance (P = 0.001 and P = 0.008, respectively). The mean TC and LDL-c of group C were not significantly different from the control group (P = 0.398 and P = 0.574, respectively). The mean HDL-c was significantly low in group A and without a significant difference in groups B and C compared with the control group (P = 0.002, P = 0.096, P = 0.316, respectively). The mean TGs of groups A and C were significantly higher and without a significant difference in group B compared with the control subjects (P = 0.002, P = 0.019, and P = 0.019, respectively). These results are demonstrated in Table (1).

Table (1): Clinical & biochemical parameters of each hypothyroidism group versus the Control group

Variable	Groups		*p*-value
Age *ys*	**Controls** 36.15±10.654	**Group A (Overt)** 35.60 ±9.185	0.862
		Group B (SCH) 35.10±11.210	0.664
		Group C (Low N) 36.50±13.407	0.885
BMI *Kg/m*	**Controls** 25.690±1.9963	**Group A (Overt)** 27.335±3.0521	**0.002**
		Group B (SCH) 26.735±2.1478	0.119
		Group C (Low N) 26.105±3.509	0.364
TSH *mIU/mL*	**Controls** 2.92±0.44	**Group A (Overt)** 61.64±5.01	**< 0.001**
		Group B (SCH) 7.82±1.74	**< 0.001**
		Group C (Low N) 4.36±0.14	**< 0.001**
FT4 *ng/dL*	**Controls** 1.57±0.23	**Group A (Overt)** 0.57±0.14	**< 0.001**
		Group B (SCH) 1.33±0.29	**0.006**
		Group C (Low N) 1.25±0.15	**0.008**
FT3 *pg/mL*	**Controls** 3.64±0.633	**Group A (Overt)** 1.25±0.45	**< 0.001**
		Group B (SCH) 3.09±0.59	**0.007**
		Group C (Low N) 3.49±0.38	0.73
TC *mg/dL*	**Controls** 167.15 ± 28.31	**Group A (Overt)** 221.95 ± 42.76	**< 0.001**
		Group B (SCH) 189.95 ± 22.79	**0.008**
		Group C (Low N) 175.90 ± 35.99	0.398
LDL-c *mg/dL*	**Controls** 96.80 ± 21.44	**Group A (Overt)** 158.65 ± 5.19	**< 0.001**
		Group B (SCH) 121.38 ± 23.14	**0.008**
		Group C (Low N) 102.89 ± 6.24	0.574
HDL-c *mg/dL*	**Controls** 55.05 ± 8.46	**Group A (Overt)** 44.10 ± 11.00	**0.002**
		Group B (SCH) 50.85 ± 7.028	0.096
		Group C (Low N) 52.60 ± 6.67	0.316
TGs *mg/dL*	**Controls** 90.20 ± 14.39	**Group A (Overt)** 112.20 ± 15.63	**0.002**
		Group B (SCH) 97.10 ± 14.46	0.139
		Group C (Low N) 101.70 ± 15.21	**0.019**

Serum MDA and urinary allantoin levels were higher in group A, B, and C compared to the control group ($P < 0.001$). The upper limit value in the control group was 11.03 nmol/mL for MDA and 68.6 ng/mL for allantoin. MDA and allantoin had significant positive correlation with each of TC, LDL-c, and TGs and a significant negative correlation with HDL-c in our patients (A, B, and C as one group, $P < 0.01$). Also Serum MDA and allantoin levels showed a positive significant correlation with TSH and a negative significant correlation with FT4 and FT3 ($P < 0.001$). No correlation was detected between MDA and allantoin with BMI, HbA1c, or patient age. These results are shown in tables (2) and (3).

Table (2): Comparison of serum MDA and urinary Allantoin levels in each Hypothyroid group VS the control group

Variable	Group		% of change	p-value
Serum MDA *nmol/mL*	**Controls** 7.86 ± 1.17	**Group A (Overt)** 50.37 ± 3.18	540.17	< **0.001**
		Group B (SCH) 27.61 ± 4.97	250.93	< **0.001**
		Group C (Low N) 15.45 ± 3.03	96.44	< **0.001**
Urinary Allantoin *ng/mL*	**Controls** 53.95 ± 4.657	**Group A (Overt)** 124.36 ± 12.8	130.4871	< **0.001**
		Group B (SCH) 105.50 ± 14.9	95.53	< **0.001**
		Group C (Low N) 69.63 ± 12.48	29.06	< **0.001**

Table (3): Correlation between oxidative markers and TSH, FT4, FT3, TC, LDL-c, HDL-C, and TGs

Variables		95%Confidence interval of means		Correlation coefficient *r-value*	*p*-value
		Lower bound	Upper bound		
MDA	**TSH**	0.42	0.71	0.586	< **0.001**
	FT4	−0.74	−0.46	−0.618	< **0.001**
	FT3	−0.83	−0.63	−0.751	< **0.001**
	TC	0.42	0.42	0.585	< **0.001**
	LDL	0.71	0.71	0.625	< **0.001**
	HDL	−0.59	−0.23	−0.427	< **0.001**
	TGs	0.07	0.47	0.286	**0.010**
Allantoin	**TSH**	0.41	0.71	0.583	< **0.001**
	FT4	−0.74	−0.46	−0.620	< **0.001**
	FT3	−0.79	−0.56	−0.697	< **0.001**
	TC	0.24	0.60	0.435	< **0.001**
	LDL	0.30	0.64	0.492	< **0.001**
	HDL	−0.63	−0.29	−0.481	< **0.001**
	TGs	0.10	0.50	0.317	**0.004**

The **Receiver Operating Characteristic (ROC)** curve was performed in an attempt to detect the cut-off point for TSH, FT4 & FT3 associated with increased oxidative stress biomarkers and in turn increased cardiovascular disease risk. For predicting urinary allantoin elevation. **TSH** value of ≥ 4.2 was associated with 87.72% sensitivity, 82.61% specificity, area under the curve (AUC) = 0.904. **FT4** value of ≤ 1 was associated with 42.11% sensitivity and 95.65% specificity**,** AUC = 0.757. Whereas **FT3** value of ≤ 3.06 was associated with 56.14% sensitivity and 86.96% specificity, AUC = 0.797. These results are demonstrated in Figure (1).

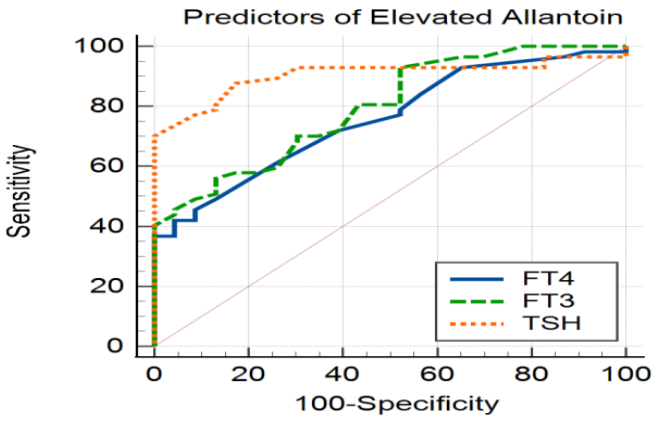

Figure (1): ROC curve used to predict elevated Urinary Allantoin levels. Sensitivity = 87.72%, 42.11%, 56.14%, Specificity = 82.61%, 95.65%, 86.96% and AUC = 0.904, 0.757. , 0.797 for values of TSH ≥ 4.2, FT4 ≤ 1 and FT3 ≤ 3.06, respectively.

- For predicting serum **MDA** elevation, **TSH** value of ≥ 3.6 was associated with 94.92% sensitivity, 80.95% specificity**,** AUC = 0.937. **FT4** value of ≤ 1.3 was associated with 76.27% sensitivity, 76.19% specificity**,** AUC = 0.815. **FT3** value of ≤ 3.08 was associated with 57.63% sensitivity, 85.71% specificity**,** AUC = 0.771. These results are demonstrated in **Figure (2)**.

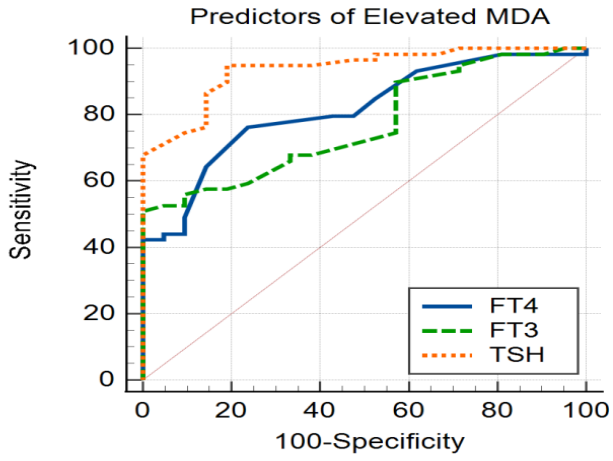

Figure (2): ROC curve used to predict elevated serum MDA levels. Sensitivity = 94.92%, 76.27%, 57.63% 80.95%, Specificity = 80.95%, 76.19%, 85.71% and AUC= 0.937, 0.815, 0.771 for values of TSH ≥ 3.6, FT4 ≤ 1.3, FT3 ≤ 3.08, respectively.

A multivariate regression analysis model was used to detect potential independent predictors of elevated MDA and allantoin. Potential factors entered into the model included TC, LDL-c, HDL-c, TGs, TSH, FT4, and FT3 and revealed FT3 as an independent predictor for MDA and allantoin, while HDL-c as an independent predictor for allantoin only, as shown in Table (4).

Table (4): Multivariate regression analysis to detect potential independent predictors of elevated MDA and Allantoin

	MDA	**Allantoin**
TC *mg/dl*	β = 0.301	β = 0.443
	Beta = 0.576	Beta = 0.551
	p = 0.244	*P = 0.307*
TGS *mg/dl*	β– = -0.096	β = -0.050
	Beta = -0.079	Beta = -0.027
	p = 0.402	*p = 0.791*
HDL *mg/dl*	β = -0.379	β = -1.111
	Beta = -0.177	Beta = -0.338
	p = 0.211	*p =* ***0.030***
LDL *mg/dl*	β = -0.158	β = -0.391
	Beta = -0.326	Beta = -0.526
	p = 0.542	*p = 0.367*
TSH *µIU/ml*	β = 0.075	β = 0.157
	Beta = 0.111	Beta = 0.152
	p = 0.295	*p = 0.190*
FT4 *ng/dl*	β = -4.595	β = -13.084
	Beta = -0.098	Beta = -0.181
	p = 0.409	*p = 0.162*
FT3 *Pg/ml*	β = -8.960	β = -11.466
	Beta = -0.481	Beta = -0.401
	p = ***< 0.001***	*p =* ***0.002***

DISCUSSION

Our study revealed that patients with overt hypothyroidism and SCH had significantly higher TC and LDL-c levels, and patients with overt hypothyroidism had significantly higher TGs and lower HDL-c levels compared to the control group.

A statistically significant difference was not detected in the TC and LDL-c levels between subjects with low-normal thyroid states and the controls. Conversely, TG levels were significantly higher in the same group compared to that of controls.

This may indicate a much lower degree of dyslipidemia in this specific subset of patients. Similar to our results, previous studies have demonstrated a highly significant elevation of TC, TG, and LDL-c in patients with overt hypothyroidism and SCH compared with healthy controls [7, 8, 9]. Another study reported a positive correlation of TSH with TC, TG, and LDL-c. Contrary to our results, TSH had a negative correlation with HDL-c in patients with overt hypothyroidism and SCH [10].

These previous studies demonstrated the occurrence of dyslipidemia in overt hypothyroidism and SCH. However, data on low-normal thyroid states are less solid. **Van Tienhoven and Dullaart** [3] retrieved nine studies that included > 90,041 subjects and evaluated the impact of low-normal thyroid states on **apo B, A-1**lipoprotein levels. Similar to our study, plasma TGs were positively correlated with TSH in one of these studies [11]. A positive significant correlation between TC, LDL-c, and TSH was also demonstrated in three studies [12, 13, 14], while another 2 studies didn't detect such correlation [15, 16].

Parameters of Oxidative Stress in hypothyroid subjects and control group: In this study, serum MDA and urinary allantoin levels were higher in the three studied groups than that of the control group, including subjects with low-normal thyroid function, with a highly statistically significant difference. Moreover, both serum MDA and urinary allantoin were significantly positively correlated with TSH, TGs, TC, and LDL-c, and negatively correlated with FT4, FT3, and HDL. The lack of significant dyslipidemia, apart from TGs, and the presence of a significant increase in oxidative markers may indicate that low-normal thyroid states can cause oxidative damage in a way that is independent of dyslipidemia. Particularly, oxidative stress may occur earlier than expected, with even subtle impaired thyroid functions.

Following our results, a previous study reported significantly higher plasma concentrations of MDA in SCH compared to controls, and MDA was positively correlated with TC, LDL-c, and TGs. However, unlike in our study, MDA was not correlated with TSH and FT4 [2].

Similarly, MDA was significantly higher in patients with hypothyroidism and was positively correlated with TSH [17]. Additionally, consistent with our study, MDA level was significantly increased in SCH and overt hypothyroidism in a study conducted to evaluate the oxidative status and serum lipid profile in hypothyroidism [7]. **Masullo *et al.*** [18] and **Erem *et al.*** [19] reported that patients with overt hypothyroidism and SCH showed significantly higher levels of MDA, TC, LDL-c, and TGs and significantly lower HDL-c levels compared to controls.

Allantoin was previously proposed as an oxidative damage marker and was strongly associated with carotid intimal medial thickness, suggesting allantoin as a potential marker for subclinical atherosclerosis [20]. Hence, the presence of significant allantoin elevation in our patients, including those with low-normal thyroid states, may indicate that these patients, who are considered normal, are at risk of endothelial damage and atherosclerosis.

In 2015, **Campos and Casada** [21] arranged to define markers of oxidative stress, including serum MDA and urinary allantoin in patients with Down syndrome and thyroid dysfunction. Since then, to our best knowledge, our study is the first to correlate serum MDA levels and urinary allantoin as oxidative stress biomarkers in varying degrees of hypothyroidism and specifically low-normal thyroid levels in the general population.

The multivariate regression analysis model in this study proved FT3 as an independent predictor for MDA ($P < 0.001$) and HDL-c and FT3 as independent predictors for allantoin ($P = 0.030$ and $P = 0.002$, respectively). Therefore, elevated urinary allantoin and serum MDA levels are strongly associated with the thyroid profile, even when corrected for other parameters. This indicates that oxidative stress is seen in patients with variable degrees of hypothyroid dysfunction regardless of lipid levels. Following our study, **Santi *et al.*** [22] reported increased MDA levels that significantly correlated with FT3, even after TC correction. MDA elevation was shown in both overt hypothyroidism and SHC. **Bakhtiari *et al.*** [23] reported that MDA levels independently showed a significant correlation with HDL-c, and TGs. Against our results, 2 previous studies attributed increased MDA in SHC to hypercholesterolemia [2, 22]. The ROC curve analysis for oxidative stress markers in the current study revealed TSH of ≥4.2 and 3.6, FT4 of ≤1 and 1.3, and FT3 of ≤3.06 and 3.08 as predictors of urinary allantoin and serum MDA, respectively. Interestingly, all of these cut-off values are within the normal reference range of thyroid hormone levels, which indicates the occurrence of oxidative stress early in the process of hypothyroidism. Current recommendations are to initiate treatment for patients with TSH levels exceeding 10 mU/L, and treatment is indicated in certain situations for the subset of patients with TSH levels below 10 mU/L who are under age of 70 years, pregnant or infertile, with hypothyroidism symptoms, goiter, presence of anti-thyroid peroxidase antibodies, or at increased cardiovascular disease risk [24].

Winther and colleagues [25] concluded that levothyroxine treatment for 6 months was associated with health-related quality of life amelioration in patients with autoimmune thyroid disease, both overt and subclinical, and their study included 78 patients. Conversely, a larger placebo-controlled TRUST trial, including 737 adults diagnosed with SCH, concluded that levothyroxine treatment did not offer any symptomatic improvement [26].

Our study demonstrated that patients with overt hypothyroidism, SCH and even more patients with low-normal thyroid function had higher levels of oxidative stress biomarkers, serum MDA and urinary allantoin, higher TC and LDL-c, and lower HDL-c levels.

CONCLUSION

Variable grades of hypothyroidism, including subjects with low-normal thyroid function, are in a condition of accelerated oxidative stress (evidenced by high serum MDA and urinary allantoin levels). Thereby, increasing the risk for increased

cardiovascular morbidity and mortality in this population. Possible lower TSH levels, even within the normal range, may indicate levothyroxine treatment. Both these biomarkers are very sensitive in detecting oxidative stress.

Our recommendations to give more concern to the missed low-normal thyroid state. We encourage further studies with a larger sample population, including patients with low-normal thyroid function to define new cut-off thyroid hormone values to initiate levothyroxine treatment and antioxidant supplements to attenuate the progression of oxidative stress damage and cardiovascular complications. Additionally, the possible construction of a combined model that merges the different oxidative stress biomarkers to increase the accuracy and precision of assessment could be postulated.

Our study was limited by the small number of subjects.

ACKNOWLEDGMENTS

We appreciate all the participants in this work.

REFERENCES

1. **Valko M, Leibfritz D, Moncol J *et al*. (2007):** Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol., 39 (1): 44-84.
2. **Cheserek M, Wu G, Ntazinda A *et al*. (2015):** Association between thyroid hormones, lipids and oxidative stress markers in subclinical hypothyroidism. J Med Biochem., 34 (3): 323-331. DOI: 10.2478/jomb-2014-0044.
3. **van Tienhoven-Wind L, Dullaart R (2015):** Low-normal thyroid function and novel cardiometabolic biomarkers. Nutrients, 7 (2): 1352-1377. DOI: 10.3390/nu7021352.
4. **von Hafe M, Neves J, Vale C, Borges-Canha M *et al.* (2019):** The impact of thyroid hormone dysfunction on ischemic heart disease. Endocr Connect., 8 (5): R76-R90. DOI: 10.1530/EC-19-0096. PubMed: 30959486. PubMed Central: PMC6499922.
5. **Nanda N, Bobby Z, Hamide A (2008):** Oxidative stress and protein glycation in primary hypothyroidism. Male/female difference. Clin Exp Med., 8 (2): 101-108.
6. **Tolun A, Zhang H, Il'yasova D *et al.* (2010):** Allantoin in human urine quantified by ultra-performance liquid chromatography-tandem mass spectrometry. Anal Biochem., 402 (2): 191-193. DOI: 10.1016/j.ab.2010.03.033.
7. **Kumar V, Kshitiz K (2017):** International Journal of CurrentResearch in Medical Sciences Study of oxidative stress and lipid profile in hypothyroid patients'. Int J Curr Res Sci., 3 (3). DOI: 10.22192/ijcrms.2017.03.03.007.
8. **Haghi A, Solhjoo M, Tavakoli M (2017):** Correlation between subclinical hypothyroidism and dyslipidemia. Iran J Pathol. Spring, 12 (2): 106-111.
9. **Maleki N, Kazerouni F, Hedayati M *et al.* (2016):** Assessment of cardiovascular risk factors in patients with subclinical hypothyroidism. Acta Cardiol., 71 (6): 691-697. DOI: 10.2143/AC.71.6.3178188. PubMed: 27920457.
10. **Hariharan S, Padhi S, Sahoo J *et al.* (2015):** Dyslipidemia in hypothyroid subjects with Hashimoto's thyroiditis. Int J Med Sci. Public Health, 4 (9): 1307-1309. DOI: 10.5455/ijmsph.2015.03042015266.
11. **Taylor P, Razvi S, Pearce S *et al.* (2013):** Clinical review: a review of the clinical consequences of variation in thyroid function within the reference range. J Clin Endocrinol Metab., 98 (9): 3562-3571. DOI: 10.1210/jc.2013-1315. PubMed: 23824418.
12. **Asvold B, Bjøro T, Platou C *et al.* (2012):** Thyroid function and the risk of coronary heart disease: 12-year follow-up of the HUNT study in Norway. Clin Endocrinol (Oxf), 77 (6): 911-917. DOI: 10.1111/j.1365-2265.2012.04477.x.
13. **Roos A, Bakker S, Links T *et al.* (2007):** Thyroid function is associated with components of the metabolic syndrome in euthyroid subjects. J Clin Endocrinol Metab., 92 (2): 491-496. DOI: 10.1210/jc.2006-1718.
14. **Kim B, Kim T, Koh J *et al.* (2008):** Relationship between serum free T4 (FT4) levels and metabolic syndrome (MS) and its components in healthy euthyroid subjects. Clin Endocrinol (Oxf), 70 (1): 152-160. DOI: 10.1111/j.1365-2265.2008.03304.x.Epub.
15. **Lu L, Wang B, Shan Z *et al.* (2011):** The correlation between thyrotropin and dyslipidemia in a population-based study. J Korean Med Sci., 26 (2): 243-249. DOI: 10.3346/jkms.2011.26.2.243.
16. **Wang F, Tan Y, Wang C *et al.* (2012):** Thyroid-stimulating hormone levels within the reference range are associated with serum lipid profiles independent of thyroid hormones. J Clin Endocrinol Metab., 97 (8): 2724-2731. DOI: 10.1210/jc.2012-1133.
17. **Chakrabarti S, Ghosh S, Banerjee S *et al.* (2016):** Oxidative stress in hypothyroid patients and the role of antioxidant supplementation. Indian J Endocrinal Metab., 20 (5): 674-678. DOI: 10.4103/2230-8210.190555.
18. **Masullo L, Magalhães R, Lemes R *et al.* (2018):** Levothyroxine replacement improves oxidative status in primary hypothyroidism. Front Endocrinol (Lausanne), 9: 655. DOI: 10.3389/fendo.2018.00655.
19. **Erem C, Suleyman A, Civan N *et al.* (2016):** The effect of L-thyroxine replacement therapy on ischemia-modified albümin and malondialdehyde levels in patients with overt and subclinical hypothyroidism. Endocr Res., 41 (4): 350-360. DOI: 10.3109/07435800.2016.1163722.
20. **Dąbrowska N, Wiczkowski A (2017):** Analytics of oxidative stress markers in the early diagnosis of oxygen DNA damage. Adv Clin Exp Med., 26 (1): 155-166. DOI: 10.17219/acem/43272.
21. **Campos C, Casado Á (2015):** Oxidative stress, thyroid dysfunction and Down syndrome. Indian J Med Res., 142 (2): 113-119. DOI: 10.4103/0971-5916.164218.
22. **Santi A, Duarte M, Moresco R *et al.* (2010):** Association between thyroid hormones, lipids and oxidative stress biomarkers in overt hypothyroidism. Clin Chem Lab Med., 48 (11): 1635-1639.
23. **Bakhtiari A, Hajian-Tilaki K, Omidvar S *et al.* (2017):** Association of lipid peroxidation and antioxidant status with metabolic syndrome in Iranian healthy elderly women. Biomed Rep., 7 (4): 331-336. DOI: 10.3892/br.2017.964.
24. **Simon C, PA-C, Weidman-Evans *E et al.* (2020):** Subclinical hypothyroidism: to treat or not to treat? J Am Acad Phys Assist., 33 (5): 21-26. DOI: 10.1097/01.JAA.0000660120.03250.55.
25. **Winther K, Cramon P, Watt T *et al.* (2016):** Disease-specific as well as generic quality of life is widely impacted in autoimmune hypothyroidism and improves during the first six months of levothyroxine therapy. Plos One, 11 (6): e0156925.
26. **Stott D, Rodondi N, Kearney P *et al.* (2017):** Thyroid hormone therapy for older adults with subclinical hypothyroidism. N Engl J Med., 376 (26): 2534-2544.