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Inference of oxidative stress in patients with hypothyroidism

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ABSTRACT

Introduction and aim. Oxidative stress is one of the complications that accompany defects in thyroid hormone levels. This study was designed to evaluate oxidative stress markers in patients with hypothyroidism.

Material and methods. This case control study was comprised of forty-two hypothyroid patients aged 36–46 years and forty age matched (35–43 years) healthy control participants randomly selected from the Endocrine Clinic of Al-Yarmook Hospital in Iraq. Blood levels of thyroid hormones malondialdehyde, glutathione, and paraoxonase-1 were assessed. Body mass index was calculated for each patient and control participant.

Results. Regarding the data of oxidative stress markers in hypothyroid patients compared to controls, a significant elevation was reported in blood levels of malondialdehyde and a significant reduction was found in blood levels of glutathione ($p=0.031$). On the other hand, the blood levels of paraoxonase-1 were significantly different in hypothyroid patients compared with the control.

Conclusion. Elevated blood levels of malondialdehyde and declined blood levels of glutathione in hypothyroid patients are a signal of oxidative stress and consequently increase the risk of cardiovascular complications.

Keywords. glutathione, hypothyroidism, malondialdehyde, oxidative stress, paraoxonase-1

Introduction

Thyroid hormone is an endocrine hormone released from the thyroid gland under the influence of thyroxine stimulating hormone. It regulates many processes inside the body that have an effect on metabolism, growth, and basal metabolic rate. Imbalance between the oxidative state and the anti-oxidative state leads to oxidative stress, which is mostly associated with thyroid diseases.[1]

Many studies reported elevated levels of lipid peroxidation and malondialdehyde in patients with hypothyroidism disorders.[2,3] Inconsistency between production and removal of reactive oxygen species that occur during the hypometabolic state may lead to oxidative stress, causing damage to proteins, DNA, and lipid damage.[4]

Oxidative stress may cause injury to endothelial cells leading to inflammation of the vascular cells, which could cause many cardiovascular complications by enhancing the release of cytokines such as osteoprotegerin and receptor activator of nuclear factor kappa-b ligand (RANKL).[5] Furthermore, the release of inflammatory cytokines may cause stimulation of angiotensin II type-1 receptors. Treatment with angiotensin II type-1 receptor blocker drugs may diminish these events in addition to reducing blood pressure.[6] Dyslipidemia and lipid peroxidation, another risk of hypothyroidism, affect endothelial cells by causing inflammation of the vascular cells which may progress to coronary artery disease and arteriosclerosis.[7,8]

Aim

The aim of this study was to quantify the levels of thyroid hormones and oxidative stress markers such as malondialdehyde, glutathione, and paraoxonase-1 in hypothyroid patients and apparently healthy individuals.

Material and methods

Study design, locus, and patient selection

In this case-control study, a total of 42 patients (19 males and 23 females) with hypothyroidism aged 36±10 years and 40 apparently healthy individuals (18 males and 22 females) aged 35±8 years, were recruited from the Endocrine Clinic of Al-Yarmook Hospital in Iraq. Questionnaires for inclusion in this study included many details such as age, gender, previous diseases, medications, and existence of chronic diseases in the family. The exclusion criteria included patients with other chronic diseases or disorders. The rules of this study obeyed instructions of the Helsinki Declaration for research and approved by the College Ethical Committee of the Pharmacy College (2/4/1236 on 22-11-2021). All participants received printed informed consent with enclosed study details and agreements.

Sample collection and laboratory measurements

After 8–12 hours of fasting, blood samples were collected from each subject and dispensed into anticoagulant bottles containing lithium heparin and centrifuged at 3000 rpm for 10 minutes. After which the plasma samples were isolated and kept at -20°C until the assay. Body mass index (BMI) was calculated for all participants by dividing the weight of subjects in kilograms by height in squared meters.

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The hormones tri-iodothyronine (T3) and tetra-iodothyronine (T4) were quantified using a radioimmunoassay kit (Institute of Isotopes, Budapest), while thyroid stimulating hormone (TSH) was quantified using an immunoradiometric assay kit (Institute of Isotopes, Budapest) according to the manufacturer's instructions. Malondialdehyde (MDA) was estimated using the thiobarbituric acid reactive substance method, while glutathione (GSH) was estimated using Ellman's reagent (5,5´-dithiobis- (2-nitro benzoic acid), DTNB), measured spectrophotometrically at 412 nm.[9,10] The activity of paraoxonase-1 (PON-1) was assessed using the substrate 4-nitrophenyl phosphate measured spectrophotometrically at 410 nm.[11]

Statistical analysis

Data were presented as mean ± standard deviation. Continuous variables evaluated by unpaired student t-test, two-tails with $p<0.05$. Pearson correlation analyses was used for assessing the correlation between variables with $p<0.05$. All statistical analyses were done using Microsoft Excel and the software Statistical Package for Social Sciences (SPSS) version 25 (IBM, Armonk, NY, USA).

Results

Table 1 shows the demographical properties of the studied groups and the clinical data of biomarkers and hormones. Concerning the blood levels of hormones, the hypothyroid patients had significantly decreased plasma levels of T3 ($p<0.0002$), and T4 ($p<0.0002$) and had significantly increased plasma levels of TSH ($p<0.001$) compared with the control individuals. Body mass index values was significantly elevated ($p=0.035$) in patients with hypothyroidism compared with control individuals.

Results of oxidative stress markers in hypothyroid patients compared with control showed a significant elevation ($p<0.0002$) in plasma levels of MDA and a significant reduction ($p=0.031$) in plasma levels of GSH, while plasma PON-1 levels showed no significant differences.

Figure 1 shows the number of variable changes in hypothyroid patients versus normal control; the mean blood level of MDA was 2.67-fold higher in hypothyroid patients compared to control. On the contrary, the mean blood level of GSH was decreased 0.77-fold in hypothyroid patients compared to normal control.

Our data in Figure 2 demonstrates that there is a highly significant positive correlation ($r=0.924$, $p=0.001$) between MDA levels and BMI for hypothyroid patients and a significant positive correlation ($r=0.364$, $p<0.05$) between MDA levels and BMI for control individuals.

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Table 1. Demographic outline and clinical data for all participants. [a]

Variables	Control	Hypothyroid patients	p
Number (n)	40	42	
Gender (male/female)	18/22	19/23	
Age (years)	35±8	36±10	
T3 (nmol/L)	2.2±0.22	0.28±0.9	<0.0002
T4 (nmol/L)	117.8±18.5	35.4±15.2	<0.0002
TSH (μ IU/mL)	1.9±0.41	26.6±12.1	<0.001
BMI (Kg/m^2)	28.34±2.62	33.8±2.75	0.035
MDA (μmol/L)	0.76±0.07	2.03±0.24	<0.0002
GSH (mg/dL)	40.5±3.5	31.5±4.3	0.031
PON-1 (IU/L)	6.25±0.42	6.18±0.5	

[a] Values are set as mean (±SD) for continuous variable, T3 – tri-iodothyronine, T4 – tetra-iodothyronine, TSH – thyroid stimulating hormone, BMI – body mass index, MDA – malondialdehyde, GSH – glutathione, PON-1 – paraoxonase

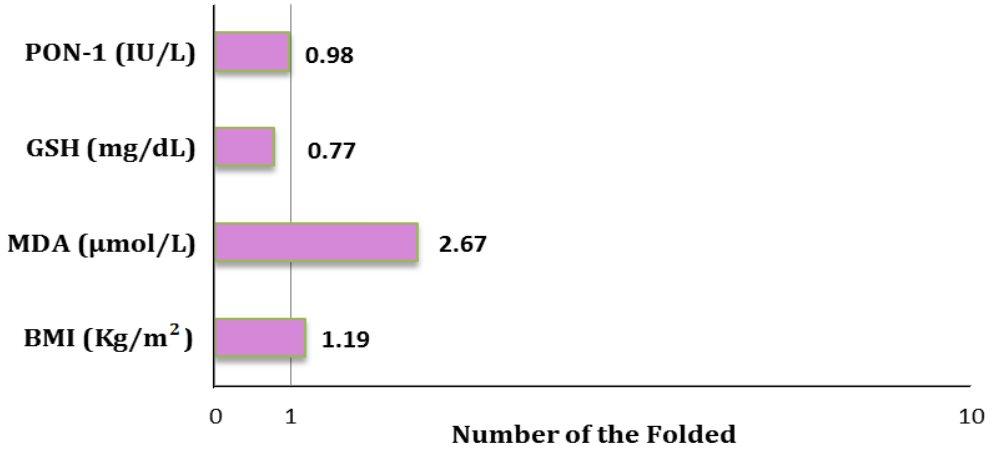

Fig. 1. The changes in the studied variables in hypothyroid patients versus controls.

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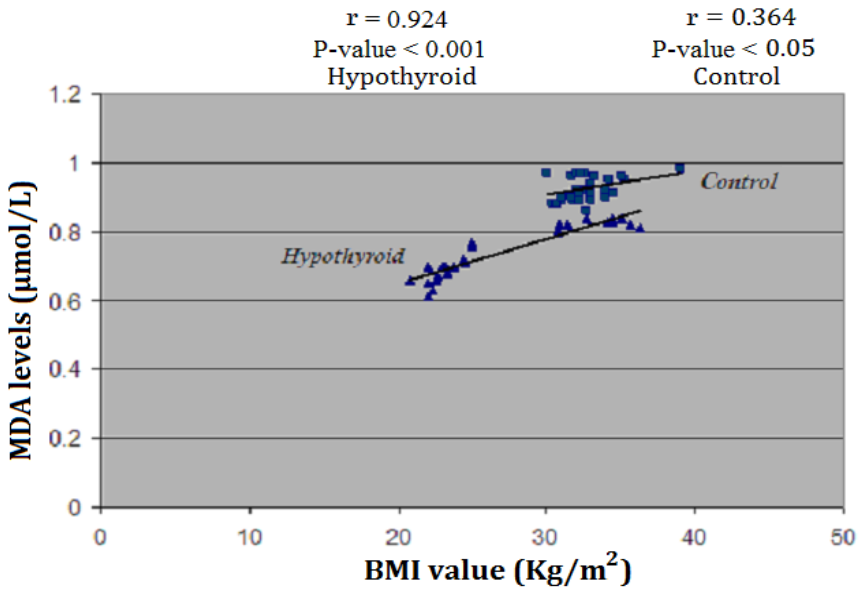

Fig. 2. The positive correlation coefficient between BMI value and MDA levels for control and hypothyroid groups.

Discussion

Oxidative stress is a state of disproportion of oxidative to antioxidative status that occurs in many disorders such as thyroid gland dysfunction and can lead to increases in the levels of reactive oxygen species such as hydrogen peroxide that cause protein damage and subsequent illness.[12] In hypothyroid patients, the risk of dyslipidemia, metabolic syndrome, and atherosclerosis are elevated because of an increased incidence of oxidative stress. In this state, lipid peroxidation, as consequence of hypercholesterolemia and elevated levels of low-density lipoprotein (LDL) cholesterol, are increasingly vulnerable to lipid peroxidation by free radicals.[13,14]

Decreased levels of thyroid hormone may diminish the rate of conversion of β-carotene- LDL to vitamin A – LDL that serve as antioxidants and subsequently enhance oxidation of LDL.[15] Dyslipidemia in hypothyroidism leads to an increase in the number and size of adipocytes (hypertrophy), and consequently, cytokine production such as IL-1, IL-6 and TNF-α by adipocytes will be escalated and enhance the incidence of pro-inflammatory states and oxidative stress.[16]

As noted in this study, BMI values for hypothyroid patients were positively correlated with MDA levels. This finding is in concordance with previous studies.[17] Reactive oxygen species cause lipid peroxidation and consequently the production of MDA indicates oxidative damage and oxidative stress inside the body.[18] Therefore, MDA can be used for diagnosis of oxidative stress accompanied hypothyroidism and for monitoring of hypothyroidism after treatment with L-thyroxine, as levels decline with treatment as documented by many studies.[19,20]

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As verified in this current study, the level of GSH in hypothyroid patients was significantly less than those in normal controls; this outcome agreed with a study by Pasupathi, et al.[21] As thyroid hormone is important for the synthesis of antioxidant agents such as GSH, the decreased level of thyroid hormone causes a decline in biosynthesis of GSH.[22] Moreover, reduced levels of GSH can occur by other factors such as decreased levels of superoxide dismutase leading to accumulation of superoxide and oxidation of GSH.[23] Likewise, Ustundag and colleagues inferred that with an escalation of oxidative stress, the levels of antioxidant activity of superoxide dismutase and glutathione peroxidase diminish in corpulent humans.[24]

PON-1 is an enzyme present in high-density lipoprotein (HDL) and has antioxidant properties. Levels of PON-1 decline in many diseases related to autoimmune and connective tissue.[25] Concerning the level of paraoxinase-1 in this study, there was no significant reduction in the levels of PON-1 for hypothyroid patients compared with control individuals. This event was consistent with the studies of Roshni, et al. and Kebapcilar, et al.[26,27] In contrast, Duman, et al., and Cebeci, et al. documented a significant decline in the level of PON-1 in hypothyroid patients.[28,29] This variation may be due to genetic differences in enzyme expression or due to differences in blood levels of HDL.

Study limitations were the number of the subjects (sample size), the retrospective design of the study and one-center analysis. Therefore, additional studies with larger sample sizes in multiple centers are suggested.

Conclusion

Thyroid hormone is important for maintaining a normal oxidative status in the body. Therefore, any defect in this hormone causes imbalance between the oxidative and anti-oxidative state and may progress to oxidative stress. Our data confirms that overproduction of MDA, and a reduction of glutathione in hypothyroid patients, provides a signal of oxidative stress escalation since these markers are related to increased free radical formation causing lipid peroxidation and decreased antioxidant enzyme levels. Therefore, these biomarkers can be used for monitoring this impairment in hypothyroidism and to overcome complications.

Declarations

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The author has no commercial interest or financial interest. The costs of the research were provided by the author.

Author contributions

Conceptualization, F.R.; Methodology, F.R.; Software, F.R.; Validation, F.R.; Formal Analysis, F.R.; Investigation, F.R.; Resources, F.R.; Data Curation, F.R.; Writing – Original Draft Preparation, F.R.;

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Writing – Review & Editing, F.R.; Visualization, F.R.; Supervision, F.R.; Project Administration, F.R.; Funding Acquisition, F.R.

Conflicts of interest

The author declares no conflict of interest.

Data availability

The datasets used and/or analyzed during this study are available from the corresponding author upon reasonable request.

Ethics approval

The rules of this study followed the Helsinki Declaration for research on humans and was approved by the College Ethical Committee of the Pharmacy College (2/4/1236 on 22-11-2021).

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