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The role of some antioxidants in reducing oxidative stress in female hypothyroidism patients with type II diabetes: a randomized controlled clinical trial

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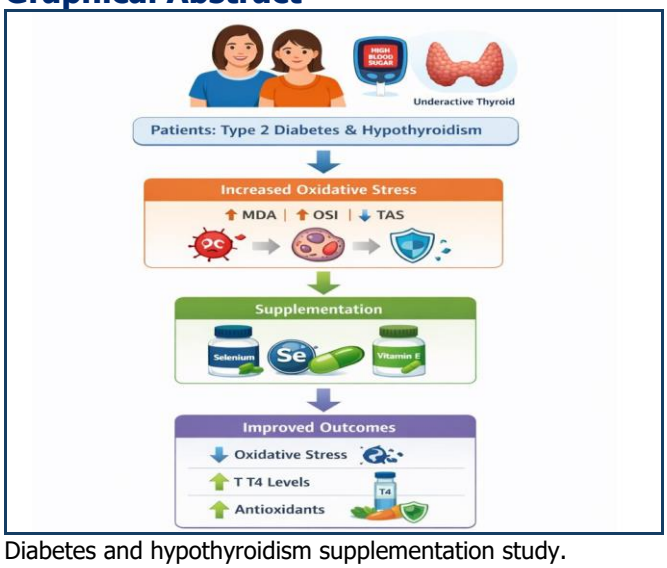
Abstract

Background: Hypothyroidism and type 2 diabetes are common conditions worldwide, with hypothyroidism affecting 4–10% of the population and type 2 diabetes affecting approximately 10% of adults globally. The increasing co-occurrence of these two conditions may contribute to increased oxidative stress and associated complications**.** Low levels of antioxidants are linked to hypothyroidism with type II diabetes because of the growth and production of free radicals, which makes patients more susceptible to oxidative stress. **Objective:** It was to assess how low antioxidants may exacerbate the pathogenic state of type 2 diabetic hypothyroid patients. **Methods:** 140 hypothyroid individuals with type 2 diabetes, ages 25 to 60, were assigned to take supplements every day with either intervention group before Eltroxin,(*n* = 50) (intervention group after Eltroxin, *n*=50) (placebo group, *n*=40) for twelve weeks, take two capsules of vitamin E (268 mg, 400 IU), and one pill of selenium (200 mcg). Serum malondialdehyde (MDA), total antioxidant status (TAS), glycated haemoglobin (HbA1c), and fasting serum glucose (FSG) were measured using laboratory kits. Meanwhile, thyroid hormones were measured using (ELISA) technique. Estimates of selenium) Se) and vitamin E were obtained using atomic absorption spectroscopy and high-performance liquid chromatography (HPLC), respectively. **Results:** The levels of MDA, TAS, thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), FSG, HbA1c, and oxidative stress index (OSI) did not differ significantly ($p>0.05$) between the patient group and placebo participants, according to this study. Vit E and Se levels in the placebo group are not markedly distinct from those in the sick team ($p>0.05$). Increased degrees of MDA, TAS, OSI, and TSH noticeably ($p<0.05$) were observed in the placebo group. After taking the medicine, the T4 level was lower noticeably ($p<0.05$ than that of the patients after taking the medicine. **Conclusion:** Individuals with hypothyroidism with type 2 diabetes may prevent hypothyroidism by consuming dietary supplements containing antioxidants, which reduce oxidative stress and promote overall health.

Keywords: Antioxidant. Type II diabetes mellitus. Hypothyroidism. Oxidative stress. Selenium. Vitamin E.

Graphical Abstract



Diabetes and hypothyroidism supplementation study.
Source: Own authorship.

Introduction

Type 2 diabetes is one of the most prevalent chronic diseases globally, with an estimated 537 million people affected in 2021, projected to rise to 783 million by 2045, representing a prevalence of nearly 10% of adults worldwide. Hypothyroidism is also a common endocrine disorder, particularly in women, and there is an increasing co-occurrence of these two conditions, which may contribute to increased oxidative stress and associated complications **[1]**.

Situated just below the Adam's apple in the front of the middle neck, one of the most important endocrine glands in the human body is the thyroid gland, the human body's most incredible functionally specialized endocrine organ. Its primary role is to secrete the appropriate and sufficient levels of T4 hormone while releasing less T3 hormone. It also controls various biological processes, such as regulating and stabilizing temperature and energy **[2]**.

Approximately 93% of the hormones that the thyroid gland releases that are metabolically active are T4, while T3 makes up the remaining 7%. In the end, tissue-level conversion of all T4 results in T3 production. They are all regarded as operationally necessary **[3]**. Although T3 is believed to be four times more powerful than T4, its blood levels are substantially lower and its half-life is shorter. Despite the two hormones' qualitative similarity, they differ in the speed and intensity of their impact **[4]**.

Rarely, hormone production could be adequate but not enough for their peripheral effects. Hashimoto's thyroiditis is one autoimmune condition that can cause acquired or congenital hypothyroidism. Although it can affect anyone at any age, hypothyroidism is more prevalent in older adults. In this instance, it manifests subtly and could be challenging to identify **[5]**. For a hypothyroid person, diseases affecting the thyroid gland are the major cause, whereas diseases affecting the hypothalamus or pituitary gland are the secondary causes. Weight gain, tiredness, and cold sensitivity are common clinical findings. Myxedema coma and heart problems are the most severe symptoms, and if untreated, they can be deadly **[6]**.

Any material that may compete with other oxidizing substrates at different concentrations and slow down or stop the oxidation of these substrates is considered an antioxidant **[3]**. Based on their physical and chemical characteristics, antioxidants can be divided into two groups: molecules that dissolve in fat and molecules that dissolve in water **[3]**. Vitamin E is one of the fat-soluble antioxidants, an all-purpose word for tocopherol and tocotrienol, of which there are eight variants, including alpha tocopherol. This vitamin is one of the primary fat-soluble antioxidants in human plasma and red blood cells. Tocopherol may scavenge hydroxyl peroxide and is found in lipoproteins and membranes. By stopping the spread of oxidative stress-induced lipid peroxidation, it serves a protective function. Vitamin C is primarily responsible for the reconversion of the oxidized vitamin E **[7,8]**.

Mineral antioxidants include selenium, an uncommon element in the brain, testicles, pancreas, thyroid, kidney, and spleen, among other body tissues. Glutathione peroxidase is an enzyme that functions as an antioxidant in cells and contains selenium. Its function is comparable to that of vitamin E. The detoxification of free radicals produced by cell metabolism depends on this antioxidant activity **[9]**.

Oxidative stress is a syndrome resulting from an imbalance between antioxidant defense systems and free radical production **[3]**. It is an unusual state that occasionally develops in cells or tissues when subjected to oxidative free radical generation that exceeds their capacity to withstand antioxidants, either internally or externally. This imbalance could be caused by an endogenous overproduction of antioxidants, a nutritional shortage in antioxidants, or exposure to environmental pro-oxidant causes. Peripheral tissues undergo changes associated with atherosclerosis, cancer, cardiovascular disease, inflammatory disorders, and aging, primarily in the form of deiodination **[10]**.

It is an abnormal state that occasionally develops in cells or tissues when subjected to oxidative free radical generation that exceeds their capacity to withstand antioxidants, either internally or externally. This imbalance could be caused by an endogenous overproduction of antioxidants, a nutritional shortage in antioxidants, or exposure to environmental pro-oxidant causes **[11]**. TSH has a general metabolic effect of accelerating basal metabolism relative to other metabolic processes, including an increase in the rate of anabolic and catabolic responses. The rate of breathing, oxygen intake, energy expenditure, and heat creation all rise after this. Although the mitochondria play a significant role in forming reactive oxygen species (ROS), TSH also influences the antioxidant status of the cell. They are not directly responsible for the respiratory state of the mitochondria **[3,12]**.

Thyroid dysfunction affects blood sugar levels as well as glucose regulation **[5,13]**. Many things, including food and thyroid health, can impact blood sugar levels. The two endocrine illnesses that are most frequently encountered are diabetes mellitus (DM) and thyroid disease **[1,5]**. DM and thyroid problems have an impact on one another. Thyroid hormones accelerate nearly every part of metabolism, including the use of glucose. They accomplish this by improving the liver's glycogenolysis and gluconeogenesis, the absorption of

glucose from the digestive tract, and the oxidation of glucose in the liver, fat, and muscle **[5]**. This imbalance can lead to insulin resistance in hypothyroid conditions **[13]**.

The study's objective is to assess how low antioxidants may exacerbate the pathogenic state of type 2 diabetic hypothyroid patients.

Material and Methods

Volunteers

All volunteers for this work are females who suffer from hypothyroidism with type 2 diabetes. They visit the Endocrinology and Diabetes Center (n=140) volunteers in Baghdad. Their ages span from 25 to 60 years old. BMI (body mass index) of less than 32 kg/m^2. All participants in this study receive thyroxine replacement therapy and metformin tablets. From March to September of 2023, the study was carried out. Pregnant and breastfeeding women, as well as smokers, were excluded from the study. To prevent bias in the study results, women who took nutritional supplements containing the components under investigation, such as vitamin E and selenium, were not allowed to participate.

Ethical Approval

Ethical approval was granted by the Scientific Research Ethical Committee at the University of Baghdad. Department of Chemistry. College of Education for Pure Science (Ibn Al-Haitham), Baghdad, Iraq, according to Declaration of Helsinki.

Informed Consent

Written informed consent was obtained from all participants prior to their inclusion in the study.

Inclusion Criteria

Participants must be female individuals diagnosed with hypothyroidism and type II diabetes and should fall within a specific age range, typically adults aged 25 to 60.They must have a confirmed diagnosis of hypothyroidism and type II diabetes based on medical records and diagnostic criteria and must be on stable medication regimens for hypothyroidism and type II diabetes for a specified duration (e.g., at least three months). Participants should exhibit signs of oxidative stress, which may be assessed through biomarkers such as MDA levels or TAS. To take part in the study, participants or their legal guardians must give their informed consent.

Exclusion Criteria

Male individuals are excluded from the study to focus specifically on females with hypothyroidism and type II diabetes. Participants with thyroid disorders other than hypothyroidism (e.g., hyperthyroidism) are excluded. Individuals with type I diabetes are excluded, as the focus is on type II diabetes. Also, pregnant or lactating females are excluded due to potential confounding factors and risks to the fetus or infant. Volunteers with recent changes in medication regimens for hypothyroidism or type II diabetes are excluded. Individuals with severe comorbidities or medical conditions that may confound the study results or pose additional risks are excluded. Participants with a history of thyroidectomy (surgical removal of the thyroid gland) are excluded due to potential differences in thyroid hormone metabolism. Individuals with a history of cancer or undergoing cancer treatment are excluded due to possible effects on oxidative stress markers. Current smokers are excluded due to potential confounding effects on oxidative stress levels. Individuals with a history of alcohol or substance abuse are excluded due to the possible impacts on oxidative stress and overall health. Individuals who refuse to engage in the study or who cannot give informed consent are not allowed to participate.

Work design

This study was designed, prepared, and written in accordance with CONSORT's guidelines for randomized clinical trials. After explaining the study and its purpose to all participants and obtaining approval from them, we divided them into the study group (n=50) with Eltroxin, another study group (n=50) before taking Eltroxin, and the placebo group (n=40). Nutritional supplements in the form of pills and capsules were given to the patient group. Vitamin E is 268mg (400 IU), two capsules per day, and selenium (200mcg) is one pill daily for 12 weeks. In the placebo group, they were given the same shape and color of pills and capsules as the supplements, but they were free of active ingredients. Participants were instructed to maintain the diet and exclude nutritional supplements containing the antioxidants under study.

Blood sampling

Samples of blood were drawn from each group. An approximately 10-milliliter disposable syringe was used to extract blood from a vein. After putting the blood in unadorned test tubes devoid of ethylene diamine tetra acetic acid (EDTA), it was allowed to sit at room temperature for fifteen minutes before being centrifuged for ten minutes at 3000 rpm. The serum was maintained at -20 °C in order to conduct additional research studies.

Measurement of laboratory parameters

Malondialdehyde was determined by using a kit

supplied by Northwest. Total antioxidant status was determined using a kit provided by Randox. ELISA technique was utilized to calculate (TSH T3 and T4). Moreover, Serum glucose was measured using a total enzymatic colorimetric technique from a kit supplied by LINEAR Chemicals, Barcelona, Spain. HbA1c was measured using a kit from a RANDOX in the United Kingdom. The furnace atomic absorption spectrophotometry model (AA670) Shimadzu was used to examine samples of selenium. Serum samples were analyzed at (196 nm) after being diluted (1:4) with deionized water for Se. Vitamin E was estimated using HPLC.

Statistical analysis

Version 21 of the statistical analysis package (SPSS) was utilized for this project. To compare the results of the study groups, we used the student t-test. The data was expressed using the mean and standard deviation (mean±SD), as the tables demonstrate. A significance level of p-value less than 0.05 was used. The sample size was determined based on the availability of eligible participants during the study period and was considered sufficient to detect statistically significant differences.

Results

It was believed this is the first study in which two antioxidants (vitamin E and selenium) were used as an experimental treatment to support the antioxidant system. This study found no significant differences (p >0.05) in the MDA, TAS, and OSI levels between the results of placebo subjects and patients after the eltroxin group. These results can be observed in Figure 1. Table 1 shows no significant difference (p>0.05) in the level of TSH, T3, T4, FSG, HbA1c, Se, and vitamin E for the placebo group compared to patients, respectively.

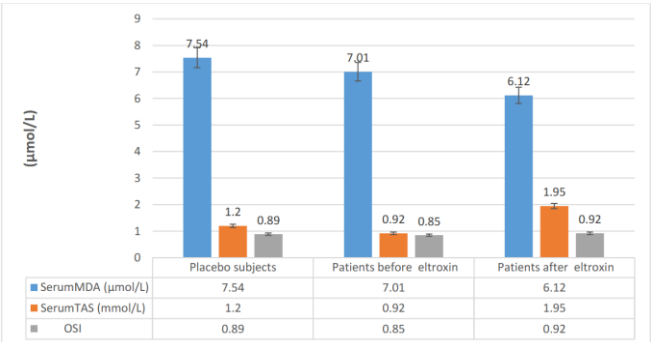


Figure 1. Serum levels of oxidative stress functions in placebo subjects compared to hypothyroidism patients with type II diabetes. Note: MDA: Malondialdehyde; TAS: Total Antioxidant Status; OSI: Oxidative Stress Index. The X-axis represents the study groups, and the Y-axis represents the measured levels of oxidative stress markers. Source: Own authorship.

Table 1. Diagnostic functions and serum levels of antioxidants in placebo subjects compared to hypothyroidism patients with type II diabetes.

Parameters	Placebo subjects	Patients before Eltroxin	Patients after Eltroxin	p- value
TSH (nmol/L)	2.214±0.617	2.3±1.035	2.801±1.041	0.6 *
T3 (nmol/L)	2.018±0.217	2.1±0.65	2.921±0.510	0.07*
T4 (nmol/L)	120±56.051	119±1.05	116±14.765	0.09*
FSG (mg/dL)	125.302±9.502	124.12±7.34	120.22±20.53	0.053*
HbA1c%	7.35±0.150	7.2±2.47	6.812±2.033	0.073*
Se (ppm)	0.091±0.003	0.09±0.02	0.071±0.005	0.095*
Vit E (mg/dL)	1.301±0.211	1.21±0.032	0.891±0.202	0.09*

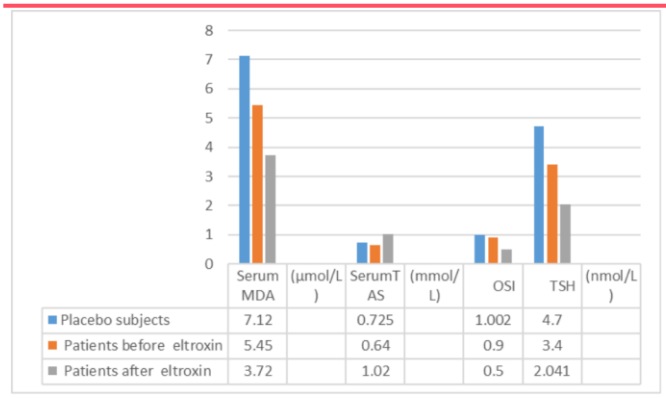
Note: Data are expressed as mean ± SD. *indicated no significant differences between groups (p >0.05). TSH: Thyroid Stimulating Hormone; T3: Triiodothyronine; T4: Thyroxine; FSG: Fasting Serum Glucose; HbA1c: Glycated Hemoglobin; Se: Selenium; Vit E: Vitamin E. Source: Own authorship.

Table 2 and Figure 2 show that the placebo group's MDA, TAS, OSI, and TSH levels were considerably (p<0.05) greater than the patient group's. Concurrently, the T4 level in the placebo group was substantially (p<0.05) lower than in the ill group. After twelve weeks of experimental treatment (200 mcg) of Se one tablet per day with (400IU)of vit E two capsules per day and placebo treatment for the two study groups, we obtained impressive results represented by an increase in the level of TAS (1.02±0.01)for the patient group compared to the placebo group(0.725 ±0.1), which reflected positively on the oxidative stress index for the patient group(0.50±0.01), as it decreased significantly (p-value=0.005) compared to the placebo group(1.002 ±0.21). All of this led to a decrease in the level of TSH for the patient group (2.041±0.017) compared to the placebo group (4.7±0.114), which led to a significant improvement in the health condition of the patient group.

Table 2. Changing in oxidative stress functions and diagnostic parameters levels after receiving treatment to placebo subjects compared to hypothyroidism patients with type II diabetes.

Parameters	Placebo subjects	Patients before Eltroxin	Patients after Eltroxin	p- value
Serum MDA (µmol/L)	7.12 ±1.31	5.45±0.24	3.72±1.23	0.003**
SerumTAS (mmol/L)	0.725 ±0.1	0.64±0.02	1.02±0.01	0.001**
OSI	1.002 ±0.21	0.90±0.05	0.50±0.01	0.005**
TSH (nmol/L)	4.7±0.114	3.40±0.09	2.041±0.017	0.002**
T4 (nmol/L)	90±10.081	104±7.59	121±12.547	0.001**

Note: Data are expressed as mean ± SD. **indicated a significant difference between groups (p<0.05). MDA: Malondialdehyde; TAS: Total Antioxidant Status; OSI: Oxidative Stress Index; TSH: Thyroid Stimulating Hormone; T4: Thyroxine. Source: Own authorship.



	Serum MDA	(µmol/L)	SerumTAS	(mmol/L)	OSI	TSH	(nmol/L)
Placebo subjects	7.12		0.725		1.002	4.7	
Patients before eltroxin	5.45		0.64		0.9	3.4	
Patients after eltroxin	3.72		1.02		0.5	2.041	

Figure 2. Changing in oxidative stress functions and diagnostic parameters levels after receiving treatment to placebo subjects compared to hypothyroidism patients with type II diabetes .MDA: Malondialdehyde; TAS: Total Antioxidant Status; OSI: Oxidative Stress Index; TSH: Thyroid Stimulating Hormone .The X-axis represents the study groups, and the Y-axis represents the measured biochemical parameters .Source: Own authorship.

Discussion

According to the data from the participants, they were not accustomed to taking dietary supplements containing vitamin E or selenium **[7,14]**. However, they opted for alternate treatment for hypothyroidism together with metformin to manage blood sugar levels **[12]**. The individuals' antioxidant systems declined because they did not consistently take antioxidant supplements along with their standard treatment **[3,15]**. The levels of MDA and OSI were increased in both the placebo and patient groups. Both groups had reduced levels of antioxidants, suggesting a negative correlation between the oxidative stress system due to low enzymatic and non-enzymatic antioxidants and the defense system provided by antioxidants **[16]**.

Several studies have shown that hypothyroidism leads to elevated oxidative stress, lipid peroxidation, and mitochondrial respiratory chain disruption due to low antioxidant levels. As a result, the formation of free radicals is boosted and heightened oxidative stress in patients with hypothyroidism **[9,11]**. Oxidative stress is exceedingly united to the disturbance of thyroid hormones T3 and T4 **[11]**. This stress arises from an imbalance between (ROS) and (RNS), causing heightened reactivity of lipid peroxides, leading to increased levels of MDA and OSI **[3]**. T3 and T4 activate the function of the insulin hormone . Decreased thyrotropin-releasing hormone (TRH) production can reduce thyroid hormone levels in diabetic's group **[5]**. Thyroid hormone imbalances in diabetic's groups may result from the usage of diabetes medicines **[13]**.

Insulin reduces triiodothyronine levels by inhibiting the liver's conversion of T4 to T3 while simultaneously increasing free tetraiodothyronine (FT4) levels **[5]**. Selenium is a trace element that acts as an antioxidant in the body and is essential for forming thyroid proteins. It is present in the forms of selenite, selenate, and selenomethionine **[17]**. Selenium is crucial for enhancing the antioxidant properties of α-tocopherol and reducing the harm caused by vitamin E in the thyroid gland. Eighteen Selenium includes crucial enzymes like glutathione peroxidase, which safeguard the cell membrane from harmful free radicals, preventing oxidative stress and lipid peroxidation by neutralizing the toxicity of hydrogen peroxide and lipid peroxides **[18]**. Selenium plays a crucial function in lowering oxidative stress on the thyroid gland in patients with hypothyroidism and type 2 diabetes **[19]**. Vitamin E acts as an antioxidant in the cells and tissues of the thyroid gland **[3]**.

Research has shown that reactive oxygen species (ROS) hinder the function of the enzyme that converts the T4 hormone to the T3 hormone **[6,20]**. Thus, keeping vitamin E levels high inhibits and decreases reactive oxygen species (ROS) activity, which is known to hinder this enzyme **[3]**. Hydroperoxides have a milder impact on patients with hypothyroidism and type 2 diabetes **[9]**. Vitamin E inhibits the growth of lipid peroxide by converting peroxyl radicals into hydroxyl radicals **[3]**. THBI (Thyroid Hormone Binding Inhibitor) disrupts thyroid hormone levels and inhibits the enzyme that converts T4 to T3. This disorder frequently occurs in individuals with unmanaged diabetes, potentially resulting in pituitary gland dysfunction **[5,13]**.

The study's fundamental discovery is the increase in T4 hormone levels and the decrease in oxidative stress and malnourishment observed in the study group compared to the placebo group **[19]**. We were intrigued by the reduction in antioxidants and the rise in oxidative stress in the placebo group compared to the research group. This highlights the important function of antioxidants in enhancing the efficiency of the thyroid gland and reducing inflammation, therefore preventing dysfunction **[7,8]**. Previous studies showed that giving 200 µg of selenium and 30 mg of zinc led to elevated T4 levels and reduced oxidative stress **[18]**.

After a comprehensive review and scientific investigation based on a small group of studies, it was found that the effect of vitamins, whether from their food sources or as a synthetic drug, is not clinically significant on the severity of inflammation **[7]**. One scientific paper showed positive results for thyroid stimulating hormone after taking supplements **[19]**. Supplementing with selenium significantly improved the effectiveness of thyroid hormones and lessened the intensity of inflammation **[18]**. It is likely that other supplements reduce the severity of inflammation,

reduce the state of oxidative stress, increase the performance of thyroid hormones, and improve the state of the antioxidant system [7].

Each antioxidant was tested in only one scientific study, and in any case, these studies were not sufficient to achieve the required efficiency to increase the defense system against oxidative stress [8]. In theory, dietary supplements may benefit individuals undergoing allergy immunotherapy and/or hormone therapy who have nutritional deficiencies. These illnesses are linked to different inadequacies in nutrition [2]. Three research examined the connection between inflammation levels and thyroid parameters using selenium yeast pills, whereas two investigations utilized selenomethionine [21]. Selenomethionine is proposed to be a more potent supplement form compared to non-organic forms. In this systematic review, notable outcomes were observed in studies that utilized selenomethionine, which is a type of selenium found in selenium yeast and can contain up to 90% of selenium.

However, it is important to remember that the results of the other studies that used selenomethionine or selenium yeast were not as positive [22]. A meta-analysis of eleven trials yielded conflicting results on the routine use of selenium supplementation for individuals with acute interstitial pneumonia (AIT) [23]. Depending on the person's selenium levels, think about supplementing with selenium [20]. Only 20% of members of the European Thyroid Society think that the available evidence supports the effectiveness of selenium supplementation for patients with AIT, despite the fact that 65% of members recommend it for these patients [24]. Recommendations for using these dietary supplements to improve oxidative stress, antioxidant status, or inflammatory markers can be made based on the data gathered [25].

Study limitations

There are some limitations to this study, as it was conducted on female patients without males, and therefore the study sample does not include the general population structure. Also, the short duration of the study and the difficulty of following up with patients for long periods make it difficult to follow up on the results resulting from taking antioxidant supplements.

Conclusion

It was concluded that antioxidant supplementation with selenium and vitamin E is associated with a reduction in oxidative stress markers in patients with hypothyroidism and type 2 diabetes. The findings of this study suggest a potential role of these supplements in improving oxidative stress status and thyroid function These results are consistent with previous studies highlighting the importance of antioxidants; however, further well-designed studies are required to confirm these findings and to better understand their clinical implications.

Abbreviations

AIT	Amiodarone-Induced Thyrotoxicosis
BMI	Body Mass Index
DM	Diabetes Mellitus
EDTA	Ethylene Diamine Tetra Acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
FSG	Fasting Serum Glucose
FT4	Free Tetra iodothyronine
HbA1c	Glycated Haemoglobin
HPLC	High-Performance Liquid Chromatography
MDA	Malondialdehyde
OSI	oxidative stress index
ROS	Reactive Oxygen Species
RNS	reactive nitrogen species
RPM	Revolution Per Minute
T3	Triiodothyronine
T4	Thyroxine
TAS	Total Antioxidant Status
THBI	Thyroid Hormone Binding Inhibitor
TRH	Thyrotropin-Releasing Hormone
TSH	Thyroid Stimulating Hormone

CrediT

Author contributions: Conceptualization; Data curation; Formal Analysis; Investigation; Methodology; Project administration; Supervision; Writing - original draft; Writing-review & editing- Abdulrahman R. Mahmood and Mohamad R. Abdullah.

Acknowledgment

Not applicable.

Ethical Approval

Ethical approval was granted by the Scientific Research Ethical Committee at the University of Baghdad. Department of Chemistry. College of Education for Pure Science (Ibn Al-Haitham), Baghdad, Iraq, according to Declaration of Helsinki.

Informed Consent

It was applicable.

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profit sectors. It was self-funded by the authors.

Data Sharing Statement

The necessary data to support the findings of this study can be provided by the responsible author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate[@].

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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