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## Effects of Lipid Peroxidation, Thyroid Hormones, and Some Vitamins in Type 2 Diabetic Patients

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### Abstract

One of the most common forms of diabetes is Type-2 that occurs due to the failure of cells in recognizing and responding to insulin if not accurately treated. The aim of this work is to evaluate the relations of thyroid hormones, vitamins, and lipid peroxidation with the glycemic index in patients experiencing Type-2 diabetes. Some tests of biochemical parameters and vitamins were conducted on 35 patients experiencing Diabetes Mellitus (DM) and 35 healthy subjects. The results indicated the increase in the levels of MDA ( $3.86 \pm 0.97 \mu\text{mol/L}$ ), HbA1c ( $8.27 \pm 1.66 \%$ ), FBS ( $198.34 \pm 32.41 \text{ mg/dl}$ ) and TSH ( $5.67 \pm 0.34 \text{ mIU/L}$ ) in the blood of diabetic subjects in comparison to the controls at a P value lower than 0.05. These increases resulted in decreasing the levels of GSH ( $3.68 \pm 1.21 \mu\text{M/mL}$ ), T3 ( $0.91 \pm 0.03 \text{ ng/ml}$ ), vitamin E ( $0.66 \pm 0.15 \text{ mg/dl}$ ), T4 ( $3.67 \pm 0.46 \mu\text{g/dl}$ ), and vitamin D3 ( $16.78 \pm 4.32 \text{ mg/L}$ ) in diabetic subjects at the same P value. The present study concludes that there is relationship between thyroid hormones and oxidative stress in type 2 diabetes mellitus. In addition, there is a negative correlation between the levels of vitamin E, D, and HbA1c. Therefore, diabetics should monitor their levels of thyroid hormones and vitamins E and D.

**Keywords:** Type 2 DM, oxidative stress, thyroid hormones, vitamin D, vitamin E.

## تأثير تأكسد الدهون, هرمونات الغدة الدرقية و بعض الفيتامينات في مرضى السكري النوع الثاني

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### الخلاصة

واحدة من الاشكال الشائعة لمرض السكري هو النوع الثاني والذي توصف بفشل الخلايا بالتميز والاستجابة للأنسولين اذا لم يتم علاجه بدقة. الهدف من هذا العمل هو تقييم هرمونات الثايرويد، بعض الفيتامينات و تأكسد الدهون المتعلقة بالمحتوى السكري في مرضى السكري من النوع الثاني. بعض الاختبارات من المعايير الكيموحيوية وبعض الفيتامينات اجريت على 35 مريض من داء السكري. أشارت النتائج على ان الزيادة في مستوى MDA, HbA1c, FBS, TSH في مرضى السكري مقارنة بمجموعة السيطرة الاصحاء ( $P < 0.05$ ) وأدى الى انخفاض في مستوى GSH, T3 و فيتامين D3 في الاشخاص المصابين بالسكري ( $P < 0.05$ ). الدراسة الحالية تستنتج بأن هنالك علاقة بين هرمونات الغدة الدرقية والجهد التأكسدي في داء

السكري النوع الثاني. بالإضافة هنالك علاقة سلبية بين فيتامين E. D و السكر التراكمي, لذلك في مرضى السكري يجب مراقبة هرمونات الغدة الدرقية و فيتامين E و D.

## Introduction

Diabetes mellitus (DM) may be defined as one of the complex metabolic and endocrine disorders caused by interactions between environmental and genetic factors, that is causing various degrees of alteration in the functionality of insulin on the peripheral tissues and pancreatic  $\beta$  cell. In addition, the underlying pathological conditions, such as obesity and excess weight, are the major aspects favoring DM2 development [1, 2] With regard to this terms, the insulinemia related to subjects experiencing diabetes might be comparable to that related to euglycemic individuals until now inappropriate in hyperglycemia states. Decreased insulin level, for hormone's determined levels, is referred to as insulin resistance (IR). In the case when  $\beta$  cells are undergoing insulin resistance, there will be insulin hypersecretion that compensates the absence of hormonal activity. Also, hyperglycemia occurs only when there are relative insulin low secretion to glucose stimulus [3]. In addition, the mechanisms of lipotoxicity and glucotoxicity, associated to DM2, were introduced in the year 1990 and supported via experimental researches in animals that were after that verified in humans. Glucotoxicity can be defined as the negative impact created by chronic hyperglycemia on cell's function and structures, while hyperglycemia could cause inhibition of hormone synthesis via a reduction in insulin mRNA; thus, glucose has the ability to induce damages at genetic information level that is of high importance for adequate synthesis of insulin [4]. Other mechanisms related to glucotoxicity might include the low activity of phospholipase C, which is one of the enzymes needed for creating inositol phosphates that are participating in the secretion of insulin via elevating intracellular calcium levels. Also, the cytotoxicity to  $\beta$  cells by glucose, which acts as a free radical, is likely, resulting in an increased  $\beta$  cell apoptosis. In the year 1963, Randle suggested that increasing free fatty acid (FFA), due to triglycerides' degradation, results in blood stream More FFA mobilization because of increased lipolysis induces an increase in the oxidation of FFA in liver and muscles, with low utilization of the glucose in the first and high hepatic gluconeogenesis. This results in hyperglycemia in addition to the inhibition of the secretion of insulin, which will cause an increase in the levels of serum glucose. Furthermore, the FFAs are deposited in muscles as triglycerides, With regard to  $\beta$  cells, there will be an increased level of reactive oxygen species (ROS), therefore decreasing insulin gene expression [5]. Thus, a dual approach is recognized in relation to lipotoxicity in DM2 pathogenesis; it is favoring insulin resistance and has a direct deleterious impact on  $\beta$  cells. Possibly, (ROS) is cause low secretion of insulin because of low glucose transport 2 (GLUT 2) activity. The relation between thyroid disorders and DM is specified via complex inter-dependent interactions. Also, elevated incidence of subclinical hypothyroidism complications, nephropathy, cardiovascular diseases, and retinopathy is indicated in T2DM [6]. In addition, subclinical hypothyroidism was reported to be the major frequent disorder in T2DM [7]. Thyroxine (T4) and triiodothyronine (T3) secretion is primarily maintained by thyroxine stimulated hormones(TSH), which is secreted from the anterior pituitary gland [8], while the thyroid hormones are required for normal glucose metabolism. Hypo- or hyper-secretion of thyroid hormones can alter glucose homeostasis (6). Furthermore, the thyroid disorders are not only worsening the metabolic control, but also impacting the diabetes management [9]. Thus, patients experiencing diabetes should be screened for thyroid dysfunctions. The American Diabetic Association (ADA) recommended that individuals experiencing diabetes should be tested for thyroid disorders [10].

DM is specified as one of the free radical-related diseases propagating complications with elevated free radical formation [11]. Possible pathways by which hyperglycemia might cause increased lipid peroxidation and free radicals formation are: 1) direct auto oxidation of

glucose, 2) activation of glycation pathways and the receptor for advanced glycation end products (RAGE), 3) promotion of the interactions of nitric oxide with superoxide anions for producing peroxynitrites as well as hydroxyl radicals, 4) polyol pathway activation, 5) NADPH oxidase activation 6) activation and induction of different lipoxygenase enzymes, and 7) stimulate of Protein Kinase C (PKC) pathway [12]. Enhanced oxidative stress in T2D has many significant impacts in atherogenesis, such as lipoprotein oxidation, particularly LDL oxidations. Also, the lipid peroxidation of polyunsaturated fatty acids (PUFA), a free radical reaction in vivo, might be suitably reflecting elevated oxidative stress in the diabetes [13]. Elevated lipid peroxidation is damaging the membrane function via reducing its fluidity and modifying the activities of membrane-bound enzymes and receptors [14], while Malondialdehyde (MDA) is one of the stable end-products of lipid peroxidation [15]. It might be defined as a 3 carbon aldehyde which might be existing in different forms in aqueous solutions. MDA was utilized as a bio-marker to lipid peroxidation, while also acting as an indicator for free radical damages [16]. Diabetes and vitamin D deficiency are endemic diseases [17] and might be related to osteoporosis as well as metabolic syndromes [18]. The major risk factors include decreased physical activities, obesity, and aging [18]. Old men experiencing deficiency of vitamin D are releasing more insulin following glucose absorption [18]. Currently, a lot of studies indicated that serum levels of 25-hydroxyvitamin D are considered as an indicator of long-term complications of diabetes, such as renal and cardiovascular diseases [19]. Thus, it might be indicated that the patients experiencing diabetes with renal and/or liver diseases have vitamin D deficiency [17]. Also, it was demonstrated that 25-hydroxy-vitamin D is reduced in Type-2 diabetes, obesity, and gestational diabetes, while the insulinogenic indices were enhanced with the supplementations of vitamin D [18].

The aim of the present study is to evaluate lipid peroxidation indicators, represented by MDA, thyroid hormones, and vitamins E and D, in type 2 diabetes mellitus and find the correlation between high HbA1c level and deficiency of vitamin E and D in diabetic patients.

### **Materials and methods**

The presented work was carried out in the Dept. of Biotechnology, College of Science, University of Baghdad. The study group includes 35 DM patients with an age range of 25-40 years, who were either attending the diabetic clinic or being admitted in the department. In addition, 35 healthy subjects (25-40 years) were enrolled in this study to serve as a control group. Samples were analyzed in the Chemical Laboratory, Department of Biotechnology, College of Science, University of Baghdad. Samples were collected in the morning after 16 hours of fasting. A needle and syringe were utilized for collecting 5 ml of blood samples from diabetic and healthy control subjects (males and females).

The blood sample was divided into two portions. The first portion was dispensed in a tube containing ethylene diamine tetra acetic acid (EDTA) and used for the estimation of HbA1C, while the second portion was dispensed in a gel tube and left to clot at room temperature. The gel tube was centrifuged at 3000 r.p.m for 10 minutes to collect serum which was used for the estimation of fasting blood sugar (FBS), vitamin E (S.VITE), vitamin D (S.VITD3), thyroxin (T4), triiodothyronine (T3), thyroid stimulating hormone (TSH), malondialdehyde (MDA), and reduced glutathione (GSH).

### **Determination of Serum Vitamin E**

Serum Vitamin E has determined by using an ELISA Kit for Human Vitamin E(VE).Cat.No:E0922Hu.

### **Determination of Serum Vitamin**

Serum vitamin D was determined by ichroma Kit Human Vitamin D No:INS-VD-EN(Rev.00).

The test applies a competitive immune detection approach. A target material in the sample binds to the fluorescence (FL) labeled detection antibody in the buffer solution, creating a complex sample mixture. This complex was loaded for migrating into the nitrocellulose matrix, in which a covalent couple 25(OH)D<sub>3</sub>, as well as bovine serum albumin (BSA), were immobilized on a test strip, and interference with the binding which is related to target material as well as FL Labeled anti-body. In the case when more target materials are existing in blood, then less detection antibodies will be accumulated, causing a lower fluorescence signal.

#### **Determination of FBS**

Fasting blood sugar was estimated in serum enzymatically by utilizing glucose oxidase GOD PAP(Kit)(Liquid)GL2624.

#### **Determination of HbA1c**

Glycated hemoglobin (HbA1c) was determined by Stan bio Glyco hemoglobin pre-fil-procedure NoP350, with a quantitative colorimetric determination of glycohemoglobin in whole blood using tubes with EDTA.

#### **Determination of thyroid hormones**

The levels of T<sub>3</sub>, T<sub>4</sub>, and TSH were determined by Beckman coulter AU analyzer.

#### **Determination of MDA**

Based on Aust and Buege approach [20], MDA concentration was detected in the serum. MDA, which is formed from the breakdown of poly-unsaturated fatty acids is serving as a convenient index of the peroxidation reactions. In addition, thiobarbituric acid was utilized for estimating MDA, where these two materials react with each other to provide a pink color that is read at  $\lambda$  max 535 nm.

#### **Determination of GSH**

The concentration of serum thiol was estimated based on Ellman's assay [21].

#### **Statistical analysis**

The data generated were resolved using the statistical software SPSS 17.0, which was utilized for analyzing differences between means of two groups.

#### **Results and discussion**

##### **Effects of VITE and VITD in type-2 diabetic patients**

Overall, 35 patients experiencing DM and 35 control subjects were recruited to the presented work. The values of biochemical parameters in type-2 diabetics and control subjects are shown in (Table1).

**Table 1-Biochemical parameters in type 2 diabetic and control subjects**

Parameters	Control subject (n=35)	Type 2 diabetic subject (n=35)	P- value
FBS ( mg/dl)	87 ± 10	198.34 ± 32.41	<0.05
HbA1c %	5.1 ± 0.55 %	8.27 ± 1.66 %	<0.05
S.VITE (mg/dl)	1.33 ± 0.14	0.66 ± 0.15	<0.05
S.VITD3 (mg/L)	26.87 ± 4.76	16.78 ± 4.32	<0.05

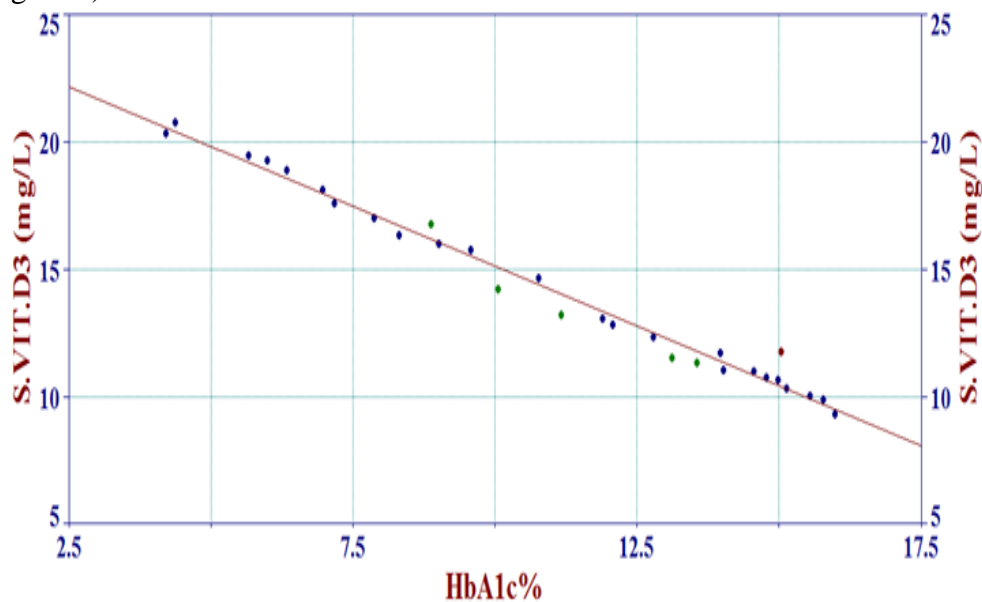
The present study investigated the degree of involvement of a few biochemical parameters in the complications related to DM. Fasting blood sugar (FBS), glycated hemoglobin(HbA1c), serum vitamin E (S.VITE), and serum vitamin D<sub>3</sub> (S.VITD<sub>3</sub>) levels were examined. Fasting blood sugar (FBS) and HbA1c levels indicated significantly higher concentrations ( $p < 0.05$ ) in the sera of type-2 diabetics in comparison to controls (Table 1). The values indicated in this work might be contributing to diabetic conditions.

Our study also showed that vitamin D levels tend to be lower in diabetics ( $16.78 \pm 4.32$  mg/L) as compared to control group ( $26.87 \pm 4.76$  mg/L). This result agrees with that of Tajik and Amirasgari [22] who reported an association between type-2 diabetes and vitamin D,

represented by the impact of vitamin D on insulin secretion, resistance, and sensitivity. The 25-hydroxylase enzyme in the intestinal microsomes and the liver catalyzes the initial phase of vitamin D activation [22]. This process is very essential, since only small amounts of vitamin D in the liver or small intestine can lead to the detection of small amounts of pro-vitamin D in the blood-stream [22]. The absence of vitamin D might decrease the secretion of insulin, while its supplementations in animals might maintain insulin secretion [17].

Serum vitamin E level was 0.66 mg/dl (SD = 0.15) in DM patients, whereas it was 1.33 mg/dl (SD = 0.14) in the controls. This reduction in the levels of vitamin E in DM is highly significant ( $p < 0.05$ ), suggesting higher oxidative stress in comparison to controls. Our result for vit.E is in agreement with Odum *et al.*, [23] who mentioned that the level of VIT.E in diabetic subject was  $15.33 \pm 4.05 \mu\text{mol/l}$  while in the control group it was  $(31.22 \pm 6.20 \mu\text{mol/l})$ . A study conducted for 3 months on the supplementations of vitamin E and vitamin C indicated that blood glucose level in patients was reduced, whereas the levels of glutathione and SOD were increased [24].

In the present study, there was a negative correlation between HbA1c and VIT.D and VIT.E. For all participants, vitamin D deficiency was identified as being associated with high HbA1c levels (Figure 1).



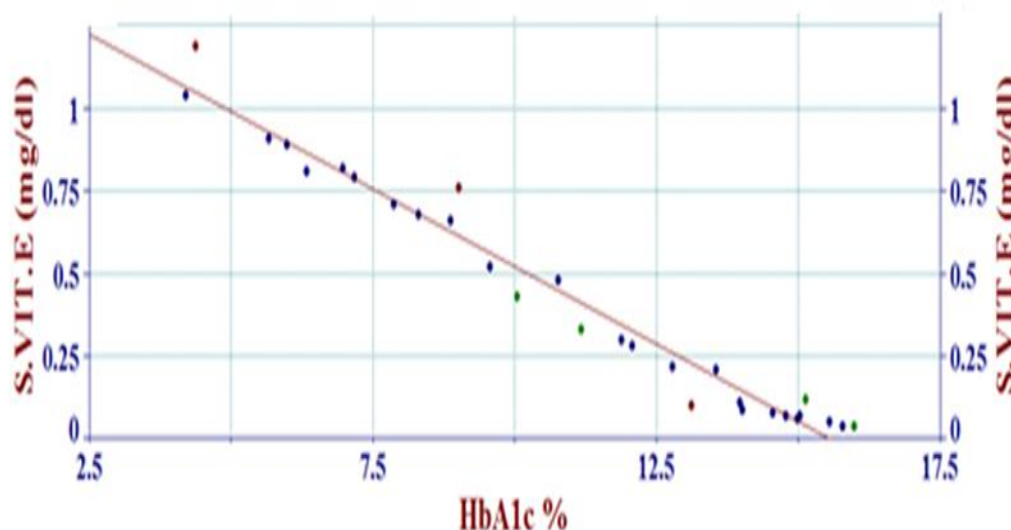
**Figure 1**-Linear correlation of glycosylated hemoglobin and 25-hydroxyvitamin D levels in all participants with type 2 diabetes mellitus,  $r=0.984$ ,  $P \leq 0.05$ .

Serum 25(OH) D levels are an indicator of vitamin D status and are related to a variety of diseases, such as DM, breast cancer, and multiple sclerosis. This may be due to the universal expression of vitamin D receptor in a variety of tissues and cells, such as adipose tissue [25] breast [26] and nerve cells [27].  $\beta$ -cells within the pancreatic islets have receptors for active vitamin D [28], enabling vitamin D to regulate the insulin response to elevated blood glucose levels. In addition, these cells express  $1\alpha$ -hydroxylase, which can convert the biologically inert 25(OH) D to active vitamin D. Vitamin D can also promote insulin-mediated responses by suppressing inflammation [29].

Previous studies have explored the relationship between vitamin D and blood glucose levels. One study performed on adolescents (aged 12–17 years) in the United States found that vitamin D levels were negatively correlated with fasting blood glucose levels, but were not related to HbA1c in male subjects [30]. Munasinghe *et al.* [31] found that individuals with a 25(OH) D level  $\geq 20\text{ng/mL}$  had a 0.74-fold less possibility of exhibiting elevated HbA1c levels compared with individuals with a 25(OH) D level  $< 20\text{ng/mL}$  in a non-diabetic

population. Another study showed that 25(OH) D levels are negatively correlated with HbA1c levels in patients with T2DM [32]

Also in the present study, there was a negative correlation between HbA1c and VIT.E in type 2 diabetic patients (Figure 2).



**Figure 2-**Linear correlation of glycosylated hemoglobin and VIT.E in all participants with type 2 diabetes mellitus,  $r=0.969$ ,  $P<0.05$ .

Our result supports data from some studies which suggest that Vitamin E supplements may lower HbA1c levels in patients with inadequate glycemic control [33]. In general, antioxidant vitamins A, C, and E are found to be decreased in diabetic subjects, possibly due to an increased need to control the excessive oxidative stress produced by abnormalities in glucose metabolism [34]. This alteration in antioxidant vitamins metabolism is related to increased values of glycosylated hemoglobin.

#### Effects of thyroid hormones in type2 diabetic patients

The mean level of serum TSH was significantly higher ( $p<0.05$ ) in type 2 diabetic patients ( $5.67 \pm 0.34$  mIU/L) than that in the control group ( $2.97 \pm 0.06$  mIU/l), while the serum T4 ( $3.67 \pm 0.46$   $\mu$ g/dl) and T3 ( $0.91 \pm 0.03$  ng/ml) levels were significantly lower ( $p<0.05$ ) in type 2 diabetic patients than those in the control group (Table 2).

**Table 2-** The values of thyroid hormones in diabetic and control subjects

Parameters	Control subjects (n=35)	Type2 diabetic subjects (n=35)	P-Values
S.TSH (mIU/L)	$2.97 \pm 0.06$	$5.67 \pm 0.34$	$<0.05$
S.T4 ( $\mu$ g/dl)	$6.87 \pm 0.24$	$3.67 \pm 0.46$	$<0.05$
S.T3 (ng/ml)	$1.78 \pm 0.04$	$0.91 \pm 0.03$	$<0.05$

In this work, the level of serum TSH was found to be increased; however, the levels of serum T4 and T3 were found to be considerably decreased in type-2 diabetes mellitus as compared to the control subjects. Such finding is in consistency with several earlier studies [35-38]. On the other hand, a number of other authors found no considerable serum TSH changes in the T2DM [39]. One study has even indicated lower serum TSH levels and higher serum T4 and T3 levels in the T2DM compared with the control group [40]. It was proposed that the changed status of the thyroid in the T2DM is related to the hypothalamus-pituitary-thyroid axis toleration, which causes a decrease in the production of T4 and T3. In addition, a lower AMPK (5'-Adenosine MonoPhosphate activated Protein Kinase) activation was described in type-2 diabetes mellitus [36], which resulted as well in a decrease in the production of thyroid

hormones [36]. Decreased thyroid hormone levels result in increasing the release of the TSH from the anterior pituitary gland, with a feedback mechanism [41]. The majority of Type2 Diabetes Mellitus patients have been obese, with the possibility of having increased leptin levels. Such an increase develops leptin resistance centrally, which results in a decrease in the production of the thyroid hormones and an increase in the secretion of TSH by a feedback mechanism in Type2 Diabetes Mellitus [36].

### Effects of oxidative stress in type2 diabetic patients

Oxidative stress parameters (MDA and GSH) in type2 diabetic and healthy control groups are listed in Table 3.

**Table 3-**Comparison of mean serum levels of MDA and GSH in type2 diabetic and healthy control subjects

Parameter	Control subject (n=35)	Type 2 diabetic subjects (n=35)	P-Values
MDA ( $\mu\text{mol/L}$ )	1.47 $\pm$ 0.27	3.86 $\pm$ 0.97	<0.05
GSH ( $\mu\text{M/mL}$ )	5.92 $\pm$ 0.27	3.68 $\pm$ 1.21	<0.05

The results of the present work showed a considerable increase ( $P \leq 0.05$ ) in MDA levels in patients experiencing T2DM in comparison with the controls. These results indicate that hyperglycemia causes increased ROS production, which could be due to an increased oxidative stress in type-2 diabetics because of being exposed to a prolonged elevation in glucose level, bringing the hyperglycemic index to maximum. In addition, there was a significant reduction ( $P \leq 0.05$ ) in GSH levels in type2 diabetes patients as compared to the controls. The sources of oxidative stress in the diabetics could involve glucose auto oxidation, shift in the redox balance, and reduced tissue concentration of the anti-oxidants that have low-molecular weights, including decreased glutathione (GSH). The results of this work confirm that diabetes patients are vulnerable to blood glucose levels and this has a relation with free radical-mediated lipid peroxidation, which acts as one of the indicators of oxidative stress [42]. In the case where ROSs are accumulated due to oxidative stress, they are generated in a faster rate than that of their neutralization via the *in vivo* non-enzymatic anti-oxidant defense mechanisms, such as GSH. This explains the low-levels of antioxidants in diabetic patients, whereas MDA, as well as the other markers of oxidation, are found in higher levels, resulting in pathological conditions [43]. Also, diabetes induces changes in the activities of the enzyme glutathione reductase which could break the chain reactions of the free radicals. The alterations in the levels of these radicals render the cells susceptible to oxidative stresses and therefore result in their injury [44]. Superoxide, hydroxyl, and  $\text{H}_2\text{O}_2$  are the major significant free radicals causing oxidative stress. In humans, there are several anti-oxidant enzymes, such as GSH, that act in scavenging the free radicals for the purpose of protecting the body [45]. Increased GSH activity is of a high importance to protect the cells from oxidative damages via the neutralization of free radicals. Oxidative stress is defined as one of the common pathogenic factors in DM, resulting from a decreased intercellular and extracellular antioxidant levels. GSH is a non-enzymatic anti-oxidant that delays or inhibits oxidative processes via various approaches. Generally, the levels of the anti-oxidant enzymes are especially sensitive to oxidative stress in type 2 DM.

### Conclusions

The present study concludes that there is relationship between thyroid hormones and oxidative stress in type 2 diabetes mellitus. In addition, there is a negative correlation between the levels of vitamins E and D and HbA1c. Therefore, diabetics should monitor thyroid hormones and vitamins E and D.

### Ethical clearance

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

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