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ORIGINAL RESEARCH

Independent Association of 25[OH]D Level on Reduced Glutathione and TNF-α in Patients with Diabetes and/or Hypertension

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Purpose: Oxidative and inflammatory pathways play a significant role in the pathophysiology of a wide variety of non-communicable diseases such as type 2 diabetes mellitus (T2DM) and hypertension. However, the effect of serum 25-hydroxyvitamin D (25[OH]D) on these pathways is still controversial. To evaluate the association of 25[OH]D on antioxidant and pro-inflammatory biomarkers, reduced glutathione (GSH) and tumor necrosis factor (TNF)-α, in T2DM and hypertensive patients.

Patients and Methods: This is a cross-sectional study of a consecutive sample of patients attending the the Family Medicine clinic at King Abdullah bin Abdulaziz University Hospital (KAAUH). Participants were screened for eligibility according to the following criteria: aged above 18 years and diagnosed with T2DM and/or hypertension for at least one year. Patients receiving any kind of vitamin D or calcium supplements within the last three months were excluded, as were those with a history of renal failure, cancer, liver, thyroid, or any other chronic inflammatory diseases.

Results: In total 424 T2DM and/or hypertensive patients (mean age 55±12 years) were recruited. In addition to routine physical and laboratory examinations, levels of serum 25[OH]D, GSH and TNF-α were measured. The prevalence of 25[OH]D deficiency (<50 nmol/L) was 35.1%, which was independent from GSH and TNF-α levels. In T2DM, hypertensive and patients having both diseases, GSH levels were 349.3±19, 355.4±19 and 428.8±20 µmol/L, respectively. Uncontrolled T2DM and hypertension patients showed significantly higher GSH compared with the controlled group. Males showed slightly higher level of TNF-α compared with females and uncontrolled hypertensive patients had relatively higher TNF-α level when evaluated against controlled hypertensive patients.

Conclusion: 25[OH]D level is independent of oxidative stress and inflammation, assessed by levels of GSH and TNF-α, respectively, in T2DM and hypertensive Saudi patients.

Keywords: vitamin D, diabetes, hypertension, reduced glutathione, TNF-α

Introduction

Type 2 diabetes mellitus (T2DM) is considered as one of the most widespread non-communicable diseases worldwide, particularly in Saudi Arabia where its prevalence has drastically increased from 8.5% in 1992 to 39.5% in 2022 among Saudi population.[1] Hypertension is also considered another global major non-communicable disease, with an alarming overall estimated prevalence of nearly a quarter of the adult population in Saudi Arabia.[2] Several studies implicated that oxidative stress and inflammatory pathways are incriminated in the initiation, progress and/or complications of both diseases.[3,4] Deficiency in reduced glutathione (GSH), one of the strongest antioxidants that largely contributes as a pivotal player in body antioxidant defense systems, has an undeniable role in the pathogenesis of T2DM as well as hypertension.[5,6] Oxidative stress caused by GSH

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deficiency may trigger the induction of inflammatory markers such as tumor necrosis factor (TNF)-α that may further induce other inflammatory mediators contributing to the progress of T2DM and hypertension.[7,8] Still, it is possible that inflammatory process precedes oxidative stress, as the initiative/cause crosstalk between these pathways in T2DM and hypertension is a relationship that is too complex to define.[9]

Deficiency in vitamin D, or its hydroxylated active form 25-hydroxyvitamin D (25[OH]D), is another global health issue affecting more than 1 billion people worldwide and is prevalent in nearly 60% of Saudi population.[10] An association has been found between 25[OH]D deficiency and several chronic diseases, including autoimmune disorders, inflammatory bowel diseases, hepatic inflammation, bronchial asthma, neuro-cognitive dysfunctions and malignancies.[11] Still, a link between vitamin D status with T2DM and/or hypertension is not fully established. Several studies indicated the presence of a correlation between the initiation, progression, or complications of these diseases with 25[OH]D deficiency,[12–15] while other studies showed that there was no correlation.[16,17] Consequently, the relationship between 25[OH]D deficiency and the oxidant/inflammatory status in T2DM and/or hypertension also remains controversial. Thus, the objective of the current study was to explore the prevalence of 25[OH]D deficiency among T2DM and hypertensive Saudi patients, and to evaluate the impact of such deficiency on the patients' oxidative and inflammatory status, represented by serum levels of GSH and TNF-α, respectively.

Materials and Methods

Study Design, Participants, and Setting

This is a cross-sectional study of a consecutive purposive sample of patients attending Family Medicine Clinic at King Abdullah bin Abdulaziz University Hospital (KAAUH) from January 2020 to December 2021. Participants were screened for eligibility according to the following criteria: aged above 18 years and diagnosed with T2DM and/or hypertension for at least one year. Patients receiving any kind of vitamin D or calcium supplements within the last three months were excluded. In addition, exclusion criteria included patients with a history of renal failure, cancer, liver, thyroid, or any other chronic inflammatory diseases.

The current study was approved by the institutional review board (IRB) from Princess Nourah bint Abdulrahman University (PNU), with number of approval 18–0136 on 17/04/2018. An approval was also taken from KAAUH (R0-2019-K-002). In addition, a written consent and study information were disseminated to each patient and filled by the recruiting physician.

Data Collection and Study Variables

Eligible patients were booked for clinic visits with assigned family physicians, in which consent, patient's data, and baseline physical examinations were recorded. Additionally, an initial set of clinical laboratory investigations were ordered during that visit. Each patient who agreed to participate and completed baseline data and physical examination was requested to go to the outpatient phlebotomy area located in the same hospital for blood sampling. Moreover, to assure confidentiality, easy identification, and processing of blood samples, each patient was given a code number to hand over to the assigned phlebotomist at the time of drawing of his/her blood sample. Study data were collected and managed using the Research Electronic Data Capture (REDCap),[18] which is a secure electronic data capture tool hosted at PNU. The tool was designed based on type of collected data and was filled by assigned family physicians. It consists of three parts: sociodemographic data, clinical findings, and laboratory results. The sociodemographic data involved: age, gender, and smoking history. In addition, patients were inquired about other co-morbidities. Blood pressure was also recorded, where patients with systolic blood pressure (SBP) more than 140 mmHg or diastolic blood pressure (DBP) more than 90 mmHg were classified as uncontrolled hypertension.[19,20] Patients whose Hemoglobin A1c (HbA1c) exceeded 7.5% were considered as uncontrolled T2DM. Furthermore, patients' weights and heights were taken to calculate their body mass index (BMI) according to the following equation: BMI = Weight in kg/square of the height in meters.

Clinical Laboratory Investigations

Vitamin D has two biologically relevant forms; namely D_3 and D_2, that are converted by hydroxylation into 25[OH]D. The level of the latter is measured in serum to determine vitamin D status of all the patients after their outpatient-clinic visit. Measurement is done using commercial vitamin D kit (Abbott Diagnostics) compatible with Abbott Architect Analyzer i2000 SR (Abbott

Laboratories, IL, USA). The principle of this technique is quantitative delayed one-step competitive chemiluminescent microparticle immunoassay (CMIA). Vitamin D status was defined according to IOM (US) reference range[21] and was considered normal (sufficient) when 25[OH]D levels were > 50 nmol/L. Other routine laboratory investigations were also done for each patient including complete blood count (CBC) using ADVIA 2120i Hematology System (Siemens Healthcare Diagnostics Inc., NY, USA), as well as fasting blood glucose (FBG), C-reactive protein (CRP) and calcium (Ca) according to the commercial kit manufacturer's instructions. Level of glycosylated hemoglobin (HbA1c) was measured using Beckman Coulter Unicel DxC Synchron 800 (Beckman Coulter, CA 92821, USA), where patients with HbA1c more than 7.5% were considered as uncontrolled diabetics.[22] Using the latter equipment, fasting lipid profile was also measured, including total cholesterol, triglycerides, low-density lipoprotein (LDL) and high-density lipoprotein (HDL).

Assessment of GSH and TNF-α Using ELISA technique

The leftovers of serum samples that were collected from patients during routine laboratory investigations were stored at −80 °C until used. Measurements of GSH and TNF-α were performed using Evolis Fully Automated ELISA Processor (Bio-Rad Laboratories, CA, USA). For GSH assessment, colorimetric GSH assay kit was used (ab239727; BioVision/Abcam), whose action is based on an enzymatic cycling method in the presence of GSH and a chromophore. The reduction of the latter produces a stable compound that can be detected kinetically at 450 nm. For determination of TNF-α in serum, human TNF-α SimpleStep ELISA Kit (ab181421; BioVision/Abcam) was used, which is a sandwich ELISA technique that quantitatively measures TNF-α by capturing the antibodies conjugated to an affinity tag recognized by the monoclonal antibody coating the ELISA plate, forming antibody-analyte sandwich complex that can be detected kinetically at 450 nm.

Statistical Analysis

Shapiro–Wilks Normality Test was used to assess the statistical normality assumption of the continuous variables. As variables were not following the normal distribution, mean ± standard error along with median and 95% confidence intervals were used to express continuous variable. Frequency and percentage were used for categorically measured variables. Spearman's correlations test was used to assess the correlations between metric variables. The Mann–Whitney U test and Kruskal–Wallis H-test were used to compare continuous variables among groups. The SPSS IBM statistical analysis program (Version#21, Armonk, NY: IBM Corp) was used for the statistical data analysis. The statistical significance level (*P*-value) was considered at 0.05 if achieved.

Results

Sociodemographic, Physical and Clinical Investigation Findings

Among the total sample recruited in this study, 120 (28.3%) had T2DM, 155 (36.6%) had hypertension and 149 (35.1%) suffered from both T2DM and hypertension. The average age of the total cohort was 54.9±0.6 years and patients with hypertension were significantly older than the other two groups (60.8±0.97 versus 51.9±0.96 and 51.7±0.80). The three groups had comparable gender distribution, smoking habits as well as BMI. As expected, patients with T2DM only showed significantly lower averages in systolic and diastolic blood pressure readings, while they had significantly higher glycemic parameters (FBG and HbA1c). Co-morbidities in terms of cerebrovascular accident/transient ischemic attack (CVA/TIA), cardiovascular disease (CVD), dyslipidemia, and osteoarthritis were significantly more frequently reported among patients with both T2DM and hypertension (Table 1).

Most patients had normal 25[OH]D level (275, 64.9%) while 44 (10.4%) had deficiency as shown in Figure 1. For this reason, we have used 25[OH]D level of 50 nmol/l as a cut-off level between sufficiency and insufficiency.

Analysis of CBC did not differ significantly among the groups with exception of white blood cells (WBC) that was at its highest level in patients with both T2DM and hypertension and the lipid profile parameters of this group, except for triglycerides, seemed significantly at a lower level when compared with patients with either T2DM or hypertension. The average level of 25[OH]D concentration in the total cohort was 63.6±1.4 nmol/L, with higher level recorded among patients with both T2DM and hypertension (70.1±2.5 nmol/L). Additionally, GSH average was 380.3±11 μmol/L and

Table 1 Sociodemographic, Physical, and Clinical Characteristics of the Studied Population

	Total N=424	Diabetes Only N=120 (28.3%)	Hypertension Only N=155 (36.6%)	Diabetes and Hypertension N=149 (35.1%)	*P*-value
Sociodemographic Characteristics					
Age	54.9±0.6	51.9±0.96	60.8±0.97	51.7±0.80	<0.01
Gender					
Male	137 (32.3)	29 (24.2)	53 (34.2)	55 (36.9)	0.07
Female	287 (67.7)	91 (75.8)	102 (65.8)	94 (63.1)	
Smoking	37 (8.7)	10 (8.3)	13 (8.4)	14 (9.5)	0.93
Obesity	105 (24.8)	27 (23.5)	34 (21.9)	44 (29.5)	0.24
Clinical profile					
BMI (kg/m^2)	31.4±0.3	31.0±0.5	31.1±0.5	32.1±0.5	0.21
SBP (mmHg)	136.3±0.8	129.6±1.2	137.4±1.3	140.6±1.4	<0.01*
DBP (mmHg)	77.3±0.5	73.7±0.9	80.2±0.9	77.2±0.9	<0.1*
Co-morbidities					
Hypothyroidism	47 (11.1)	15 (12.5)	10 (6.5)	22 (14.8)	0.05
CVA/TIA	8 (1.9)	0 (0.0)	1 (0.6)	7 (4.7)	<0.01*
CVD	15 (3.5)	1 (0.8)	3 (1.9)	11 (7.4)	<0.01*
Osteoarthritis	61 (14.4)	10 (8.3)	20 (12.9)	31 (20.8)	<0.01*
Hyperlipidemia	155 (36.6)	43 (35.8)	45 (29.0)	67 (45.0)	0.02*
Laboratory findings					
Hb (g/L)	132.2±0.8	131.9±1.5	132.7±1.6	131.7±1.1	0.87
HCT (%)	0.41±0.0	0.41±0.0	0.42±0.0	0.41±0.0	0.64
MCV (fL)	86.7±0.4	86.2±0.6	86.5±0.7	87.5±0.5	0.26
RBC ($x10^{12}/L$)	4.8±0.03	4.8±0.04	4.8±0.05	4.7±0.04	0.26
WBC ($x10^9/L$)	7.3±0.11	7.4±0.17	6.9±0.20	7.7±0.18	<0.01*
Platelets ($*10^9/L$)	278.7±3.7	276.3±6.7	281.8±6.7	277.5±5.9	0.82
RDW (CV%)	14.0±0.7	14.1±0.9	13.9±0.1	14.0±1.7	0.37
Cholesterol (mmol/L)	4.6±1.2	4.7±4.1	4.7±0.1	4.4±3.7	<0.01*
LDL (mmol/L)	2.7±1.1	2.9±2.3	2.9±1.8	2.6±1.8	0.02*
HDL (mmol/L)	1.2±0.02	1.2±0.03	1.2±0.03	1.1±0.03	<0.01*
TG (mmol/L)	1.4±0.13	1.4±1.0	1.3±0.8	1.4±0.6	0.22
FBG (mmol/L)	7.0±0.02	7.7±0.3	5.6±0.8	7.9±0.2	<0.01*
HbA1c (%)	6.7±0.13	7.1±0.14	5.7±0.06	7.4±4.3	<0.01*
25[OH]D (nmol/L)	63.6±1.4	63.1±2.5	57.8±2.2	70.1±2.5	<0.01*
Ca (mmol/L)	4.1±1.0	2.4±0.01	2.3±2.2	3.9±1.6	0.88
CRP (mg/L)	7.28±0.9	6.72±1.05	8.48±2.2	6.50±0.8	0.60
GSH (μmol/L)	380.3±11	349.3±19	355.4±19	428.8±20	0.03*
TNF-α (pg/mL)	153.2±5	153.3±10	148.8±8	157.5±8	0.77

Notes: Data presented as mean± standard error or as frequency (%). *Significance indicated when *P*-value is less than 0.05.

was substantially higher in the same group (428.8±20 μmol/L) when compared with the patients with only T2DM or hypertension. TNF-α average was 153.2±5 pg/mL and was similar in all three groups.

Association of Vitamin D Status on GSH and TNF-α in T2DM and/or Hypertensive patients

Table 2 displays the independent association of vitamin D status (deficiency versus normal levels of serum 25[OH]D) on GSH and TNF-α among various groups in the studied population. Younger patients (below 60 years) and females had

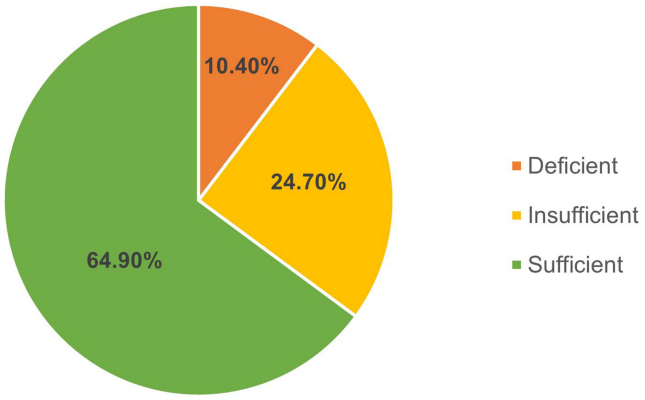

Figure 1 Vitamin D status (25[OH]D) among study population.

lower levels of GSH whether they were suffering from 25[OH]D deficiency or not. Among patients with normal 25[OH]D levels, patients with uncontrolled T2DM showed a significantly higher level of GSH (420.7 µmol/L (95% CI: 355.3–486.0)) compared with controlled group (367.9 µmol/L (95% CI: 337.9–397.8)) and similarly in patients with 25[OH]D deficiency (uncontrolled T2DM 432.8 µmol/L (95% CI: 324.6–523.1)) versus controlled T2DM (351.7 µmol/L (95% CI: 310.3–393.2), respectively). In the meantime, the GSH level was higher among patients with uncontrolled hypertension (400.1 µmol/L (95% CI: 340.7–459.4)) compared with those with controlled hypertension (332.9 µmol/L (95% CI: 283.9–381.9)), but this difference did not reach the statistically significant level ($P>0.05$). The GSH showed significantly higher level among patients with both T2DM and hypertension when compared with those suffering from only one of the conditions ($P=0.04$). Moreover, older patients, males, and those with normal 25[OH]D levels had relatively higher GSH level across all categories of co-morbidities. Patients with uncontrolled T2DM had higher levels of GSH whether they were suffering from 25[OH]D deficiency or not, while the GSH level did not differ between patients with either controlled or uncontrolled hypertension even if they have vitamin D deficiency. Likewise, the independent association of vitamin D status on TNF-α among various groups was evaluated (Table 2) showing no specific defined pattern when comparing patients with 25[OH]D deficiency and those with normal level across all subgroups (age, gender, controlled vs uncontrolled T2DM/hypertension or even co-morbidities). However, males showed slightly higher level of TNF-α compared with females and uncontrolled hypertensive patients had relatively higher TNF-α levels when evaluated against controlled hypertensive patients.

Correlation Between 25[OH]D, GSH and TNF-α Levels with All Tested Parameters

Pairwise correlations were investigated between all laboratory workup and 25[OH]D concentration, GSH and TNF-α. The 25[OH]D concentration was positively correlated with MCV (r = 0.128, $P<0.01$). The level of GSH was also positively correlated with HbA1c (r = 0.146, $P<0.01$) and WBC (r = 0.143, $P<0.01$), while negatively correlated with HDL (r = −0.124, $P<0.01$). TNF-α did not show any significant correlations in the pairwise comparisons with other parameters (Table 3).

Discussion

The prevalence of 25[OH]D deficiency is one of the alarming health concerns worldwide due to its association with several chronic non-communicable, and even communicable diseases.[23] Such deficiency is prevalent in different societies to various extents depending on several factors, including duration of sunlight exposure, skin pigmentation, 25[OH]D precursor availability from dietary nutritional sources, genetic polymorphisms, physical activity, and vitamin D pharmaceutical supplementation.[24–26] The association between the prevalence of 25[OH]D with diabetes and/or hypertension also seemed to have ethnic bases.[27] In Saudi Arabia, 25[OH]D deficiency was a major concern, as some studies reported nearly 70% prevalence among T2DM patients.[13] In the current study, the prevalence of 25[OH]D deficiency was 35.1% in T2DM and/or hypertensive patients. It is noteworthy that patients suffering from hypertension only had lower levels of

Table 2 Reduced Glutathione (GSH) and Tumor Necrosis Factor (TNF)-α Levels Among different Categories According to Vitamin D Status

	GSH		*P*-value	TNF-α		*P*-value
	Sufficient 25[OH]D N=165 (38.9%)	**Insufficient 25[OH]D N=259 (61.1%)**		**Sufficient 25[OH]D N=165 (38.9%)**	**Insufficient 25[OH]D N=259 (61.1%)**	
Age						
Below 60	375.0 (340.1, 409.9)	363.5 (314.8, 412.1)	0.67	155.9 (138.1, 173.8)	147.2 (131.3, 163.1)	0.78
60 and above	387.4 (342.4, 432.5)	373.1 (312.2, 433.9)		148.3 (131.1, 165.5)	160.9 (125.7, 196.2)	
P-value	0.61			0.86		
Gender						
Males	396.8 (342.6, 451.2)	409.7 (345.9, 473.4)	0.08	160.0 (132.9, 187.2)	159.7 (130.7, 188.7)	0.28
Females	373.5 (341.5, 405.4)	338.4 (290.4, 386.4)		150.5 (136.0, 165.1)	145.6 (129.1, 162.2)	
P-value	0.66			0.81		
Diabetes						
Controlled	367.9 (337.9, 397.8)	351.7 (310.3, 393.2)	0.03*	154.1 (138.9, 169.2)	146.7 (131.1, 162.3)	0.53
Uncontrolled	420.7 (355.3, 486.0)	432.8 (324.6, 523.1)		149.7 (125.9, 173.4)	166.8 (124.0, 209.5)	
P-value	0.82			0.70		
Hypertension						
Controlled	380.3 (344.1, 416.5)	332.9 (283.9, 381.9)	0.16	146.5 (129.4, 163.7)	150.2 (129.8, 170.3)	0.87
Uncontrolled	379.2 (337.9, 420.4)	400.1 (340.7, 459.4)		159.1 (140.2, 178.1)	152.2 (129.5, 174.9)	
P-value	0.58			0.47		
Comorbidity						
Diabetes	355.1 (312.8, 397.4)	342.9 (270.7, 415.1)	0.04*	143.8 (120.0, 167.6)	161.8 (127.0, 196.6)	0.57
Hypertension	346.6 (296.1, 397.1)	364.1 (306.5, 421.8)		158.2 (131.2, 185.1)	137.7 (122.3, 153.2)	
Both	418.2 (372.2, 464.2)	392.0 (316.4, 467.6)		155.9 (137.7, 174.0)	166.6 (128.7, 204.5)	
P-value	0.85			0.79		

Notes: Data are presented as median (95% Confidence Interval). *Significance indicated when *P*-value is less than 0.05.

25[OH]D. The prevalence of 25[OH]D deficiency reported in the current study is slightly lower than the previously reported levels, which is in line with the documented constant decrease in 25[OH]D deficiency prevalence in this region of Saudi Arabia over the recent years.[28,29] This might be attributed to the constant efforts to improve the awareness, knowledge, attitude, and practices towards maintaining normal levels of 25[OH]D among Saudi population.[30,31] Moreover, different populations, assays and definitions of deficiency could influence these results.

To date, it was still debatable whether there was an effect of 25[OH] deficiency on the antioxidant and/or inflammatory status of T2DM and/or hypertensive patients. On one hand, some studies showed a relationship between 25[OH]D and the alteration of GSH levels in T2DM.[32,33] While one meta-analysis showed that 25[OH]D

Table 3 Correlation Between Reduced Glutathione (GSH) and Tumor Necrosis Factor (TNF)-α with Other Laboratory and Clinical Findings

	BMI	Hb	HCT	MCV	RBC	WBC	Platelets	RDW	Choles.	LDL	HDL	TG	FBG	HBA1c	25[OH]D	Ca	CPR	TNF	GSH
BMI	1																		
Hb	−0.152*	1																	
HCT	−0.125*	0.850**	1																
MCV	−0.086	0.296**	0.261**	1															
RBC	−0.054	0.567**	0.727**	−0.399**	1														
WBC	0.106*	−0.058	−0.058	−0.075	−0.015	1													
Platelet	0.091	−0.240**	−0.263**	−0.169**	−0.108*	0.261**	1												
RDW	0.172**	−0.369**	−0.321**	−0.499**	0.072	0.033	0.176**	1											
Choles.	0.073	−0.040	−0.033	−0.010	−0.026	−0.060	0.118*	0.034	1										
LDL	0.040	−0.025	−0.028	−0.005	−0.013	−0.045	0.116*	0.029	0.893**	1									
HDL	−0.001	−0.119*	−0.120*	0.037	−0.132**	−0.162**	0.054	0.014	0.373**	0.163**	1								
TG	0.025	0.066	0.087	−0.063	0.063	0.107*	0.048	0.010	0.232**	0.064	−0.295**	1							
FBG	−0.014	0.032	0.011	−0.043	0.043	0.088	−0.025	−0.035	−0.013	−0.002	−0.108*	0.141**	1						
HBA1c	0.042	0.036	0.031	−0.052	0.073	0.104*	−0.008	−0.013	−0.021	−0.014	−0.135**	0.161**	0.819**	1					
25[OH]D	−0.009	−0.060	−0.074	0.128**	−0.168**	−0.017	−0.038	−0.072	−0.031	−0.037	0.064	−0.018	0.012	−0.030	1				
Ca	0.115*	0.079	0.080	0.005	0.074	−0.042	0.080	0.016	0.012	−0.005	−0.033	0.086	0.030	0.030	0.108*	1			
CPR	0.097	−0.017	−0.020	−0.038	0.012	0.259**	−0.018	0.041	0.084	0.087	0.012	−0.038	0.006	0.018	−0.068	−0.091	1		
TNF	0.014	0.017	−0.006	0.020	−0.001	0.011	0.062	−0.034	−0.020	0.009	−0.066	−0.058	−0.023	0.016	0.035	−0.015	−0.069	1	
GSH	−0.041	0.049	0.063	−0.005	0.066	0.143**	0.030	0.055	−0.087	−0.051	−0.124*	0.080	0.093	0.146**	0.051	−0.072	0.021	−0.075	1

Notes: Significance indicated when (*) *P*-value less than 0.05, (**) *P*-value less than 0.01.

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supplementation had a beneficial effect improving most of the oxidative stress parameters among diabetic patients.[34] Furthermore, a study reported that severe 25[OH]D deficiency was accompanied by an increase in TNF-α in diabetic patients suffering from painful peripheral neuropathy.[35] For hypertension, 25[OH]D/calcium supplementation was reported to cause an increase in GSH and a decrease in blood pressure in women at risk of hypertension with pregnancy.[36] To the contrary, some studies showed that 25[OH]D had no effect. For example, one study reported that 25[OH]D deficiency was not accompanied by an elevation in systemic inflammation in T2DM patients.[37] As for hypertension, an animal study performed on spontaneously hypertensive rats showed that 25[OH]D supplementation did not alter the level of GSH.[38] Similarly, another study showed that 25[OH]D supplementation failed to improve hypertension clinically in patients by decreasing their blood pressure[39] or by decreasing their systemic inflammatory markers.[40] In line with the results of the latter studies, in the present study, 25[OH] deficiency was independent from oxidative stress represented by GSH level or inflammatory pathway represented by TNF-α levels in both T2DM and hypertensive Saudi patients. Nevertheless, the alarming prevalence of more than one third of a tested population suffering from 25[OH]D deficiency still should not be taken lightly, as it is a serious co-morbidity when added to the hazards faced by T2DM and hypertensive patients.

In the current study, the absolute level of GSH was around 380 μmol/L on average among all tested groups, which is considered within the normal range reported for GSH levels in humans (290–490 μmol/L).[41] In previous studies, the level of GSH was reported to be lower in T2DM[42] and hypertensive patients[43] compared with normal controls, owing to the oxidative stress induced during the pathogenic course of these diseases. Nevertheless, some studies reported that there was no significant difference between GSH levels in T2DM or hypertensive patients compared with their respective controls.[44,45] It is noteworthy that, in the current study, we did not compare T2DM/hypertensives with controls, but rather we investigated a correlation between the level of GSH among diabetics and/or hypertensive patients, correlating it with vitamin D status. Indeed, some studies indicated a crosstalk between 25[OH]D and GSH, where GSH was reported to positively up-regulate the bioavailability of 25[OH)D,[46] and, on the other hand, 25[OH)D was shown to up-regulate GSH production in vitro in U937 monocytes exposed to high glucose.[47] Still, it seems that such in vitro and animal preliminary studies do not reflect significance in clinical situations, as it did not attain significant medically relevant correlation in the present study. Independent from 25[OH]D levels, the current study showed that the level of GSH had a trend of being relatively higher in patients with uncontrolled T2DM or hypertension compared with controlled patients. In addition, GSH was higher in patients suffering concomitantly from both T2DM and hypertension compared with those having one of the diseases alone. It is possible that such an increase in GSH is a part of a feedback protective mechanism of the reactive oxygen species scavenging system. This feedback effect was accentuated by the fact that GSH level in the current study also increased in correlation with age and with the increase in WBC, indicative of infection, while decreased in correlation with HDL, which is considered the "good" cholesterol.

The pro-inflammatory cytokine TNF-α was considered one of the biomarkers indicative of the severity of T2DM, as higher levels of circulating TNF-α receptors were correlated with higher levels of mortality in these patients.[48] In hypertensive patients, TNF-α was also shown to have higher levels compared with controls.[49] Supplementation of T2DM patients with 25[OH]D was shown to cause an improvement of their inflammatory status.[50] Similarly, in hypertensive patients, a correlation was established between 25[OH]D insufficiency and SBP in hypertensive patients,[51] which was contrary to the results of the current study where there was no correlation of the level of 25[OH]D with TNF-α in either T2DM or hypertensive patients. An explanation for such discrepancy may be due to the increment of GSH seen in the present study that might blunt the oxidative stress, which might be a main determinant factor of induction of inflammatory pathway. Nevertheless, in the current study slightly higher levels of the pro-inflammatory cytokine TNF-α were seen in uncontrolled hypertensive patients compared with patients with controlled blood pressure.

Conclusions

The prevalence of 25[OH]D deficiency in T2DM and hypertensive Saudi patients was seen in about one third of the patients only, which is considered an improvement compared with previously reported studies. This was independent of the levels of GSH or TNF-α in both disease groups. GSH seemed to play a critical role as a feedback protective biomarker in uncontrolled patients of both diseases, ameliorating oxidative stress and limiting the induction of TNF-α.

Abbreviations

25[OH]D, 25-hydroxyvitamin D; BMI, body mass index; Ca, calcium; CVA/TIA, cerebrovascular accident/ transient ischemic attack; CVD, cardiovascular diseases; CRP, C-reactive protein; DBP, diastolic blood pressure; FBG, fasting blood glucose; GSH, reduced glutathione; Hb, hemoglobin; HCT, hematocrit; HDL, high density lipoprotein; KAAUH, King Abdullah bin Abdulaziz University Hospital; LDL, low density lipoprotein; MCV, mean corpuscular volume; RBC, red blood cells; RDW, red cell distribution width; SBP, systolic blood pressure; T2DM, type II diabetes mellitus; TG, triglycerides; TNF-α, tumor necrosis factor-α; WBC, white blood cells.

Ethics Approval and Informed Consent

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Princess Nourah bint Abdulrahman University, number 18-0136 on 17/04/2018, as well as King Abdullah bin Abdulaziz University Hospital (R0-2019-K-002). Informed consent was obtained from all subjects involved in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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