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Association of Vitamins D, B6, and B12 Deficiencies with Hyperlipidemia Among Jordanian Adults

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Abstract

Background: Obesity is an abnormal fat accumulation that adversely affects human health. Studies reported several vitamin deficiencies in obese patients. The current study investigates the deficiencies of vitamins D, B6, and B12 among Jordanian adults with hyperlipidemia and demonstrates the association between serum vitamin levels and metabolic and lipid profile parameters.

Methods: Sixty male subjects were divided into 40 hyperlipidemic patients (age: 45.9 yr. ±10.2) and 20 controls (age: 41.2 yr. ±10.7). The blood levels of triglycerides, total cholesterol, high density lipoprotein (HDL)-cholesterol, hemoglobin A1c, and vitamins D, B6, and B12 were measured.

Results: The hyperlipidemic patients showed significantly increased triglycerides, total cholesterol, non-HDL, cholesterol/HDL ratio, low-density lipoprotein (LDL)- cholesterol levels, and decreased HDL-cholesterol levels compared to the controls. No significant differences were found in the blood levels of vitamin D, vitamin B6, or vitamin B12 between groups. However, 50% of the hyperlipidemic patients and 54.5% of the controls exhibited vitamin D deficiency. Only the hyperlipidemic patients exhibited deficiencies of vitamins B6 and B12 in 5.4% and 3.3% of cases, respectively. In the controls, vitamin B12 level was inversely associated with total cholesterol, whereas in the hyperlipidemic patients, vitamin B6 level was inversely correlated with total cholesterol and non-HDL levels.

Conclusion: The hyperlipidemic patients exhibited vitamins D, B6, and B12 deficiencies. Additionally, vitamins B6 and B12 levels were inversely correlated with total cholesterol and non-HDL levels. Our findings highlight the importance of routine evaluation of vitamin levels in patients with hyperlipidemia.

Keywords: Cholesterol, Hyperlipidemia, Obesity, Vitamins.

Introduction

Obesity is an abnormal fat accumulation that adversely affects human health. The prevalence of obesity is continuously increasing worldwide (1). Obesity poses serious health problems, including the development of cardiovascular diseases, diabetes mellitus, cancer, and others (2, 3). A high-fat, high-calorie diet, sedentary lifestyle, and genetic predisposition are some of the fundamental etiologies of obesity (4). Studies have shown that obesity positively correlates with hyperlipidemia, including increased serum levels of triglycerides, total cholesterol, and low-density lipoprotein (LDL)-cholesterol and decreased levels of high density lipoprotein (HDL)- cholesterol (5). Remarkably, previous studies reported several nutritional deficiencies in obese patients that may arise from eating unhealthy foods with little nutritional value (6).

Vitamins are essential nutrients required for human health. They are mainly classified into two groups: fat-soluble vitamins D, A, K, and E

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and water-soluble vitamins B1, B2, B3, B5, B6, B7, B9, B12, and C (7).

Vitamin D is called sunshine vitamin as UV light is required to convert pro-vitamin D (7-dehydrocholesterol) in the skin cells to pre-vitamin D3, which is isomerized to vitamin D3 form. After that, vitamin D3 is converted by specific cytochrome p450 enzymes in the liver to 25-hydroxy vitamin D3 (25(OH)D3). The latter is hydroxylated to its final active form of 1-25-dihydroxy vitamin D3 (1,25-$(OH)_2$D3) in the kidney cells (8). Vitamin D is a vital component for the normal functions of the skin, immune system, and parathyroid gland. It is also implicated in preserving bones and teeth, cancer prevention, and xenobiotics metabolism (9). Many studies showed that obesity increases the status of vitamin D deficiency (10). On the other hand, vitamin D deficiency was reported as one of the factors that causes obesity (11).

Vitamin B6 (pyridoxine) is an essential nutrient required as a cofactor for the metabolic reactions of amino acids, nucleic acids, carbohydrates, and lipids. The metabolically active form of vitamin B6 is called pyridoxal 5-phosphate. Vitamin B6 reduces inflammation, regulates blood pressure, and controls blood clotting (12). Vitamin B6 deficiency was associated with several diseases, such as microcytic anemia, epileptiform convulsions, seborrheic dermatitis, and confusion (13).

Vitamin B12 (cobalamin) is linked to many vital processes in the body, such as DNA synthesis, hematological development, neurological functions, bone health, and fetal development (9). The serum concentration of vitamin B12 determines its status in the body, where vitamin B12 deficiency leads to megaloblastic anemia and neurological abnormalities, including depression, Parkinsonism, and Alzheimer's disease (14).

Little is known about the prevalence of vitamin deficiencies among the Middle Eastern populations and how they are affected by obesity. Therefore, the current study investigates the prevalence of vitamin D, B6, and B12 deficiencies among Jordanian adults with hyperlipidemia and healthy controls. Furthermore, the study demonstrates the possible associations between serum vitamin levels and metabolic and lipid profile parameters.

Materials and Methods

Subjects

Out of 143 Jordanian adults visiting the MedLabs Medical Laboratory, Al-Karak, for laboratory investigations from January to April 2023, only 60 subjects were recruited in the current study. Inclusion criteria were defined as males, aged 20-65 years old with absence of advanced chronic medical conditions that may interfere with the study outcomes, such as advanced kidney disease, hyperparathyroidism, and malignancies. Subjects who were taking any vitamin supplements before sample collection were excluded.

The recruited participants were divided into two groups, Hyperlipidemic patients (N=40), the subjects had serum triglyceride levels above 150 mg/dl and LDL-cholesterol levels above 100 mg/dl and Control group (N=20), the subjects were healthy and with normal levels of serum triglycerides and LDL-cholesterol.

The study was conducted in agreement with the Helsinki Principles declaration that was revised in 2000. The Ethics Review Committee of the Faculty of Pharmacy, Muath University, approved the experimental procedures of the study (Approval no: 2632023). All subjects provided written informed consent before their participation.

Biochemical analysis

Blood samples were collected from all participants after overnight fasting, and the sera were separated and kept at -80 ^{o}C freezer until analysis. All participants performed the following routine biochemical analyses using commercial kits as per the provided instructions:

Glycosylated hemoglobin A1c (HbA1c**,** Roche Diagnostics GmbH, Germany, REF: 04528182 190, V4.0), Triglycerides (Roche

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Diagnostics GmbH, Germany, REF: 20767107 322, V12.0), Cholesterol (Roche Diagnostics GmbH, Germany, REF: 03039773190, V9.0), and High-density lipoprotein (HDL, Roche Diagnostics GmbH, Germany, REF: 07528566190).

Measurement of vitamin B6 concentration

The serum level of vitamin B6 was measured using an enzyme-linked immunosorbent assay (ELISA) kit (Vitamin B6, ELISA Kit, Catalog No: MBS2502839, My BioSource, USA). Briefly, the assay was performed as follows: Each well of a 96-well plate was pipetted with 50 µl of sample or standard, followed by the addition of 50 µl of biotinylated detection antibody, and incubated for 45 min at 37°C. The plate was washed three times, and excess solutions were aspirated. Subsequently, 100 µl of horseradish peroxidase (HRP) conjugate was pipetted, and the plate was incubated at 37°C for 30 min before being washed five times. 90 µl of substrate reagent was added, and the wells were incubated for another 15 min at 37°C. After the necessary incubation, 50 µl of stop solution was added immediately. The produced color was read spectrophotometrically at a wavelength of 450 nm using a microplate spectrophotometer (Multiskan Skyhigh, Thermo Scientific, USA). Unknown sample concentrations were calculated after the construction of a standard curve.

Measurement of vitamins D and B12 concentrations

Vitamin D level was measured by Elecsys Vitamin D total III, Roche Diagnostics GmbH, Germany, REF: 09038086190, V1.0) kit which detects 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3. Elecsys Vitamin B12 II (Roche Diagnostics GmbH, Germany, REF: 07212771190, V6.0) was used for the measurement of vitamin B12 level in the sera samples. The assay was briefly performed as follows: The serum sample (15 µl) was incubated with one g/l of dithiothreitol and 57.5 g/l of sodium hydroxide to liberate the bound vitamin. Ruthenium-labeled vitamin D binding protein (VDBP) or intrinsic factor for measuring vitamin D or B12, respectively, was incubated with the resulting mixture to form a complex. A biotin labeled vitamin D or B12 and streptavidin-coated microparticles were added to allow binding to the solid phase. When the mixture was drawn into a measurement cell, the microparticles were magnetically trapped on the electrode's surface. ProCell II M was used to extract any unbound chemicals from the solution. A photomultiplier was used to detect the resulting chemiluminescent emission. The concentrations were obtained via a calibration curve, using 2-point calibration, and a standard curve.

Statistical analysis

All raw data were coded before entry into the statistical SPSS software (version 28, IBM Corp., USA). Data were expressed as mean ± standard deviation (SD). An unpaired Student t-test was used for comparing groups with quantitative and normally distributed variables. A non-parametric Mann-Whitney test was used for comparing groups with quantitative and non-normally distributed variables. Spearman correlation coefficient was used to determine significant correlations between quantitative variables. The receiver operating characteristic (ROC) curve was employed to detect the best cutoff value of significant parameters. *P*-values less than 0.05 were considered significant**.**

Results

Demographic and biochemical characteristics of subjects

This study recruited 40 hyperlipidemic patients and 20 healthy controls. All subjects were gender (males) and age matched. No significant difference in age between the hyperlipidemic patients (45.9 yr. ±10.2) and the controls (41.2 yr. ±10.7) was detected (p=0.106). Remarkably, the hyperlipidemic patients showed significantly increased levels of triglycerides, total cholesterol, non-HDL, LDL-cholesterol, and cholesterol/HDL ratio compared to the controls. The hyperlipidemic patients showed significantly decreased HDL

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levels compared to the controls. The HbA1c level showed no significant difference between groups (Table 1). Interestingly, total cholesterol, triglycerides, non-HDL, total cholesterol/HDL ratio, and LDL showed a sensitivity of 95%, 100%, 97.5%, 97.5%, and 100%, respectively, in detecting hyperlipidemia. They all achieved 100% specificity. However, HDL achieved 82.5% sensitivity and 60% specificity (Table 2).

Table 1. Demographic and biochemical characteristics of subjects.

	Controls	**Hyperlipidemic patients**	
	Mean ± SD	**Mean ± SD**	***P*-value**
Age (yr.)	41.2 ±10.7	45.9 ±10.2	0.106
Total cholesterol (mg/dl)	144.4 ±13.8	213.8 ± 33.3	**< 0.001***
Triglycerides (mg/dl)	94.5 ± 32.9	252.1 ± 113.9	**< 0.001***
HDL (mg/dl)	44.7 ± 9.1	36.1 ± 6.0	**< 0.001***
Non-HDL (mg/dl)	99.7 ±13.2	177.7 ± 32.1	**< 0.001***
Cholesterol/HDL Ratio	3.3 ± 0.6	6.1 ±1.5	**< 0.001***
LDL (mg/dl)	80.8 ± 13.1	132 ± 28.1	**< 0.001***
HbA1c	6.1 ± 2.0	6.4 ± 1.8	0.620

yr.: year, *: statistically significant.

Table 2. The sensitivity and specificity of the lipid profile parameters in detecting hyperlipidemia.

	AUC	***P*- value**	**95% Confidence Interval**				
			Lower Bound	**Upper Bound**	**Cut off**	**Sensitivity %**	**Specificity %**
Total cholesterol (mg/dl)	0.976	**< 0.001***	0.935	1.017	> 170	95	100
Triglycerides (mg/dl)	1.000	**< 0.001***	1.000	1.000	> 152	100	100
Non-HDL (mg/dl)	0.984	**< 0.001***	0.951	1.016	> 127	97.5	100
Cholesterol/ HDL Ratio	0.996	**< 0.001***	0.985	1.006	> 4.35	97.5	100
LDL (mg/dl)	1.000	**< 0.001***	1.000	1.000	> 100.5	100	100
HDL (mg/dl)	0.788	**< 0.001***	0.665	0.911	41.5	82.5	60

AUC: Area under the curve, *: statistically significant.

Prevalence of vitamin D, B6, and B12 deficiencies among subjects

The serum levels of vitamin D, vitamin B6, and vitamin B12 showed no significant differences between the hyperlipidemic patients and the controls (Fig. 1A-C). However, 50% of the hyperlipidemic patients and 54.5% of the controls exhibited vitamin D deficiency (estimated as blood levels less than 20 ng/ml). Only the hyperlipidemic patients

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exhibited vitamin B6 deficiency (blood levels less than 5 ng/ml) in 5.4% of cases and vitamin B12 deficiency (blood levels less than 200 pg/ml) in 3.3% of cases. No vitamin B6 or B12 deficiencies were detected among the controls. Interestingly, 5% of the hyperlipidemic patients exhibited two or more types of vitamin deficiencies (Fig. 1 D & E).

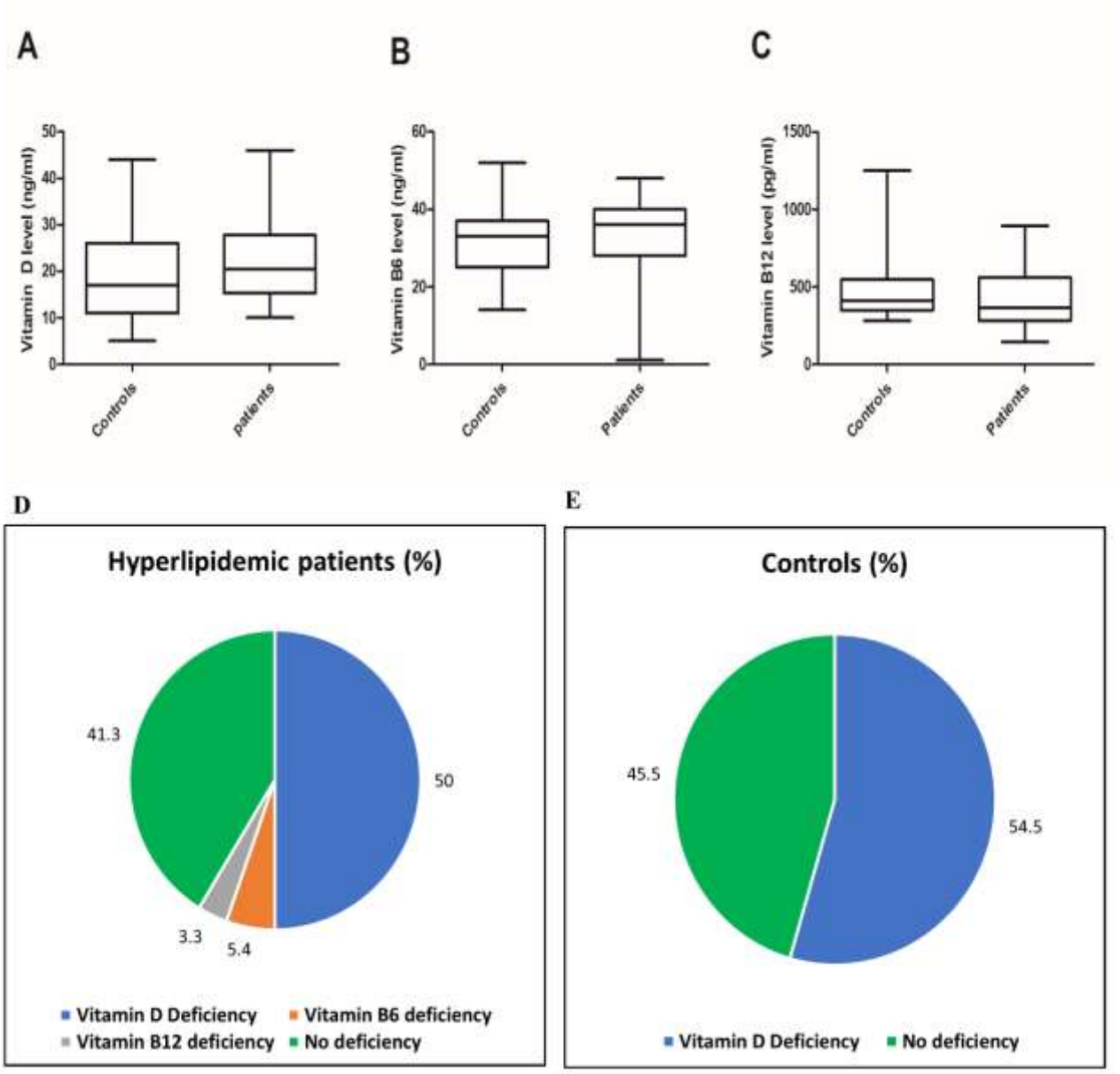

Fig. 1. Serum Levels of Vitamins in Hyperlipidemic Patients and Controls. Serum levels of vitamin D, B6, and B12 were comparable between hyperlipidemic patients and controls (A-C). Vitamin D deficiency (<20 ng/ml) was prevalent in 50% of hyperlipidemic patients and 54.5% of controls. Vitamin B6 deficiency (<5 ng/ml) occurred in 5.4% of hyperlipidemic cases, and vitamin B12 deficiency (<200 pg/ml) in 3.3%. No B6 or B12 deficiencies were observed in controls. Notably, 5% of hyperlipidemic patients exhibited concurrent deficiencies in two or more vitamins (D & E).

Correlation between serum vitamin levels and lipid profile parameters

In the control group, vitamin B12 level was inversely correlated with total cholesterol (p = 0.024) (Table 3). Notably, in the hyperlipidemic patients, vitamin B6 level was inversely correlated with total cholesterol level (p= 0.003) and non-HDL level (p= 0.005) (Table 4).

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Table 3. Correlation between the lipid profile parameters and vitamin levels in the controls.

Control group		Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	Non-HDL (mg/dl)	Cholesterol /HDL Ratio	LDL (mg/dl)
Age (yr.)	**Correlation Coefficient**	-0.299	0.390	-0.057	-0.210	-0.041	-0.429
	***P*-value**	0.201	0.089	0.811	0.375	0.865	0.059
HbA1c	**Correlation Coefficient**	-0.252	0.151	0.028	-0.469	-0.081	-0.406
	***P*-value**	0.314	0.550	0.912	**0.050***	0.750	0.095
Vitamin B12 (pg/ml)	**Correlation Coefficient**	-0.599	-0.103	-0.270	-0.422	0.007	-0.345
	***P*-value**	**0.024***	0.725	0.350	0.133	0.982	0.226
Vitamin D (ng/ml)	**Correlation Coefficient**	-0.228	0.174	-0.028	-0.229	-0.135	-0.085
	***P*-value**	0.500	0.610	0.936	0.499	0.692	0.805
Vitamin B6 (ng/ml)	**Correlation Coefficient**	-0.070	-0.051	-0.112	0.021	0.156	0.072
	***P*-value**	0.775	0.835	0.648	0.933	0.525	0.769

yr.: year, *: statistically significant.

Table 4. Correlation between the lipid profile parameters and vitamin levels in hyperlipidemic patients.

Hyperlipidemic patients		Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	Non-HDL (mg/dl)	Cholesterol /HDL Ratio	LDL (mg/dl)
Age (yr.)	**Correlation Coefficient**	0.073	0.070	0.060	0.098	0.047	0.207
	***P*-value**	0.653	0.666	0.712	0.548	0.774	0.200
HbA1c	**Correlation Coefficient**	0.198	0.056	0.098	0.197	0.116	0.356
	***P*-value**	0.239	0.744	0.563	0.242	0.495	**0.031***
Vitamin B12 (pg/ml)	**Correlation Coefficient**	-0.001	-0.173	0.158	-0.046	-0.077	-0.010
	***P*-value**	0.997	0.360	0.403	0.809	0.686	0.960
Vitamin D (ng/ml)	**Correlation Coefficient**	0.223	0.035	-0.054	0.251	0.245	0.189
	***P*-value**	0.295	0.870	0.804	0.236	0.249	0.377
Vitamin B6 (ng/ml)	**Correlation Coefficient**	-0.474	-0.169	-0.211	-0.456	-0.202	-0.307
	***P*-value**	**0.003***	0.317	0.210	**0.005***	0.230	0.064

yr.: year, * : statistically significant.

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Correlation between HbA1c and other parameters

The results revealed that HbA1c level in the control group was inversely associated with non-HDL level (p = 0.050) (Table 3). However, HbA1c level in the hyperlipidemic patients was directly associated with LDL levels (p=0.031) (Table 4). Furthermore, in the hyperlipidemic patients, HbA1c level was directly correlated with age (p <0.001) (Table 5). Interestingly, vitamin D level in the control group was inversely correlated with vitamin B6 level (p = 0.009).

Table 5. Correlation between the serum vitamin levels and other parameters.

		Age (yr)	HbA1c	Vitamin B12 (pg/ml)	Vitamin D (ng/ml)	Vitamin B6 (ng/ml)
Controls						
HbA1c	**Correlation Coefficient**	0.423	1.000	-0.157	0.295	0.234
	***P*-value**	0.080	-	0.607	0.378	0.366
Vitamin B12 (pg/ml)	**Correlation Coefficient**	0.501	-0.157	1.000	0.162	-0.171
	***P*-value**	0.068	0.607	-	0.728	0.576
Vitamin D (ng/ml)	**Correlation Coefficient**	0.451	0.295	0.162	1.000	-0.739
	***P*-value**	0.164	0.378	0.728	-	**0.009***
Vitamin B6 (ng/ml)	**Correlation Coefficient**	0.070	0.234	-0.171	-0.739	1.000
	***P*-value**	0.775	0.366	0.576	**0.009***	-
Hyperlipidemic patients						
HbA1c	**Correlation Coefficient**	0.569	1.000	0.262	-0.079	-0.113
	***P*-value**	**< 0.001***	-	0.178	0.733	0.518
Vitamin B12 (pg/ml)	**Correlation Coefficient**	0.291	0.262	1.000	-0.230	0.054
	***P*-value**	0.119	0.178	-	0.304	0.785
Vitamin D (ng/ml)	**Correlation Coefficient**	-0.059	-0.079	-0.230	1.000	0.075
	***P*-value**	0.786	0.733	0.304	-	0.741
Vitamin B6 (ng/ml)	**Correlation Coefficient**	0.014	-0.113	0.054	0.075	1.000
	***P*-value**	0.933	0.518	0.785	0.741	-

yr.: year, *: statistically significant.

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Discussion

In the present study, the hyperlipidemic patients exhibited vitamins D, B6, and B12 deficiencies. In addition, the serum levels of vitamins B6 and B12 were inversely correlated with the total cholesterol and non-HDL levels of subjects.

Several studies showed low blood levels of vitamins D, B6, B12, C, and E in morbidly obese patients compared to healthy controls, consistent with our results (15). More specifically, vitamin D deficiency was higher by three times in obese patients with accumulated fats compared with obese patients with lower fat (16). In a study by Vimaleswaran et al., it was found that each one-unit increase in body mass index (BMI) was correlated with a 1.15% reduction in vitamin D level, confirming that obesity is a major risk factor for the development of vitamin D deficiency (17). Interestingly, vitamin D level decreases with increasing age. However, outdoor physical activity can compensate for vitamin D deficiency, especially in young and middle-aged people (18).

We found no significant correlations between vitamin D levels and the lipid profile parameters. In a study performed on Jordanian subjects, vitamin D level was directly correlated with HDL-cholesterol only, with no significant associations with other lipid profile parameters, consistent with our results (19). In contrast, it was found that morbidly obese patients with vitamin D deficiency exhibited low serum HDL and high triglyceride, total, and LDL-cholesterol levels (20, 21).

In a recent study performed on Saudi Arab patients with vitamin D deficiency, administering vitamin D supplements for six months improved a 10-year risk score of atherosclerotic cardiovascular disease via increasing the blood HDL-cholesterol level (22). In contrast, vitamin D supplementation had no significant effect on the anthropometric indices of patients with type 2 diabetes (23).

In the current study, only the hyperlipidemic patients showed vitamin B6 deficiency in 5.4% of cases, and its level was inversely correlated with total cholesterol and non-HDL levels. A negative correlation between BMI and vitamin B6 concentrations was observed in obese patients, consistent with our results (24). On the other hand, a study performed on Thai subjects demonstrated that serum vitamin B6 levels were adequate in the obese and overweight subjects compared to normal adults (25). Consistent with our findings, a study was performed on Lebanese patients with hypertriglyceridemia taking vitamin B6 supplements for 12 weeks, where the plasma total cholesterol levels were reduced (26).

Interestingly, we did not find a significant difference in vitamin B12 levels between the hyperlipidemic patients and the controls, although vitamin B12 deficiency was only detected in the hyperlipidemic patients (3.3% of cases). Consistent with our results, vitamin B12 level was not significantly different between adult obese patients and the controls (25). Also, no significant difference in vitamin B12 levels was detected between obese subjects and normal controls (27). More relevant to the current study, in a study performed on 84 healthy Jordanian volunteers, 27 participants showed vitamin B12 deficiency, and the serum level of vitamin B12 was positively associated with BMI (28). In our study, vitamin B12 level in the control group was inversely correlated with total cholesterol, consistent with a previous study showing a correlation between low vitamin B12 concentrations and higher levels of total cholesterol, LDL-cholesterol, and cholesterol/HDL ratio (28).

The study's drawbacks include the small number of recruited subjects and the unidentified BMI of subjects, which hinder the generalization of the current findings. Nevertheless, the current results strongly recall the routine evaluation of vitamin status in hyperlipidemic patients to avoid future disease complications.

The hyperlipidemic patients exhibited vitamins D, B6, and B12 deficiencies. The vitamins B6 and B12 levels were inversely correlated with total- cholesterol and non-HDL

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levels. This study points to the importance of routine vitamin evaluation in patients with hyperlipidemia.

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Ethics statement
The Ethics Review Committee of the Faculty of Pharmacy, Muath University, approved the experimental procedures of the study (approval no: 2632023). All subjects provided written informed consent before their participation.

Conflicts of interest
Authors declare there are no conflicts of interest.

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