The Relationship between Vitamin D Deficiency and NAFLD in Sample of Egyptian Type 2 Diabetic Patients

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ABSTRACT

Background: vitamin D deficiency has a strong association with insulin resistance and NAFLD.
Objective: to assess vitamin D levels in patients having type2 diabetes with NAFLD and to study its relationship with insulin resistance. **Patients and methods**: a case–control study conducted on 50 subjects who were divided into **2 groups**: 35 patients having T2DM and NAFLD (**group 1**) and 15 healthy subjects served as control **(group 2)**. Fasting plasma glucose (FBG), 2 hour post prandial (2hrpp), and fasting plasma insulin (FPI) were measured with calculation of HOMA-IR. Fasting lipids, Hb A1c, calcium, phosphorus, urea, creatinine, serum alanine aminotranseferase (ALT), aspartate aminotransaminase (AST) were also measured. BMI was calculated, serum 25 (OH)D was measured with ELISA and abdominal ultrasonography was done for all participant.
Results: the study showed lower level of vitamin D in patients with T2DM and NAFLD 10.6 (5.5-21.3) as compared to control group 31 (27-39.7). While non-significant difference was found between male and female regarding 25(OH) D level and HOMA-IR**.** There was significant negative correlation between vitamin D level and HOMA-IR.
Conclusion: Vitamin D level was associated with presence of NAFLD. There was strong relation between vitamin D level and insulin resistance as vitamin D deficiency was associated with higher levels of HOMA-IR. Obesity may be related to low vitamin D level, but no difference in VD level between males and females was found.
Keywords: Vitamin D, NAFLD, T2DM.

INTRODUCTION

Type 2 diabetes (T2DM) is a chronic endocrine disease characterized by poor pancreatic cells and insulin resistance. It represents one of the major health problems worldwide due to its chronic disrupted complications which require special medical care for prevention and early treatment [(1)]. Several genetic and environmental factors have linked to T2DM development [(2)].

NAFLD is the most common liver disease in western countries and Mediterranean region, the exact mechanism of NAFLD development is unknown. However, NAFLD has the potentiality of progression to nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (HCC). It is commonly associated with insulin resistance and metabolic syndrome [(3)].

Vitamin D deficiency has been identified as one of the risk factors for type 2 diabetes development and NAFLD [(4)]. Several evidence based studies have documented the relation between vitamin D deficiency and development of T2DM [(5)]. Where vitamin D supplementation found to be associated with the decreased risk in predisposed patients [(6)].

The biological effects of vitamin D mediated after its binding to cytosolic / nuclear vitamin D receptors (VDRs) which belong to steroid hormone receptor family. These receptors spread in many tissues including hepatocytes and pancreatic β cell [(7)].

VDR upregulation occurs secondary to a pathologic stimulus. This would be consistent with the observations of VDR expression/activity in transformed hepatocytes and the inverse correlations between VDR levels and severity of non-alcoholic fatty liver disease described by studies [(8)].

AIM OF THE WORK

To assess vitamin D levels in patients having type 2 diabetes with NAFLD and to study its relationship with insulin resistance.

PATIENTS AND METHODS

The study was conducted on 50 subjects; age and sex matched, their age ranged from 30 to 50 years.

Ethical approval:
All subjects were selected after taking their verbal and written consent and after **taking approval from the ethical committee consent of Al-Azhar University.**

They were divided into **two groups**, **Group 1:** included thirty five patients having type 2 diabetes with NAFLD on oral antidiabetic treatment. Patients were selected from diabetes outpatients' clinic of Al-Zahraa University Hospital. **Group 2**: included fifteen clinically normal subjects (not having diabetes) served as control group. They were recruited from the employee and patients attending (orthopedic, general surgery, ENT) outpatient clinics of Al-Zahraa University Hospital.
The clinical part of the study was performed from October 2018 to June 2019.

Diagnosis of T2DM was based on American Diabetes Association criteria (2018). FPG≥ 126 mg/dL. Fasting is defined as no caloric intake for at least 8 h, or 2-h PG≥200 mg/dL or HbA1C≥6.5%, or in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis and a random plasma glucose ≥200 mg/dL

For defining NAFLD, the hepatic steatosis index (HSI) was calculated as follows: (8 × ALT/AST ratio

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+ body mass index (BMI) + 2 (if diabetic) + 2 (if female). The HSI cutoff point for NAFLD is 36) [9].
All patients with history of excessive alcohol consumption, hepatitis C or B infection, chronic liver disease or autoimmune hepatitis were excluded as well as patients with abnormal liver and renal function tests (more than two times the upper limits of normal), severe anemia or known hemoglobinopathy, as well as patients receiving insulin, corticosteroid or vitamin D supplementation were also excluded.

All participants were subjected to detailed history taking and clinical examination including blood pressure measurement, anthropometric measurement including weight (Bwt) kg, height (Ht) cm, and waist circumference (WC) cm. Body mass index (BMI) was calculated **{body weight (kg)/height (m^2)}** [10].
Fasting plasma glucose (FBG), 2 hour post prandial (2hrpp), fasting plasma insulin (FPI) and serum alanine aminotranseferase (ALT), aspartate aminotransaminase (AST) were measured as well as serum urea, creatinine, total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL), high density lipoprotein) HDL, serum calcium (Ca), phosphorus(PO_4). Also, 25(OH) D were measured for all subjects by enzyme-linked immunosorbent assay (ELISA) technique, HOMA-IR was calculated according the equation
(HOMA- IR = Fasting Insulin (µU/ml) x fasting glucose (mg/dl) /405) [10].

Liver sonographic examination was carried out to all subjects by a single experienced radiologist with high-resolution ultrasonography.

Statistical analysis

Data were analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Sciences (SPSS) version 16. Parametric data were expressed as mean ± SD while some of them were expressed as median (IQR) and non-parametric data were expressed as number and percentage of the total.
Comparing the mean ± SD of 2 groups was done using **the student`s t test. Chi-square test** to study the association between each variables or comparison between 2 independent groups as regards the categorized data. Measuring the mutual correspondence between two values was done using **the Spearman correlation coefficient.**
P value > 0.05 was considered non-significant and < 0.05 was considered significant
Receiver operating characteristic (ROC) curves: by using medical calculator program for complete analysis of ROC curves and criterion levels.

RESULTS

Comparison between Group 1 and Group 2 shows significant increased measurement of Bwt, WC, BMI and SBP in group I, also significant higher levels of FBS, 2hrpp, ALT,AST, TC, TG and LDL in group I as compared to group II. While there was significant lower level of HDL in group I as compared to group II. Non-significant difference was found between the 2 groups regarding serum Ca and PO_4 (Table1).

Table (1): Clinical and laboratory data of the studied groups:

Parameters	**Group** 1 N=35	**Group** 2 **N=15**	**P-value**
	Mean ± SD		
Age (Years) (30-60)	48.60 ± 9.45	45.07 ± 8.12	>0.05
Sex	Female: 22 (62.9%) Male: 13 (37.1%)	7 (46.7%)	>0.05
BWT(Kg)	84.06 ± 15.47	66.40 ± 10.31	<0.05*
WC (cm)	107.94 ± 12.64	78.67 ± 14.01	<0.05*
BMI (kg/m^2)	34.88 ± 7.50	25.51 ± 3.23	<0.05*
SBP (mmHg)	122.43 ± 14.47	114.00 ± 7.37	<0.05*
FBG (mg/dl)	143.97 ± 55.51	96.67 ± 7.83	<0.05*
PP(mg/dl)	224.00 ± 86.47	110.47 ± 12.10	<0.05*
ALT (u/l)	21.57 ± 9.74	14.20 ± 2.88	<0.05*
AST (u/l)	23.11 ± 8.67	18.00 ± 5.42	<0.05*
Serum Ca (mg/dl)	9.5±0.53	9.48±0.43	>0.05
Serum PO_4 (mg/dl)	4.62±1.14	4.06±0.62	>0.05
LDL (mg/dl)	115.01 ± 39.56	83.73 ± 23.21	<0.05*
HDL (mg/dl)	41.69 ± 8.51	54.27 ± 14.66	<0.05*
Cholesterol (mg/dl)	187.89 ± 44.0	154.73 ± 28.32	<0.05*
TG (mg/dl)	156.06 ± 67.65	83.07 ± 28.65	<0.05*

Bwt: Body weight**; BMI:** Body mass index; **WC:** Waist circumference**; SBP:** Systolic blood pressure**; FBG:** Fasting blood glucose, **PP:** post prandial blood Glucose, ALT**:** Alanin transferase **AST:** Aspartate transferase. **Ca:** serum calcium, **PO_4:** serum phosphorus, **LDL:** low density lipoprotein, **HDL**: high density lipoprotein, **TG:** triglycerides**.**

Both FPI and HOMA-IR were statistically significantly higher in group 1, while 25(OH) D was significantly lower in group 1 as compared to group 2 (Table 2).

Table (2): Comparison between group 1 and group 2 regarding FPI, HOMA-IR and 25(OH)D

Study groups / Parameters	Group 1	Group 2	P-value
	Median (IQR)		
Insulin (FPI) (µiu\ml)	6.8 (4.6 - 10.3)	5.3 (2.1-6.9)	<0.05*
HOMA IR	2.1 (1.3 - 3.7)	1.1(0.45-1.6)	<0.05*
25 (OH)D (ng\ml)	10.6 (5.5-21.3)	31 (27-39.7)	<0.05*

25(OH)D: 25 Hydroxy vitamin D, ***HOMA-IR:*** *Homeostatic model assessment of insulin resistance*. ***FPI***: fasting plasma insulin

There was non-significant difference between males and females of group 1 regarding 25(OH) vitamin level and HOMA-IR (Table 3).

Table (3): Comparison between male and female regarding 25(OH) D and HOMA-IR in group I

	Female	Male	P-value
	Median (IQR)		
25(OH) D (ng\ml) 25 (OH)D (ng\ml)	**10.55 (5 - 21.3)**	**10.6 (6 - 19.9)**	**>0.05**
HOMA IR	**2.13 (1.3 - 4.06)**	**2.1 (1.3 -3.2)**	**>0.05**

In current study significant negative correlation was found between 25(OH) vitamin D and FPI, HOMA-IR, WC, BMI, ALT and AST. While non-significant correlations were found regarding other clinical and laboratory data in group I (Table 4).

Table (4): Correlation between 25(OH)D and clinical and laboratory data in group 1

Parameters	25(OH) vitD (ng\dl)	
	R	P-value
Insulin (µIu\ml)	**-0.624****	<0.05*
HOMA IR	**-0.348***	<0.05*
Age(years)	0.026	>0.05
WC (cm)	**-0.348***	<0.05*
BMI (cm\m2)	**-0.346***	<0.05*
Duration of D.M (moths)	0.096	>0.05
SBP (mmHg)	0.167	>0.05
FBS (mg\dl)	-0.136	>0.05
ALT (u\l)	**-0.404***	<0.05*
AST (u\l)	**-0.417***	<0.05*
LDL (mg\dl)	-0.089	>0.05
HDL (mg\dl)	0.232	>0.05
Cholesterol(mg\dl)	-0.054	>0.05
TG(mg\dl)	-0.042	>0.05
Ca (mg\dl)	-0.141	>0.05
Po4 (mg\dl)	-0.012	>0.05

Correlating HOMA-IR with clinical and laboratory parameters there were significant positive correlation between HOMA- IR, WC, BMI, FPI, FBG, 2hr PP, LDL and cholesterol; while significant negative correlation was found between HOMA-IR and 25(OH)vitamin D. But, non-significant difference was found regarding other parameters (Table 5).

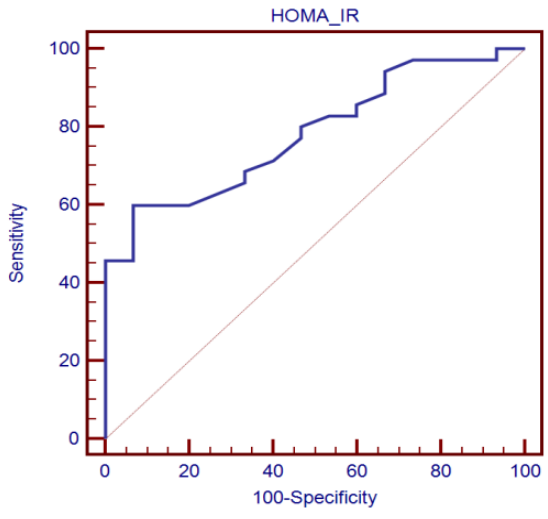
Table (5): Correlation between HOMA-IR and clinical &laboratory data in group 1.

	HOMA- IR	
	R	**P-value**
Insulin (FPI) (µiu\ml)	**0.873****	**<0.05***
25(OH) vit. D (ng\ml)	**-0.348***	**<0.05***
Age (years)	-0.003	>0.05
WC (cm)	**0.340***	**<0.05***
BMI(kg/m^2)	**0.426***	**<0.05***
Duration of DM (months)	-0.154	>0.05
SBP (mmHg)	0.146	>0.05
FBS (mg/dl)	**0.532****	**<0.05***
PP (mg/dl)	**0.382***	**<0.05***
ALT (u/ml)	0.263	>0.05
AST(u/ml)	0.130	>0.05
LDL (mg/dl)	**0.383***	**<0.05***
HDL (mg/dl)	-0.075	>0.05
Cholesterol (mg/dl)	**0.361***	**<0.05***
TG (mg/dl)	0.160	>0.05

From the ROC curve of HOMA- IR the cutoff point was 1.8, above which insulin resistance was detected and the cutoff point of 25(OH)D was 19.9, below which vitamin D deficiency was detected (Table 6).

Table (6): Receiver operating characteristic (ROC) curve of HOMA- IR and 25(OH)D

Parameter	AUC	Cutoff Point	Sensitivity	Specificity	PPV	NPV
HOMAIR	**0.783**	**>1.8**	**60.00**	**93.33**	**94.1**	**42.4**
25 (OH)D	**0.857**	**≤19.9**	**74.29**	**86.67**	**92.9**	**59.1**



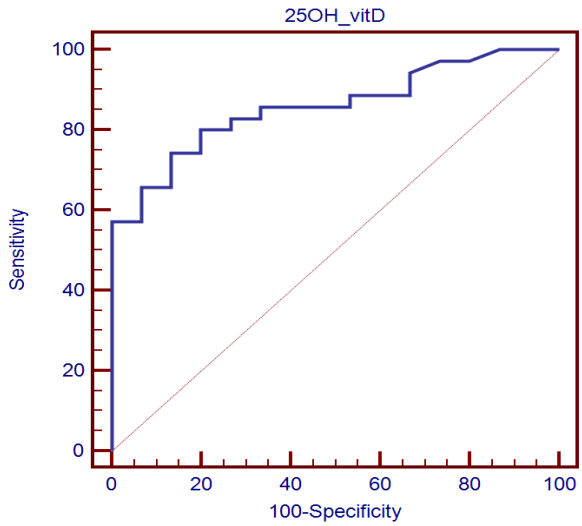


Figure (1): The ROC curve of HOMA IR **Figure (2):** The ROC curve of 25(OH)D

DISCUSSION

A case-control study that was carried out on 50 subjects they were divided into 2 groups: **Group 1** included 35 patients (T2DM and NAFLD) 22 females and 13 males. The mean age was 48.60 ± 9.45 years and their mean BMI was 34.88 ± 7.50 kg/m². **Group 2** included 15 subjects, 7 females and 8 males. The mean age was 45.07 ± 8.12 years and their mean BMI was 25.51 ± 3.23 kg/m².

The aim of the present study was to assess vitamin D levels in patients having T2DM with NAFLD and to study its relationship with insulin resistance.

In the present study, serum 25(OH) vitamin D levels were significantly lower in group I as compared to group II and this was in agreement with previous studies [11-14].

However, **Ha *et al.*** [15] in a national survey-based cross-sectional study in Korea demonstrated that vitamin D insufficiency was not associated with the presence of NAFLD in the general population, and this discrepancy because he divided participants into subgroups and then excluded diabetes and obesity.

In the present study, there were significantly higher levels of FBG, ALT and AST in group I as compared to group II. The same results were reported by similar previous studies[12,13,16,17].

On the other hand, **Elshamy *et al.*** [18] who studied the association between serum levels of 25-hydroxyvitamin D and nonalcoholic fatty liver disease in the Egyptian population and found non-significant difference between group I and group II regarding ALT and AST.

In this study, there were significantly higher levels of LDL, cholesterol, and TG while lower level of HDL in group I as compared to group II. These results are mostly due to the fact that all diabetic patients were not dispensing any lipid-lowering drugs. The same results were reported by previous studies. They found high levels of TC, TG and LDL in patients with T2DM and NAFLD[13, 16, 18, 19].

In contrast, **Barchetta *et al.*** [12] found significantly higher level of TG and significantly lower levels of HDL in patient group compared to control, while non-significant difference was found between the 2 groups regarding LDL and total cholesterol.

In the current study there was non-significant difference between male and female regarding 25(OH) vitamin D level.

This is may be due to all males and females are living in urban areas and working indoor. Also increased level of air pollution lead to decrease the amount of UVB rays available for cutaneous vitamin D synthesis [20]. Furthermore, dairy products are not fortified with vitamin D and very few are vitamin D-rich food.

Shawky *et al.* [16] and **Saedisomeolia *et al.*** [19] also, reported the same results when they studied vitamin D levels as an independent risk factor of NAFLD in a cross-sectional study of Korean healthy population and found significantly higher levels of vitamin D in males than females. These results may be due to different populations and may be due to a large number of males versus females involved in this study {7,514 subjects (5,278 men and 2,236 women)}.

Correlating 25(OH) vitamin D with FBG, FPI and HOMA-IR in group 1 showed significant negative correlation

The same results were found by **Barrea *et al.*** [20]**,** they found inverse correlations between vitamin D level and both fasting plasma insulin and HOMA IR.

These results are also in agreement with other previous studies [21- 23].

A possible connection between glycemic control and vitamin D metabolism could be due to poor chronic glycemic control directly affects vitamin D metabolism through reduction in the activity of the cytochrome P450–dependent steroid hydroxylases [23]. Also, vitamin D may affect glucose and insulin homeostasis by modulating β-cell function and immune response as well as inhibition of inflammatory cytokines and improving peripheral insulin sensitivity [24].

In contrast, **Leitão *et al.*** [25] found non-significant correlation between vitamin D level and HOMA-IR. They explained these results as their population (Portuguese) are usually exposed to more than 2500 hours of sun/year or even more in southern regions.

Correlating vitamin D and lipid profile: in this study non-significant correlation was found between 25(OH) vitamin D and Cholesterol, TG, LDL and HDL in group1. It was suggested that vitamin D has both direct and indirect effects on modifying the lipid profile through regulatory action that increases the activity of lipoprotein lipase in adiposity [26].

The same results were found by previous similar studies[19].

Also, **Amiri *et al.*** [27] who studied the effect of daily calcitriol supplementation with and without calcium on disease regression in non-alcoholic fatty liver patients following an energy-restricted diet and

showed that TG, ALT, and AST decreased significantly and HDL increased significantly.

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