

Vitamin D is it a Key in Egyptian NAFLD Pathogenesis?

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ABSTRACT

AIM: To analyze the association of serum vitamin D level assessed by 25-hydroxyvitamin D3 {25(OH)D3 } with nonalcoholic fatty liver disease (NAFLD).

METHODS: 104 urban subjects recruited from Gastroenterology Outpatients Clinic in El Sahel Teaching Hospital prospectively. The study participants divided into two groups: 57 (54.8%) cases of NAFLD (31 males, 26 females) and 47 (45%) healthy controls (21 males and 26 females). Serum 25 (OH) vitamin was measured for all participants.

RESULTS: there is significant decrease of vit D level in NAFLD group 53.53 ± 42.47 in correlation to control 97.91 ± 27.16 , $p < 0.001$. Also there is significant increased in BMI and glucose of NAFLD group than control, 33.14 ± 5.07 , 25 ± 3.69 and 111.58 ± 20.30 , 99.83 ± 10.63 , $p < 0.001$ but no statistically significant difference in TG .

CONCLUSION: Vit D deficiency is an important cause in NAFLD pathogenesis as well as MS.

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Key words: Vitamin D; Non alcoholic fatty liver disease

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a spectrum of disorders characterized by predominantly macrovesicular hepatic steatosis occurring in individuals in the absence of significant alcohol consumption. Ranging from simple steatosis to steatohepatitis (NASH), to fibrosis and cirrhosis that can progress to liver failure and hepatocellular carcinoma^[1]. Although NAFLD is strongly associated with obesity, insulin resistance, diabetes and the metabolic syndrome, (MS) its pathogenesis is incompletely understood. NAFLD is observed in 75% of type 2 diabetic patients^[2]. Recently, an increased risk of cardiovascular disease in patients with NAFLD has been also suggested^[3], based on the strong association between NAFLD and MS^[4].

Thus, NAFLD is historically considered the hepatic component of MS, insulin-resistant (IR). It is characterized by loss of the normal inhibition by insulin of adipose tissue. Lack of appropriate insulin-induced suppression of lipolysis translates into elevated

plasma fatty acids (FFA) and increased fatty acid uptake by ectopic tissues (muscles, pancreatic beta cells, liver) that results in many of clinical manifestations of the metabolic syndrome and NAFLD^[5]. Chronic excess of FFA to the liver drives hepatic gluconeogenesis in NAFLD^[6]. Vitamin D is a lipophilic molecule produced onto the skin through a UV-mediated reaction, then it is metabolized to its active 1, 25 (OH)₂ form through two consecutive hydroxylations exerted by kidney and liver, respectively^[7]. Vitamin D from the skin or from diet is metabolized in the liver to 25-hydroxyvitamin D [25(OH) D], which is the major circulating metabolite^[8]. Serum 25(OH)D correlates with overall vitamin D stores and is the most commonly used biomarker for assessing vitamin D deficiency. Deficiency is often defined by circulating 25(OH) D levels below 30 ng/mL (50 nmol/L)^[9].

25(OH) D is metabolized in the kidneys to the biologically active form 1, 25(OH)₂ D, which exerts its functions through binding to its nuclear receptor (vitamin D receptor, VDR). Within the last two decades, VDR has been shown to be present not only in primary target tissues such as bone, kidney and intestine, but also in many other tissues, including the immune and endocrine systems, muscles, brain and liver, therefore expanding the role of vitamin 1, 25(OH)₂ D, beyond the skeletal system^[10].

Numerous publications propose that low levels of 1, 25(OH)₂ D, may contribute to the development of insulin resistance, the metabolic syndrome and more recently NAFLD^[1-3]. Accordingly, there are reports to suggest increasing prevalence of 1, 25(OH)₂ D, which exerts in deficiency in parallel to the prevalence of obesity^[11]. Although the mechanisms underlying the association of 1, 25(OH)₂ D, with NAFLD are not yet fully understood, recent animal studies have shown that 1, 25(OH)₂ D, has an important role in the regulation of oxidative stress, the production of pro-inflammatory cytokines^[12,1] hepatocyte apoptosis^[2] and even liver fibrosis^[3]. As both diseases NAFLD and 1, 25(OH)₂ D, deficiency are associated with insulin resistance, type 2 diabetes and cardiovascular disease, many studies have emerged over the past few years examining the relationship of vitamin D status, reflected by 25(OH) vitamin D level, with the development of NAFLD. To this end, we aimed to systematically review and quantify the association between 25(OH)₂ D, levels and NAFLD.

PATIENTS AND METHODS

Study Population

We studied 104 urban subjects recruited from Gastroenterology Outpatients Clinic in El Sahel Teaching Hospital prospectively. The study participants divided into two groups: 57 (54.8%) cases of NAFLD (31 males, 26 females) and 47 (45%) healthy controls (21 males and 26 females). Informed consent was obtained from all participants before enrollment in the study. The study was carried out in accordance with the principles of the Declaration of Helsinki, and its appendices, and local and national laws.

Selection Criteria

To be eligible for the study, patients had to fulfill the following criteria: no history of current or past excessive alcohol drinking; negative tests for the presence of hepatitis B surface antigen and antibody to hepatitis C virus; absence of history and findings consistent with cirrhosis and other chronic liver diseases.

All subjects had a complete work-up including a clinical examination, anthropometric measurements and body mass index calculated for all subjects, laboratory tests and a liver US scan.

BMI

BMI was calculated according to the following equation: weight (Kg)/height² (m²).

Laboratory investigations

Study population underwent fasting blood sampling to assess blood glucose (FBG), total cholesterol, HDL-cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total & direct bilirubin, INR, alpha fetoprotein and creatinine by biochemical laboratory methods.

The kits for AST, ALT, total bilirubin, direct bilirubin, albumin and glucose concentrations made in USA, Synchron system and assayed using Beckman CX4 chemistry analyzer (NY, USA, supplied by the Eastern Co. For Eng. & Trade-Giza, Egypt).

25 (OH) vitamin D measurement

We measured serum 25(OH) vitamin D, the most stable circulating form of this molecule using DRG Assay quantitative determination of 25-OH Vitamin D in plasma and serum (DRG International Inc., USA).

NAFLD evaluation

Liver ultrasonography (US) scanning was performed to assess the degree of steatosis. All US were performed by the same operator using Hitachi (Voluson) ultrasound machine equipped with a convex 3.5 MHz probe. Liver steatosis was scored semi-quantitatively on a scale of 0-3: 0; absent; 1, mild; 2, moderate; 3, severe. Steatosis was graded on the basis of abnormally intense, high level echoes arising from the hepatic parenchyma, liver-kidney difference in echo amplitude, echo penetration into deep portion of the liver and clarity of liver blood vessel structure.

Data was entered into excel sheet, cleaned and edited. Statistical analysis was done using SPSS ver. 21 software. Quantitative data was presented as range, mean and standard deviation. Comparison between mean of two groups was carried out using independent t-test. Pearson correlation was done to estimate the association between quantitative data while spearman correlation was used for qualitative data. ROC curve analysis was employed to estimate the validity of using Vit D measurement to differentiate between the control and cases. In all hypothesis testing a *p* value of ≤0.05 was considered as statistically significant result. Person correlation coefficient done to correlate steatosis grades with VIT D level.

RESULTS

Blood samples from 57 cases were analyzed in comparison to Healthy subjects. Table 1 demonstrate the age, liver enzymes, Glucose, AFP, BMI, Creatinine, Lipid profile and vitamin D. There were statistically significant differences between the NAFLD and the control groups regarding all parameters except TG, INR, AST/ALT ratio and Cholesterol (*p*<0.05). These data showed that, there were significant higher levels of liver enzymes glucose, BMI, and LDL and significant lower level of Vitamin D in NAFLD group compared with the control group while table 2 showed no significant correlation was found between Vitamin D and BMI in NAFLD cases, there was a negative statistically significant correlation between Cholesterol and Vitamin D and positive statistically significant correlation between BMI and LDL and VIT D in NAFLD group. Also there was high significant correlation between Vit D and ultrasound grading of steatosis with negative person correlation coefficient (Table 3). Figure

1 represent the Receiver operating curve for Vit D showed that Area under the curve=0.749 (95% CI 0.635-0.828), $z=4.556$, $p<0.0001$, Cutoff point ≤ 40 with sensitivity =77.17% and specificity=89.36%.

DISCUSSION

Vitamin D is a secosteroid with known effects on calcium homeostasis that has recently been shown to have other significant functions regarding immune modulation, cell differentiation and proliferation, and the inflammatory response. As our understanding of the many functions of vitamin D has grown, the presence of vitamin D deficiency (VDD) association and NAFLD as well as the role of vitamin D deficiency in the pathogenesis of NAFLD and the available evidence on the clinical utility of vitamin D replacement in NAFLD populations are discussed^[13].

Numerous publications propose that low levels of vitamin D may contribute to the development of insulin resistance, the metabolic syndrome and more recently NAFLD^[1,3]. Accordingly, there are reports to suggest increasing prevalence of vitamin D deficiency in

parallel to the prevalence of obesity^[11]. Although the mechanisms underlying the association of vitamin D with NAFLD are not yet fully understood, recent animal studies have shown that vitamin D has an important role in the regulation of oxidative stress, the production of pro-inflammatory cytokines^[12,1], hepatocyte apoptosis^[2] and even liver fibrosis^[3]. As both diseases NAFLD and vitamin D deficiency are associated with insulin resistance, type 2 diabetes and cardiovascular disease, many studies have emerged over the past few years examining the relationship of vitamin D status, reflected by 25(OH) vitamin D level, with the development of NAFLD. This study was carried out prospectively in period between August 2013 and October 2014 in Gastroenterology Out-patients Clinic in El Sahel Teaching Hospital.

Table 1 Clinical & Biochemical characteristic in NAFLD & control groups.

	Control		Case		t-test	P value
	Mean	Standard Deviation	Mean	Standard Deviation		
Age	39.49	14.81	45.30	12.02	2.209	0.029
ALT	29.70	6.22	64.18	30.40	8.351	<0.001
AST	32.32	8.63	59.61	21.42	8.792	<0.001
AST/ALT	1.09	0.22	1.08	0.49	0.245	0.807
T.Bil	0.72	0.21	1.35	0.71	6.427	<0.001
D.Bil	0.14	0.05	0.33	0.20	6.721	<0.001
FBS	99.83	10.63	111.58	20.30	3.785	<0.001
Albumin	3.88	0.24	3.47	0.56	4.962	<0.001
INR	1.02	0.08	1.07	0.18	1.857	0.067
BMI	25.13	3.69	33.14	5.07	9.036	<0.001
Creatnine	0.99	0.18	0.79	0.33	3.894	<0.001
TG	153.87	33.10	150.65	37.03	0.463	0.644
CHOL	164.47	26.04	169.61	46.35	0.713	0.478
HDL	44.21	6.79	40.58	10.77	2.092	0.039
LDL	107.62	11.34	127.89	22.16	6.019	<0.001
Vit D	97.91	27.16	53.53	42.47	6.451	<0.001

All data expressed as mean±SD, *There is a significant difference between control group and patient group by using independent t-test at $p<0.05$, FBS: fasting blood sugar, AST: aspartate transaminase, ALT: alanine transaminase, T.Bil: total bilirubin, BMI: body mass index, CHOL: cholesterol, TG: triglyceride, LDL: low density lipoprotein, HDL: high density lipoprotein, Vit D: Vitamin D.

Table 2 Correlation of BMI and Vit D to clinical and biochemical characteristics in NAFLD & control group.

	Control group				Cases			
	BMI		Vit D		BMI		Vit D	
	Pearson Correlation	P value	Pearson Correlation	P value	Pearson Correlation	P value	Pearson Correlation	P value
Age	0.168	0.260	-0.235	0.111	-0.130	0.336	0.012	0.927
ALT	0.238	0.107	-0.005	0.972	0.117	0.386	-0.050	0.710
AST	0.265	0.072	0.070	0.639	-0.018	0.892	-0.018	0.896
AST/ALT	0.203	0.181	0.062	0.688	-0.022	0.870	0.159	0.239
T.Bil	0.349	0.016	0.153	0.304	0.017	0.902	-0.182	0.175
D.Bil	0.393	0.006	0.135	0.366	0.020	0.884	0.014	0.917
Glucose	0.232	0.117	0.194	0.191	-0.176	0.190	-0.083	0.540
Albumin	0.004	0.981	0.237	0.109	-0.032	0.813	-0.028	0.833
INR	0.246	0.095	-0.134	0.370	-0.051	0.707	-0.144	0.284
Creatnine	0.185	0.213	-0.157	0.292	0.089	0.509	0.013	0.926
TG	0.289	0.049	-0.041	0.782	0.066	0.626	0.057	0.674
CHOL	0.396	0.006	0.097	0.516	0.134	0.319	-0.293	0.027
HDL	0.366	0.011	0.103	0.489	0.174	0.196	0.074	0.585
LDL	0.314	0.031	-0.309	0.035	0.347	0.008	0.013	0.921
Vit D	0.045	0.763			0.229	0.086		

FBS fasting blood sugar, AST: aspartate transaminase, ALT: alanine transaminase, T.Bil: total bilirubin, CHOL: cholesterol, TG: triglyceride, LDL: low density lipoprotein, HDL: high density lipoprotein, Vit D: Vitamin D.

Table 3 Correlation between ultrasound grading of steatosis and Vit D level

	r	P
Us steatosis grading to Vit D	-0.8652	0.0001

*Correlation is significant at p value <0.05 level, vit D: Vitamin D.

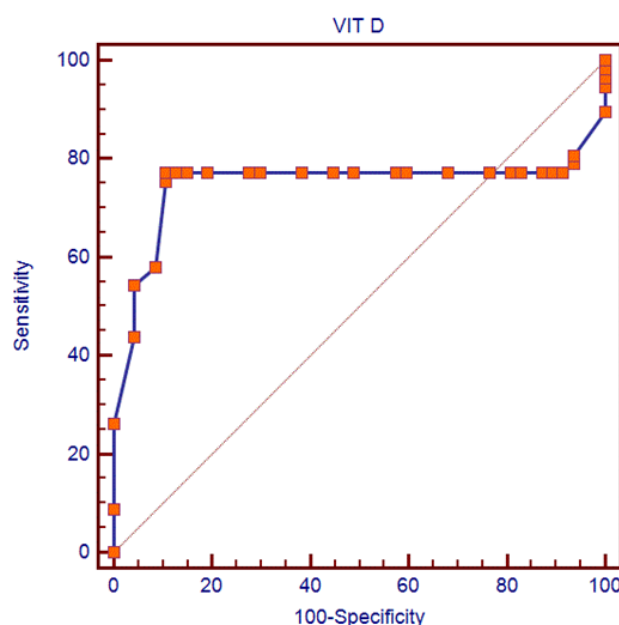


Figure 1 ROC curve of Vit D level.

In our study there was significant decrease in Vit D with mean 53.53 ± 42.47 in NAFLD group vs 97.91 ± 27.16 in control group, $p < 0.001$ and significant increase in BMI 33.14 ± 5.05 in NAFLD group vs 25.13 ± 3.69 in control, $p < 0.001$ but no significant correlation between BMI and Vit D in NAFLD group this is in agreement with Ya-ping Hao *et al.*^[14] 2014 who demonstrated that serum 25(OH)D3 levels were inversely associated with NAFLD, even in subjects with normal total body fat, suggesting a potential role of lower levels of vitamin D in the occurrence and development of NAFLD and Ilaria Barchetta *et al.*^[15] which found inverse correlation between serum 25(OH) vitamin D levels and the degree of NAFLD, independently from fatness and suggesting that vitamin D may exert a dose-dependent effect on fat accumulation into the hepatocytes. Also Kasapoglu B *et al.*^[16] who studied 613 non-obese (body mass index < 30 kg/m) with NAFLD correlated with low Vit D level.

Also M Eliades *et al.*^[17] in Meta-analysis: vitamin D and non-alcoholic fatty liver disease where Seventeen cross-sectional and case-control studies have evaluated the association between vitamin D and NAFLD and concluded that NAFLD patients have decreased serum 25(OH)D concentrations, suggesting that vitamin D may play a role in the development of NAFLD.

In this study there is significant increase in AST and ALT in NAFLD group with mean 59.61 ± 21.42 , and 64.18 ± 30.4 , $p < 0.001$ vs control 32.32 ± 8.63 and 29.7 ± 6.22 but no significant correlation between Vit D and liver enzymes which correlate with Ilaria Barchetta *et al.*^[15] study where they demonstrated a strong independent association between low 25(OH) vitamin D levels and NAFLD in a population of adults without signs of severe liver damage even when the diagnosis of fatty liver is based on routine US examination but in Kasapoglu B *et al.*^[16] study higher alanine aminotransferase levels were found to be the significant determinants for non-alcoholic fatty liver disease.

In the present study there is a negative statistically significant correlation between cholesterol and Vit D in NAFLD group $p = 0.027$ and no significant correlation with triglyceride this disagree with Kasapoglu B *et al.*^[16] study where higher triglyceride levels and higher alanine amino transferase levels were found to be the significant determinants for non-alcoholic fatty liver disease. While Rhee EJ, *et al.*^[18] detected that Age, serum calcium, and aspartate amino transferase levels showed weak but significant positive correlations with 25(OH)D3 level; total cholesterol, triglycerides, low-density lipoprotein cholesterol and fasting insulin level showed weak but significant negative correlations with 25(OH)D3 level.

In our study there was negative correlation coefficient ($r = -0.8652$) and high significant correlation ($p < 0.001$) between US grades of steatosis and 25(OH)D concentrations and this confirmed by Eliades *et al.*^[17] demonstrated that vitamin D deficiency is prevalent in NAFLD subjects, suggesting that vitamin D may play a role in the development of the disease. The anti-inflammatory and immune modulatory properties of vitamin D provide plausible mechanisms by which vitamin D may impact on disease progression and severity in NAFLD.

CONCLUSION

In our study there was significant decrease in Vit D in NAFLD group with no significant correlation to triglycerides level and BMI. Further prospective studies are needed to clarify the exact mechanism and relationships between vitamin D level, MS and NAFLD and role of Vit D therapy in managing NAFLD person's to regress its progression into NASH and liver cirrhosis. Therefore, further investigation will

be needed to determine vitamin D requirements for proper immune function.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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