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STUDY OF VITAMIN D STATUS IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

By

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a highly prevalent condition. Emerging evidence suggests that vitamin D (VD) may play a role in the pathogenesis of NAFLD.

Objective: Studying VD status in patients with NAFLD.

Patients and Methods: Seventy five patients were divided into 5 groups: Group (1) Diabetic patients with NAFLD, Group (2): Dyslipidemic patients with NAFLD, Group (3): Non-diabetic, non-dyslipidemic patients with NAFLD, Group (4): Diabetic patients without NAFLD, and Group (5): Healthy subjects (age and sex matched).

All subjects were subjected to full history taking, clinical examination and laboratory investigations including assessment of serum VD, fasting insulin, lipid profile, blood glucose, AST and ALT, and assessment of IR by determination of HOMA-IR. This were in addition to abdominal ultrasonography and using Hamaguchi score to evaluate fatty liver state.

Results: VD significantly decreased in all NAFLD patients (groups 1, 2 and 3), in addition to group 4 (diabetic patients without NAFLD), compared with group (5). This association was independent from age, sex, insulin resistance (IR), or liver functions. Also, VD significantly decreased in males of group 2 compared to females of same group. Moreover, there was significant negative correlation between VD and US score of NAFLD patients.

Conclusion: VD was deficient in patients with NAFLD and diabetic patients without NAFLD. This association was independent from age, sex, diabetes, IR or liver functions. The greater the degree of NAFLD, the greater was the degree of VD deficiency. Also, there was an inverse correlation between VD and US score.

Keywords: NAFLD, NASH, vitamin D, insulin resistance (HOMA-IR).

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a multifactorial disease and its pathogenesis is closely linked to the metabolic syndrome (MS) **(Fabbrini et al., 2010).** NAFLD has become one of the most common forms of chronic liver disease worldwide**.** NAFLD represents a continuum of hepatic injuries**,** which progress from simple fatty liver to steatohepatitis (NASH), liver cirrhosis or even hepatocellular carcinoma (HCC) **(Eliades and Spyron, 2015).** The evolu-

tion of liver inflammation in NAFLD and the progression from simple fatty liver to NASH hepatic fibrosis is more complex **(Tilg and Moschen, 2010).**

Part from the primary role of VD in bone and mineral homeostasis**,** it has potent immunomodulatory effects both on the innate and adaptive immune system **(Baeke et al., 2010).** VD has witnessed a significant scientific interest and expanded its actions to include cell differentiation and proliferation and inflammation regulation **(Unal et al., 2014).**

VD deficiency shares associations with obesity and sedentary lifestyle**.** As an understanding of the many functions of VD has grown**,** the presence of VD deficiency has become one of the most prevalent and important micronutrient deficiencies worldwide **(Eliades and Spyron, 2015).**

As an understanding in the pathogenesis of NASH continues to evolve**,** VD may play an important role in the development and progression of NAFLD**.** While clear evidence of an association between VD and liver disease exists, it remains unknown whether VD deficiency confers an enhanced risk to liver disease or whether liver disease causes VD deficiency **(Stokes et al., 2013).** However, still there are little studies about VD status among patients with NAFLD.

The present work aimed to study VD status in patients with NAFLD.

PATIENTS AND METHODS

In this study, 75 subjects were selected and classified into 5 equal groups:

- Group (1): Diabettic patients with NAFLD.
- Group (2): Dyslipidemic patients with NAFLD.
- Group (3): Non-diabetic, non-dyslipidemic patients with NAFLD.
- Group (4): Diabetic patients without NAFLD.
- Group (5): Healthy subjects (age and sex matched).

All subjects were selected from the outpatient clinic of Internal Medicine and inpatients of Internal Medicine Departments of Sayed-Galal Hospital, Al-Azhar University. Their ages ranged between 19 and 55 years old.

The study was performed during the period from May 2015 to June 2016.

All subjects were subjected to full history taking, thorough clinical examination, laboratory investigations including FPG, 2-hr-PPPG, TG, TC, HDL, LDL, ALT, fasting serum insulin level, assessment of IR by determination of HOMA-IR, serum VD level and abdominal ultrasound.

Abdominal ultrasonography was done to assess NAFLD according to **Hamaguchi et al. (2005)**.

Analysis of data was done by IBM computer using SPSS (statistical program for social science version 12): Unpaired (Independent) t-test to compare quantitative variables between groups, and one way analysis of variance (ANOVA) followed by post hoc analysis (LSD test) to compare between more than two groups regarding quantitative data with parametric distribution.

RESULTS

VD significantly decreased in all NAFLD patients groups 1, 2 and 3. In addition to, it is significantly decreased in diabetic patients without NAFLD group 4. This association was independent of age, sex, IR or liver functions. Moreover, it significantly decreased in males of group 2 compared to females of same group. VD significantly decreased in groups 1, 2, 3 and 4 compared with group 5. IR is significantly increased in group 1 and 4 compared with other groups (post hoc Tukey's test) (Table 1).

Table (1): VD & IR in the studied groups.

Groups	Group 1 (N=15)	Group 2 (N=15)	Group 3 (N=15)	Group 4 (N=15)	Group 5 (N=15)	P	LSD
Mean ± SD	15.47 ± 9.16	17.93 ± 8.22	15.13 ± 6.61	16.13 ± 7.73	43.67 ± 8.235	<0.001*	5 vs 1,2,3,4
IR	22.8 ± 7.28	5.4 ± 1.6	2.93 ± 0.78	20.13 ± 7.7	2.61 ± 0.67	< 0.001	

VD significantly decreased in males of group 2 compared to females of same group (Table 2).

Table (2): VD and sex in the studied groups.

VD (ng/dL)		Males	Females	t-value	P
Group 1	Mean ± SD	15.6 ± 8.5	15.3 ± 10.9	0.06	> 0.05
Group 2	Mean ± SD	13.1 ± 3.7	22.1 ± 8.9	-2.48	0.03*
Group 3	Mean ± SD	14.8 ± 7.9	15.7 ± 4.4	-0.25	> 0.05
Group 4	Mean ± SD	18.6 ± 8.3	13.3 ± 6.4	1.37	> 0.05
Group 5 (Controls)	Mean ± SD	42.4 ± 9.1	46.2 ± 6.4	-0.83	> 0.05

Insulin significantly increased in groups 1 and 4 in comparison to groups 2, 3 and controls. FPG and 2-hr-PPPG significantly increased in groups 1 and 4 in comparison to groups 2, 3 and 5. TG significantly increased in groups 1, 2 and 4 in comparison to controls. Also, there was a significant increase in the level of TG in group 2 in comparison to groups 1, 3 and 4. TC significantly increased in groups 1 and 2 in comparison to controls. TC increased in groups 2 when copared to

group 1, 3 and 4. HDL decreased in group 2 when copared to group 3. Also HDL significantly decreased in groups 1 and 2 in comparison to controls. LDL significantly increased in groups 2 when copared to groups 1, 3, 4 and controls (Figure 1).

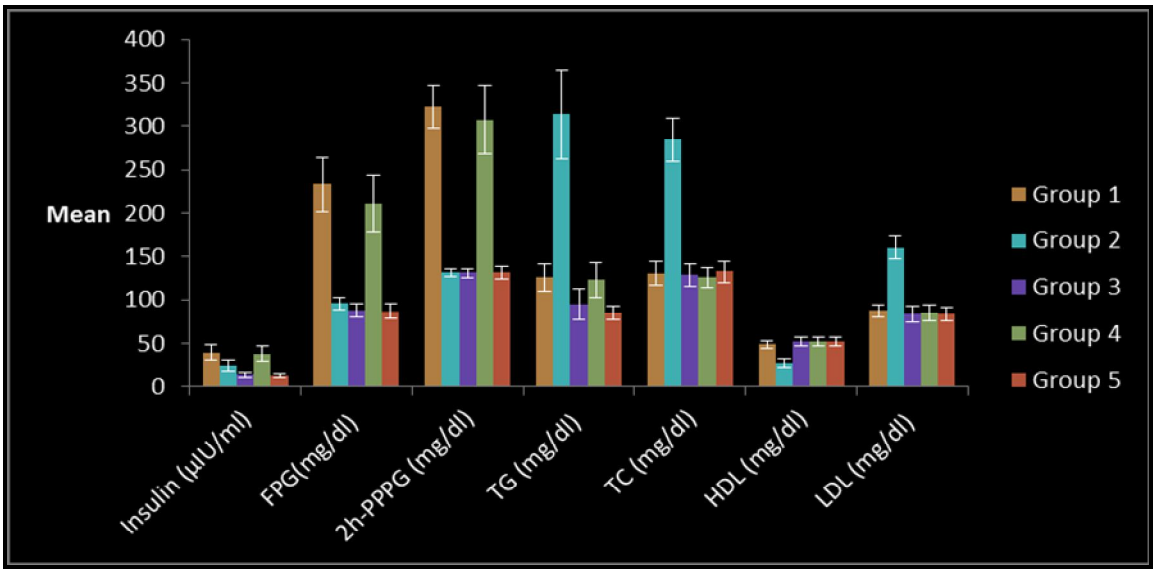

Figure (1): Bar chart comparing insulin, FPG, 2h-PPPG, TG, TC, HDL and LDL.

There was a significant negative correlations between VD and US score in NAFLD patients. The greater the degree of NAFLD, the greater was the degree of VD deficiency (**Figure 2**).

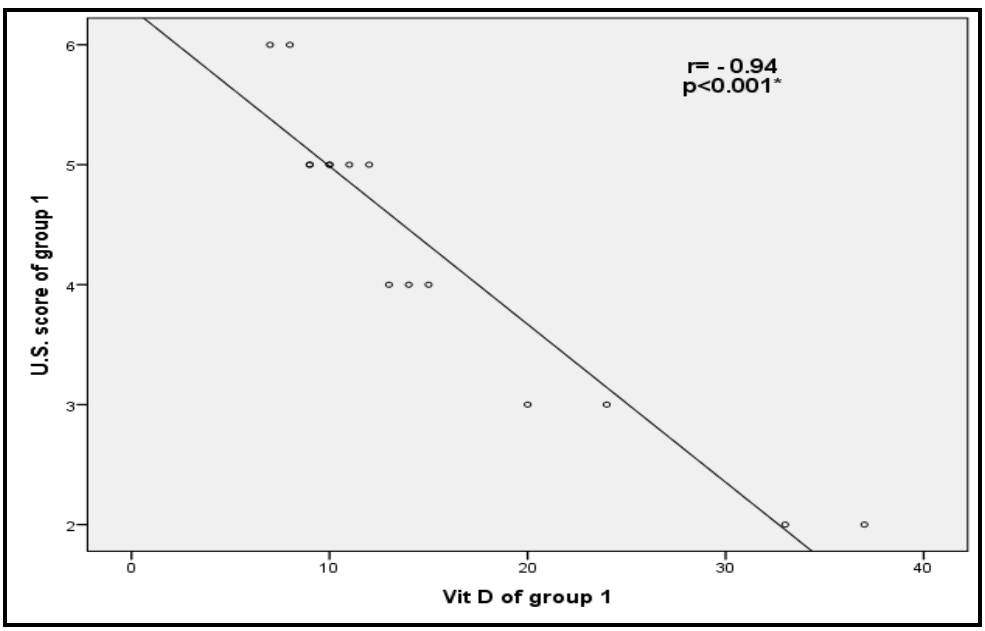

Figure (2): Scatter diagram illustrate -ve correlations of VD and US score of group 1.

DISCUSSION

VD significantly decreased in diabetic patients with NAFLD, dyslipidemic patients with NAFLD and non-diabetic, non-dyslipidemic patients with NAFLD when compared to healthy subjects**.**

More specifically, it significantly decreased in NAFLD patients compared with healthy subjects with lowest values detected in non-diabetic, non-dyslipidemic patients with NAFLD. These results were in agreement with those reported by **Barchetta et al. (2011) and Dasarathy et al. (2012),** where they noted that VD significantly decreased in patients with NAFLD when compared to healthy subjects. These results were in agreement with those obtained by **Jablonski et al. (2013)** who found that patients with NAFLD have significantly decrease of serum VD when compared to controls, suggesting that low VD status might play a role in the development and progression of NAFLD.

There was a significant -ve correlation between VD and US score of NAFLD patients in the present work. So, the greater the degree of NAFLD, the greater was the degree of VD deficiency. This result was in agreement with those documented by **Hourigan et al. (2015) and Zhai et al. (2016)** who found that negative correlation between VD and US score of NAFLD patients**.** In our study, it was found that VD significantly decreased in Diabetic patients without NAFLD. This was in agreement with that seen by **Mohammad et al. (2014)** who found that VD level significantly lower in diabetic patients than the healthy individuals.

In our study, no significant correlation was found between VD and IR in any groups. However, serum VD level was significantly lower in NAFLD patients in absence of DM and hyperlipidemia. These findings may point to possible underlying mechanisms for NAFLD other than IR. The association between low serum VD level and the presence of NAFLD in absence of IR was also found in the study of **Targher et al. (2007**). They found that low VD levels was closely associated with histologic severity of steatosis, necro-inflammation and fibrosis in NAFLD, independent of age, gender, BMI, IR score and the presence of MS. These findings have been confirmed in children with NAFLD by the study of **Manco et al. (2010).**

In the current study**,** no significant correlations were found between VD, age, gender of the patients, renal functions, blood glucose, IR and HDL in the different NAFLD groups. These findings were in agreement with findings reported by **Barchetta et al. (2011)** who revealed that the association between NAFLD and low VD levels was independent of age, FPG, AST, ALT and GGT.

CONCLUSION

VD deficiency was found in patients with NAFLD and diabetics without NAFLD. The greater the degree of NAFLD, the greater was the degree of VD deficiency. There was an inverse correlation between VD and US score.

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دراسة حالة فيتامين (د) فى مرضى التدهن الكبدى الغير كحولى

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خلفية البحث : يعتبر مرض تدهن الكبد الغير كحولى من أكثر الأمراض شيوعا وتقوم الأبحاث الحالية بإظهار دور نقص فيتامين "د" في حدوث مرض تدهن الكبد الغير كحولى.

الهدف من البحث : تقييم حالة فيتامين "د" بالدم فى مرضى تدهن الكبد الغير كحولى.

المرضي وطرق البحث : اجريت الدراسة على 75 شخصاً تم تقسيمهم إلي خمس مجموعات متساوية: مجموعة (1) مصابين بمرض السكرى و مصابين بتدهن كبدى غير كحولى ، ومجموعة (2) مصابين بخلل فى دهون الدم ومصابين بتدهن كبدى غير كحولى ، ومجموعة (3) مصابين بتدهن كبدى غير كحولى وغير مصابين بمرض السكري أو خلل بدهون الدم ، مجموعة (4) مصابين بمرض السكرى وغير مصابين بتدهن كبدى غير كحولى ، ومجموعة (5) أشخاص أصحاء للمقارنة.

وأجرى لكل الأشخاص المشاركين فى الدراسة : أخذ التاريخ الكامل و فحص إكلينيكى شامل ، وعمل تحاليل (قياس نسبة فيتامين "د" بالدم ، وقياس نسبة السكرالصائم وبعد الأكل بساعتين ونسبة الإنسولين الصائم بالدم وحساب مقاومة الإنسولين ، وتحليل إنزيمات الكبد والكرياتينين بالدم ، وقياس نسبة كثافة الدهون بالدم) ، وعمل موجات صوتية على البطن وتحديد معدل هاماجوشى للتدهن الكبدى.

النتائج :

- وجود نقص ذو دلالة إحصائية فى مستوى فيتامين "د" لدى مرضى تدهن الكبد غير الكحولى في مجموعات 1و2و3 ، ومرضى السكر بدون تدهن الكبد الغير كحولى (مجموعة 4) مقارنة بالأصحاء وهذا الإرتباط لا يعتمد على السن أو الجنس أو مقاومة الأنسولين أو وظائف الكبد .
- وجود نقص ذو دلالة إحصائية فى مستوى فيتامين "د" لدى الذكور مقارنة بالإناث في المجموعة (2).
- وجود علاقة ذات دلالة إحصائية سلبية بين فيتامين "د" ومجمع نقاط تقييم الموجات الصوتية لدرجة تدهن الكبد لدى مرضى تدهن الكبد الغير كحولى.

الإستنتاج :

- وجود نقص بفيتامين "د" لدى مرضى تدهن الكبد الغير كحولى وكذلك مرضى السكر ،وهذا الإرتباط لا يعتمد على السن أو الجنس أو مقاومة الأنسولين أو وظائف الكبد .
- كلما زادت درجة تدهن الكبد الغير كحولى ، كلما زادت درجة نقص فيتامين "د".
- وجود علاقة سلبية بين فيتامين "د" ومجمع نقاط تقييم الموجات الصوتية لدرجة تدهن الكبد لدى مرضى تدهن الكبد الغير كحولى.