Effect of Intramuscular Injection of Vitamin D on 25-Hydroxyvitamin D Levels, Glycaemic Control, and Liver Enzymes in Libyan Patients with Type 2 Diabetes Mellitus

Hafsa M. Alemam[1]   Mouna M. ElJilani[2]   Abdulla M. Bashein[3]

[1] Department of Environment, Food, and Biological Applications, Libyan Centre for Biotechnology Research, Tripoli, Libya
[2] Department of Genetic Engineering, Libyan Centre for Biotechnology Research, Tripoli, Libya
[3] Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tripoli, Tripoli, Libya

Address for correspondence Abdulla M. Bashein, PhD, Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Tripoli, Tripoli, Libya
(e-mail: abashein@gmail.com; a.bashein@uot.edu.ly).

Abstract

Background   Vitamin D is a fat-soluble hormone that plays an important role in glycaemic control. In addition, it has a positive effect on improving liver enzyme function.

Aim   This study was performed to examine the effect of intramuscular injection of vitamin D on serum 25-hydroxyvitamin D [25(OH)D] levels, glycemic control, and liver enzymes in Libyan patients suffering from type 2 diabetes mellitus (T2DM) with vitamin D deficiency.

Methods   This cross-sectional study enrolled 100 T2DM (50 males and 50 females). Their serum 25(OH)D, fasting blood glucose (FBG), and liver enzymes were measured at the baseline and 12 weeks after treatment with vitamin D (200,000 IU) injection monthly for 3 months. Data analysis involved the estimation of mean $\pm$ standard error (SE) and comparison of means between pre and post-treatment values using paired t-test. Independent t-test was used to compare the means between males and females. The level of significance was set at $p < 0.05$.

Results   Females had a lower 25(OH)D blood levels than males at baseline (7.03 ± 0.25 ng/mL versus 7.86 ± 0.26 ng/mL, respectively $p < 0.02$). 25(OH)D levels in both sexes was increased significantly from 7.45 ± 0.18 ng/mL to 26.69 ± 0.24 ng/mL after 12 weeks of vitamin D injections ($p < 0.001$), with no significant differences between male and females. FBG levels in both sexes was significantly decreased from 144.68 ± 1.84 mg/dL to 85.96 ± 0.34 mg/dL post treatment ($p < 0.001$). Alanine aminotransferase (ALT) was increased from 10.24 ± 0.17 U/L at baseline to 20.34 ± 1.15 U/L post treatment ($p < 0.001$). Similarly, aspartate aminotransferase (AST) was increased from 11.23 ± 0.21 to 20.57 ± 0.22 U/L ($p < 0.001$), and alkaline

phosphatase (ALP) was decreased from 124.95 ± 1.15 U/L to 111.17 ± 1.27 U/L ($p < 0.001$). There were no significant differences between male and female liver enzymes either pre- or post-vitamin D injections

Conclusion Treatment with vitamin D injection showed a significant increase in 25 (OH)D accompanied by decreased FBG and ALP levels and increased ALT and AST levels. Vitamin D levels should be monitored and adjusted in diabetic patients.

ملخص المقال باللغة العربية

تأثير الحقن العضلي لفيتامين (د) على مستويات 25 هيدروكسي فيتامين (د)، والتحكم في نسبة السكر في الدم و أنزيمات الكبد في المرضى الليبيين المصابين بداء السكري من النوع الثاني

المؤلفون: حفصة الإمام، متى الجيلاني، عبد الله باشين1، المركز الليبي لأبحاث التكنولوجيا الحيوية، قسم الكيمياء الحيوية والبيولوجيا الجزيئية كلية الطب، جامعة طرابلس 1- ليبيا.

المؤلف المسؤول: عبدالله باشين، جامعة طرابلس، بريد إلكتروني: abashein_95@yahoo.com

الأهداف: فيتامين (د)، هو هرمون قابل للذوبان في الدهون، يلعب دوراً مهماً في التحكم في نسبة السكر في الدم، بالإضافة إلى أن له تأثير إيجابي في تحسين وظيفة إنزيمات الكبد. أجريت هذه الدراسة لفحص تأثير الحقن العضلي لفيتامين (د) على مستوي 25-هيدروكسي فيتامين (د) في الدم، وضبط نسبة السكر في الدم وأنزيمات الكبد في المرضى الليبيين الذين يعانون من نقص فيتامين (د) وكذلك داء السكري من النوع الثاني.

المواد والطرق: شملت هذه الدراسة المقطعية مئة مريض (50 ذكور و50 إناث)، تم قياس محتوي الدم لكل من 25-هيدروكسي فيتامين (د)، وجلوكوز الدم الصائم، وإنزيمات الكبد قبل العلاج وبعد 12 أسبوعًا من العلاج بفيتامين (د) (200000 وحدة دولية) شهرياً لمدة ثلاثة أشهر.

التحليل الإحصائي، تضمن تحليل البيانات تقدير متوسط ± الانحراف المعياري، ومقارنة قيم ما قبل المعالجة وبعدها باستخدام اختبار (ت) لعينتين مرتبطتين، كما تم استخدام اختبار (ت) لعينتين مستقلتين لمقارنة النتائج بين الذكور والإناث، تم تحديد مستوى الدلالة الإحصائية عند0.05>م.

النتائج: كان لدى الإناث مستويات دم من 25-هيدروكسي فيتامين (د) أقل مقارنة بالذكور عند بداية العلاج (7.03±0.25) مقابل (7.86±0.26) نانوجرام/مليليتر على التوال (0.02>م). تم زيادة مستويات 25-هيدروكسي فيتامين (د) في كلا الجنسين بشكل معنوي من (7.45± 0.18) إلى (26.69 ± 0.24) نانوجرام/ مليليتر بعد 12 أسبوعًا من حقن فيتامين (د) (0.001> م) ، مع عدم وجود فروق دلالية بين الذكور والإناث. انخفضت مستويات جلوكوز الدم في كلا الجنسين بشكل ملحوظ من (144.68±1.84) إلى (85.96 ± 0.34) مليجرام/لتر بعد العلاج (0.001>م). حدث ارتفاع في مستوي ناقلة أمين الألانين (ALT) من (10.24± 0.17) قبل العلاج إلى (20.34± 1.15) وحدة/لتر بعد العلاج (0.001>م). وبالمثل، حدث ارتفاع في مستوي ناقلة أمين الأسبارتات (AST) من (11.23± 0.21) إلى (20.57± 0.22) وحدة/لتر بعد العلاج (0.001>م). وانخفض الفوسفاتاز القلوي (ALP) من (124.95±1.15) إلى (111.17±1.27) وحدة/لتر بعد العلاج (0.001>م). لم تكن هناك فروق ذات دلالة إحصائية بين إنزيمات الكبد للذكور والإناث سواء قبل أو بعد حقن فيتامين (د).

الاستنتاج: أظهر العلاج بحقن فيتامين (د) زيادة ملحوظة في مستويات 25-هيدروكسي فيتامين (د) مصحوبة بانخفاض مستويات الجلوكوز والفوسفاتاز القلوي، وزيادة مستويات ناقلة أمين الأسبارتات وناقلة أمين الألانين. يجب مراقبة مستويات فيتامين (د) وتعديلها عند مرضى السكري.

الكلمات المفتاحية: مستويات 25-هيدروكسي فيتامين (د)، الفوسفاتاز القلويALP ، وجلوكوز الدم الصائم FBG، ناقلة أمين الأسبارتاتAST، ناقلة أمين الألانين ALT، فيتامين (د)، ليبيا.

Introduction

Vitamin D is a fat-soluble multifunctional hormone that has a vast number of functions on bones and non-skeletal cells. Many studies demonstrated the expression of vitamin D receptor (VDR) in several cell types including pancreatic β cells.[1,2] Vita-min D response element was detected in insulin genes[3] and it enhances the transcription of insulin receptor genes.[4] Moreover, evidence showed that the glycemic status is affected by the receptors of vitamin D located in the human liver and kidney.[5]

Previous cross-sectional and longitudinal observational studies reported the association between vitamin D status

and risk of T2DM, and highlighted the role of vitamin D in modifying the risk of T2DM.[1] It is suggested that the positive effect of vitamin D on glucose adjustment occurs by the alteration of intracellular calcium levels, which leads to an increase in insulin sensitivity and insulin secretion.[5]

In addition, recent studies found an association between vitamin D deficiency and non-alcoholic fatty liver disease (NAFLD)[6–8]. NAFLD is considered as the most common cause of liver disease.[9] Sixty to seventy percent of patients with type 2 diabetes mellitus and greater than 34% of the population suffer from NAFLD.[10,11]

Considering the high prevalence of vitamin D deficiency worldwide and its association with an increased risk of T2DM and NAFLD, and the lack of studies related to vitamin D deficiency in Libyan diabetics, the current study was performed to evaluate the effect of the intramuscular (IM) vitamin D supplements on glycemic control and liver enzymes in Libyan patients with T2DM and vitamin D deficiency.

Materials and Methods

The present study is a cross-sectional study performed from September to December 2019, at public health care centers in Tripoli, Ghadames, and Al-Zawiya in the western region of Libya. In total, 100 male and female patients, who came for regular checks, with a history of taking anti-diabetic medications and suffered from vitamin D deficiency, participated in the study. Each participant was informed about the study's nature, gave consent for participation and completed a self-reported questionnaire before the study was commenced. The study was approved by the Bioethics Committee of Libyan Center for Biotechnology Research, Tripoli, Libya, and conducted in accordance with the Helsinki Declaration.

Individuals' body weight and height was measured to calculate the body mass index (BMI), height was measured without shoes and while wearing light clothing in a standing position. BMI was calculated using the formula of weight (kg)/height (m)^2. Other health-related factors including education level, smoking habit, physical activity, and place of residence were among the questions as well.

Study Design

At the beginning, baseline (pre-treatment) plasma concentrations for 25(OH)D, FBG and liver enzymes (ALT, AST, and ALP) of the participants were measured. Then, the participants under supervision of a medical doctor, received an IM dose of 200,000 IU/month of vitamin D (Cholecalciferol Pharma Developpement Chemin de Marcel, France) for 3 months, followed by the same measurements 1 month after the last dose of vitamin D injection. The inclusion criteria was as follows: (1) participants aged 25 to 62 years; (2) healthy individuals suffer only from T2DM for over 1 year; (3) unchanged anti-diabetic drugs. The exclusion criteria were as follows: (1) subjects with chronic diseases other than T2DM; (2) subjects with severe infections prior to the study; (3) patients using vitamin D or multi-mineral supple-

ments over the past 3 months prior to the study; and (4) pregnancy or lactation.

Biochemical Parameters Measurement

Five mL of venous blood were collected by venepuncture from each subject after eight hours fasting, transferred into a white tube, incubated in a water bath at 37°C for 15 minute and centrifuged for 10 minutes at 3000 rpm at room temperature, then sera were separated and used to measure FBG, 25 (OH)D levels, and liver enzymes including AST, ALT, and ALP. FBG was performed according to the manufacturer's instructions using a semi-automatic biochemistry analyzer Kenza Max BioChemistry, Biolabo Diagnostics, Kenza Biochemistry, France. Measurement of vitamin D was performed using ichroma II Boditech Med Incorporated, Gangwon-do, Korea. Ichroma vitamin D is a fluorescence immunoassay (FIA) for the quantitative determination of total 25(OH) D2/D3 levels in human serum/plasma. The activity of liver enzymes were measured using Biolabo SAS, Maizy kit, France.[12]

Statistical Analysis

All statistical analyses were performed using SPSS Version 22 statistical software package for Windows (SPSS Inc. Chicago, Illinois, USA). Descriptive analyses including number (N), percentage (%), mean ± standard error (SE), were performed to describe the general characteristics of the study population. Paired t-test was used to compare means between pre and post-treatment values. In addition, independent t-test was used to compare the means between males and females. $P < 0.05$ was considered statistically significant.

Results

Baseline Characteristics of Study Participants

The mean age of all participants was 45.6 ± 8.5 years with no significantly difference in age between males and females ($p = 0.88$). ►Table 1 illustrates that the majority of the patients (82%) were overweight, and there was no significant difference between males and females in this regard ($p = 0.67$). The majority of the patients were married (79%), non-smokers (63%), and all smokers were males. Most of the participants do not practice any physical activity of any kind (84%). In addition, most of the patients had university education (87%), and 58% of them reside in Tripoli.

Biochemical Parameters

The biochemical parameters were measured at the baseline (pre-treatment values) and 1 month after the last dose of vitamin D injection (post-treatment values) (►Table 2 and ►Table 3). At baseline all participants had severe vitamin D deficiency (25(OH)D <10 ng/mL-Table 2). Comparing 25 (OH)D baseline levels, females showed significant lower levels than males ($p < 0.02$; ►Table 3). 25(OH)D levels were increased significantly in both sexes 12 weeks after treatment ($p < 0.001$; ►Table 2), with no significant differences between males and females ($p < 0.66$; ►Table 3). Post-treatment values for FBG were decreased significantly in

Table 1 Baseline characteristics of study participants

Variable	Participants
N	100
Gender (n [%])	
Male	50 (50%)
Female	50 (50%)
Age (y)	45.6 ± 8.5
BMI (kg/m^2)	26.1 ± 1.9
Marital status	
Single	21
Married	79
Education level	
Illiterate	13
High school	0
University and above	87
Smoking status	
Yes	37
No	63
Physical activity	
Yes	2
No	84
Sometimes	14
City of residence	
Tripoli	58
Ghadames	40
Al-Zawiya	2

both sexes ($p < 0.001$; ►**Table 2**) with no significant differences between male and female either before or after vitamin D treatments. The post-treatment values for the liver enzymes AST and ALT were increased significantly from their pre-treatment values, while ALP values decreased significantly in both sexes after 12 weeks of treatments ($p < 0.001$; ►**Table 2**). However, there was no significant difference in liver enzymes measured between males and females neither pre nor post-vitamin D injection (►**Table 3**).

Discussion

Vitamin D deficiency is prevalent worldwide and its supplementation is simple, safe, and inexpensive.[13,14] The recent renewed interest in vitamin D results from a worsening trend of worldwide deficiency as well as novel insights regarding its effects on glucose metabolism.[1,15–19] Vitamin D deficiency was defined as a serum circulating 25(OH)D levels of <20 ng/mL based on the Endocrine Society Clinical Practice Guidelines.[20] Normal FBG was defined as blood glucose of < 110 mg/dL,[21] and elevated liver enzymes were defined as one or more measurement of AST (>34 U/L), ALT (>41 U/L),[22] and ALP (>115 U/L) in both genders.[23]

The diabetic patients included in this study had a significant vitamin D deficiency, (25(OH)D levels lower than 20 ng/mL), with female still have a significantly lower levels than males. These results are in agreement with many international reported results regarding vitamin D deficiency in T2DM.[19,24–29] Vitamin D deficiency was suggested to be associated with a decreased insulin release and insulin resistance.[28,30–32] The relationship between vitamin D deficiency and insulin resistance could develop through inflammation, as vitamin D deficiency was associated with increased inflammatory markers.[33] In addition, genetic polymorphisms of vitamin D-related genes may predispose to impaired glycemic control and T2DM.[34] However, these speculations about the mechanism responsible for the development of T2DM in vitamin D deficiency should be proven by randomized clinical trials on a large number of patients.

The administration of vitamin D to our patients resulted in a statistically significant increase in 25(OH)D levels after 12 weeks of treatment. This finding nicely agreed with previously reported results,[24–26] suggesting that the treatment protocol is successfully effective. The increase in the levels of 25(OH)D was accompanied by a significant decrease in FBG concentration, returning the FBG to the normal accepted range. Similar results were reported in a meta-analysis of Iranian diabetics.[35] However, high doses of vitamin D failed to produce any improvements in the glycemic indices in prediabetes individuals with impaired fasting glucose.[36] The decrease in FBG concentration produced by vitamin D administration might have resulted from the stimulation of insulin secretion from the pancreatic β-cell, or a decrease in insulin resistance.[37]

Table 2 Comparison between the pre- and post-treatments values of 25(OH)D, FBG, and liver enzymes in all patients ($n = 100$)

Variable	Range		Mean ± SE		p-Value
	Pre-treatment	Post-treatment	Pre-treatment	Post-treatment	
25(OH)D (ng/mL)	3.76–10	19.02–30.81	7.45 ± 1.82	26.69 ± 2.40	**<0.001**
FBG (mg/dL)	122–197	80.01–94.81	144.68 ± 18.38	85.96 ± 3.44	**<0.001**
AST (U/L)	7.99–19.78	13.84–26.08	11.23 ± 2.08	20.57 ± 2.18	**<0.001**
ALT (U/L)	5.14–17.14	13.99–33.89	10.24 ± 1.66	20.34 ± 3.66	**<0.001**
ALP (U/L)	95.99–180.21	58.07–162.01	124.95 ± 11.43	111.17 ± 12.17	**<0.001**

Table 3 Comparison between the pre- and post-treatments values of 25(OH)D, and liver enzymes between males and females ($n = 50$ patients each)

Variable	Males Mean ± SE	Females Mean ± SE	Independent t-test	p-Value
Pre-treatment 25(OH)D	7.86 ± 0.26	7.03 ± 0.25	1.97	**0.02**
Post-treatment 25(OH)D	26.79 ± 0.26	26.58 ± 0.36	1.98	0.66
Pre-treatment ALT	10.06 ± 0.22	10.43 ± 0.26	−1.10	0.27
Post-treatment ALT	19.69 ± 0.39	20.99 ± 0.61	1.79	0.08
Pre-treatment AST	10.94 ± 0.25	11.53 ± 0.29	−1.42	0.16
Post-treatment AST	20.50 ± 0.31	20.64 ± 0.32	10.32	0.75
Pre-treatment ALP	126.29 ± 1.67	123.63 ± 1.56	1.16	0.25
Post-treatment ALP	113.55 ± 1.61	108.79 ± 1.93	1.9	0.06

Liver function tests are used to determine the presence of hepatic damage or impaired function. This is done by measuring ALT, AST, and ALP levels. We found that both ALT and AST were increased by the administration of vitamin D. These increases did not exceed the normal limits allowed for both enzymes (7–55 U/L for ALT and 8–48 U/L for AST). This finding is not in agreement with other studies that vitamin D supplementation in subjects with T2DM was associated with statistically significant decreases in ALT.[8,24,38] However, another study that investigated the effect of high-dose vitamin D supplementation on serum markers of liver function among normal individuals showed that there was no significant difference in ALT and AST activity between pre- and post-treatment with vitamin D.[39]

The pre-treatment values of ALP levels in our patients were at the upper allowed limit (40–129 U/L). These values were decreased after treatment with vitamin D. Several previous studies showed that patients with vitamin D deficiency have high ALP levels.[40,41] Our result was in accordance with the findings of previous studies that showed that ALP decreased post-treatment with vitamin D.[38,42] It has been suggested that serum alkaline phosphatase might be used as a tool to screen for hypovitaminosis D,[43] but others documented that it was not a useful screening tool.[44,45] In general, treatment with vitamin D did not affect liver function considerably, which confirm the safety of the doses used of vitamin D.

Strengths and Limitations

This study had several strengths: no change in the patients' antidiabetic medications and diet during vitamin D treatment were made, as doing so could have affected the results. Moreover, all participants were vitamin D-deficient. However, this study had some limitations: first, it included relatively few participants; second, it included only the western region of Libya; and third, no placebo group was included.

Conclusion

This study suggests that with the doses used, vitamin D supplementation may improve glycemic control without affecting liver functions in patients with T2DM and vitamin D deficiency. Future longer-term, high-dose vitamin D intervention trials using a larger number of T2DM patients with vitamin D deficiency are needed to validate these results.

Conflicts of Interest
None declared.

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