Association of Serum Levels of 25 Hydroxyvitamin D and Type 2 Diabetes Mellitus: Age and Gender Dependent Study

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ABSTRACT:

BACKGROUND:

Vitamin D deficiency has been found to have an inverse relationship with the occurrence of diabetes mellitus (DM). The aims of this study were to investigate the serum levels of vitamin D in type 2 DM and to correlates the obtained values with their age and gender.

SUBJECTS AND METHODS:

This case-control study was carried out at Al-Ramadi General Teaching Hospital, and the National Diabetes Center for Treatment and Research at Al- Mustansiriya University, Iraq, during the period from December 2014 to November 2015. It involved, 80 patients with type 2 DM, and 60 healthy subjects. Investigations included serum measurement of 25 Hydroxyvitamin D (25OHD), fasting glucose, Insulin, glycated hemoglobin (HbA1c) in all patients and controls using ELISA technique.

RESULT:

The median concentration of serum 25 OHD of patients with type 2 DM (15.70 ng/ ml) were significantly lower than that of healthy controls (20.27 ng/ ml, P= 0.001). The rate of vitamin D deficiency (VDD) was significantly higher in type 2(82.5%) diabetic patients than in healthy controls (48.3%, P=0.001). Type 2 diabetic patients with age of 60 years and above and female had the highest VDD compared to others, type 2 diabetic patients with age of ≥ 60 years increased the risk of having VDD by 9.8 times compared to those with age group <18 years, but still insignificant (P =0.07).

CONCLUSION:

The results revealed significant deficiency of serum vitamin D in diabetic patients type 2. Supplementation of vitamin D may improve the control of this syndrome or even delay its incidence and complication.

KEYWORDS: age, type 2 diabetes mellitus (t 2 d m), gender, vitamin d level.

INTRODUCTION:

Diabetes mellitus (DM) is a major challenge for global public health, and it comprises a heterogeneous etiology of diseases characterized by elevated blood glucose[(1)]. Type 2 diabetes is a complex disorder characterized by imbalance between insulin resistance and insulin secretion that induce liver glucose output by preventing glycogen formation and stimulating glycogenolysis and gluconeogenesis[(2)]. Vitamin D is a group of sterols that have a hormone-like function, obtained by endogenous synthesis from 7-dehydrocholesterol in the skin by direct sunlight and/ or from food. Two main forms of vitamin D are identified; vitamin D_2 or "ergocalciferol", and vitamin D_3 or "cholecalciferol" which is produced during solar ultraviolet B radiation[3].Vitamin D deficiency and diabetes have one major trait in common: both are pandemic[4]. Numerous studies have indicated a relationship between vitamin D status and the danger of diabetes or glucose intolerance. Vitamin D has been proposed to play an important role and to be a risk factor in the development of insulin resistance and the pathogenesis of type 2 DM by affecting either insulin sensitivity or β cells function or both[(5-6)]. The connection between vitamin D and diabetes was reinforced by the discovery of vitamin D receptor (VDR) and vitamin D binding protein (DBP) in pancreatic tissue (β – cells) and also in different cell types of the immune system.

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Studies indicate that vitamin D is intimately related to diabetes and vitamin D supplementation may delay the progression of diabetes at an early stage[7]. Consequently, vitamin D has been proposed as a possible therapeutic agent in prevention and treatment of type 2 DM[8].

Vitamin D homeostasis may play a critical role in glucose metabolism. Little is known about VDD and its association with diabetes in countries of the Arab region where the population is suffering a rapid increase in the prevalence of diabetes[9].

The aims of this study was to investigate serum levels of vitamin D in a sample of Iraqi patients with type 2 DM and to correlates the obtained values with their age and gender.

SUBJECTS AND METHODS:

This case- control study was carried out at Al-Ramadi General Teaching Hospital and the National Diabetes Center for Treatment and Research at Al- Mustansiriya University during the period from December 2014 to November 2015. It involved 80 have had type 2 D M (female= 39, male= 41) and 60 were apparently healthy subjects and served as control group (female= 33, male= 27). Patients and healthy subjects were further sub grouped according to their age and gender. They were classified into four groups according to their ages; < 18 years, 18-39 years, 40-59 years, and ≥ 60 years.

Formal consent was taken from each subject. We received ethical approval from the Scientific Committee of the Biology Department, College of Science, University of Anbar, Iraq.

Five milliliter of peripheral veins blood sample was aspirated from each patient and control, allow clotting for 30 minute and then centrifuged at 2500 rpm for 10 minute. The obtained serum samples were stored at – 20 ^{0}C till the time of measurement of total 25OHD by using the kit provided from (Demeditec Company, Germany). Cata. No. DE1971. Vitamin D deficiency was defined as serum 25OHD concentration of less than 20 ng/ml, insufficiency as >20 ng/ml to <30 ng/ml and sufficiency was defined as 25OHD > 30 ng/ml[10]. Serum insulin concentration was measured according to the assay procedure of www.demeditec.com, the Demeditec insulin ELISA Kit was a solid phase Enzyme- Linked Immunosorbent Assay (ELISA) based on the sandwich principle. Glycated hemoglobin was measured by Clover $A1_C$® system, using kit catalog no. NFHS01A provided from (Infopia, Korea). The CLOVER $A1_C$ system is a fully automated boronate affinity assay for the determination of the percentage of Hemoglobin $A1_C$ (HbA1c %) in whole blood. Serum glucose was estimated by enzymatic colorimetric (GOD-PAP) method, using kit catalog No.1001191 LOT.634 supplied by Spinreact, Spain. HOMA-IR was determined using the reported equation[11].

The Statistical Package for Social Science SPSS version 21 (SPSS Inc., Chicago, IL., USA), and Minitab analysis programs were used for all statistical studies. ANOVA and Student's t-tests were used to test for statistical significance. Linear regression was utilized to test for correlation between different studied parameters, and the significance of the r-value was assessed by related t-test. P-values of less than 0.05 were considered significant.

RESULTS:

Table 1 shows the median and range of serum levels of 25OHD in the diabetic patients and controls. type 2 DM (15.7 ng/ml) were significantly lower than healthy controls (20.27 ng/ml, P=0.001). Table 2 shows vitamin D deficiency in the two studied groups. Vitamin D deficiency (VDD) was defined by the percentage of the number of patients or controls who have had serum level of 25OHD of less than 20 ng/m from the each whole group. The value of VDD was significantly higher in type 2(82.5%) diabetic patients than in healthy controls (48.3%, P=0.001).

Tables 3 and 4 illustrate the effect of age and gender on the rate of VDD on diabetic patients and controls. There was no significant difference in the VDD among healthy individuals with respect to age and gender. However, healthy subjects aged sixty years and more were associated with increased in risk of having VDD by fifteen times compared to those with age equal to or less than eighteen years (table 3). The age group 40-59 years reduces the risk of having VDD by 15 times compared to other groups of age but the difference was not statistically significant (p=0.18). Table 4 shows that type 2 diabetic patients with age of 60 years and above and female had the highest VDD compared to others. Type 2 diabetic patients with age of ≥ 60 years increased the risk of having VDD by 9.8 times compared to others, but still insignificant (P =0.07). Table 5 shows that diabetics with type 2 who were deficient for VD had significantly higher mean rank value of serum insulin levels (42.9 µIU/ml) and HOMA-IR value (43.1%) than type 2 diabetic patients who were sufficient for VD level (29.1 µIU/ml, 28.1 %, P=0.044, P=0.028, respectively).

Table 1: The Mean (±SD) or median value of serum Vitamin D and glucose metabolism in the studied groups.

Parameter	Healthy controls N= 60	Type 2 DM N= 80	P
Serum Vitamin D(ng/ml)			<0.001
Median	20.27	15.70	
Mean rank	126.2	76.7	
Hba1c%			<0.001
Mean	5.4±0.45	10.4±2.18	
Fasting plasma Glucose (mg/dl)			<0.001
Mean	89±10.2	239.3±82.9	
Serum insulin (uIU/ml)			0.036
Median	8.39	10.15	
Mean rank	77.4	94.8	
Insulin resistance % (HOMA)			<0.001
Median	1.1	1.9	
Mean rank	55.9	102.7	

Table 2: Vitamin D deficiency value in the studied groups.

	Deficient Vitamin D (<20)ng/ml										
	Insufficient / normal		Deficient		Total						
	N	%	N	%	N	%	P	OR	95% CI OR	Chi	P
Study group							<0.001				
Healthy controls	31	51.7	29	48.3	60	100					
Type 2 DM	14	17.5	66	82.5	80	100		5.04	(2.34 - 10.86)	17.27	<0.001

Table 3: Effect of age and gender on vitamin D value among Healthy controls.

	Deficient Vitamin D (<20)ng/ml										
	Insufficient / normal		Deficient		Total				Inverse OR		P (Fisher's exact)
	N	%	N	%	N	%	P	OR		95% CI OR	
Age group (years)							0.27[NS]				
<18	5	83.3	1	16.7	6	100.0		Reference			
18-39	14	46.7	16	53.3	30	100.0		5.71	**	(0.59 - 54.98)	[NS]
40-59	11	55.0	9	45.0	20	100.0		4.09	**	(0.4 - 41.67)	[NS]
60+	1	25.0	3	75.0	4	100.0		15.00	**	(0.66 - 339.7)	[NS]
Gender							0.59[NS]				
Female	16	48.5	17	51.5	33	100.0		Reference			
Male	15	55.6	12	44.4	27	100.0		0.75	1.3	(0.27 - 2.09)	[NS]

Table 4: Effect of age and gender on vitamin D value among Type 2 DM.

	Deficient Vitamin D (<20)ng/ml										
	Insufficient / normal		Deficient		Total				Inverse OR	95% CI OR	P (Fisher's exact)
	N	%	N	%	N	%	P	OR			
Age group (years)							0.07[NS]				
<18	0	0.0	0	0.0	0	0.0		Reference			
18-39	4	40.0	6	60.0	10	100.0		1.44	**	(0.07 - 29.04)	[NS]
40-59	8	18.2	36	81.8	44	100.0		4.29	**	(0.24 - 75.43)	[NS]
60+	2	7.7	24	92.3	26	100.0		9.80	**	(0.48-200.81)	[NS]
Gender							0.38[NS]				
Female	5	12.8	34	87.2	39	100.0		Reference			
Male	9	22.0	32	78.0	41	100.0		0.52	1.9	(0.16 - 1.73)	[NS]

Table 5: The median and mean rank of serum insulin and HOMA-IR according to vitamin D deficiency subgroups among type 2 DM patients.

	Deficient Vitamin D (<20)ng/ml		
	Insufficient/normal N= 14	Deficient N= 66	P
Serum insulin (uIU/ml)			0.044
Median	8.53	12.08	
Mean rank	29.1	42.9	
r=-0.038 P=0.74[NS]			
Insulin resistance (HOMA-IR)			0.028
Median	1.4	2.3	
Mean rank	28.1	43.1	
r=-0.126 P=0.26[NS]			

DISCUSSION:

A high prevalence of vitamin D deficiency in type 2 of diabetic patients was seen in the present study. This study found that 82.5 % of type 2 diabetic individuals suffered from deficiency of this vitamin. These results were similar to Lee et al[12] (2012) who found that 89% of their diabetic patients suffered from deficiency of vitamin D.

Gagnon et al [13](2011) and Taheri et al[14] (2012) found that the mean serum concentration of VD in diabetic patients was significantly lower than in non-diabetic individuals. These findings are in harmony with that obtained by the present study. The high incidence of vitamin D deficiency in DM patients may be associated with altered dietary habits or inadequate exposure to the sunshine. Moreover, VDD may be related to the occurrence of malabsorptive state among the patients with DM. It has been described that VD can prevent the death of islet cells[15].This may reflect the direct relation among dysfunction of beta cells, hypovitaminosis D, and resistance towards insulin. It has been mentioned that VD is effective for making positive improvements in the production of insulin[16]. Also,Nikooyeh et al[17] (2011), Afsaneh et al [18](2013) and Nasri et al[19] (2014) concluded that supplementation of vitamin D is associated with a lower risk of type 2 DM.

The results of the present study also showed that HOMA-IR value was significantly increased in VD deficient subgroup of type 2 diabetic patients (table5), and this finding may indicates the important role of this vitamin in maintaining normo glycemic state by influencing insulin secretion and sensitivity. In deficiency state of vitamin D, there was a decrease in insulin sensitivity and increased IR. Chronic VDD may be a predisposing reason for type 2 DM[20].

Vitamin D may have an effect on insulin sensitivity either directly, by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport, or indirectly via its role in regulating extracellular calcium and ensuring normal calcium influx through cell membranes and adequate intracellular cytosolic calcium pool.

Intracellular cytosolic calcium is essential for optimal insulin-mediated functions in insulin-responsive tissues such as skeletal muscle and adipose tissue. Changes in intracellular cytosolic calcium in primary insulin target tissues may contribute to peripheral insulin resistance[21].

Numerous cross-sectional studies have reported a significant inverse association between serum vitamin D and the presence of type 2 diabetes[22-23]. Most case-control studies have also found that patients with type 2 diabetes or impaired glucose tolerance are significantly more likely to have a lower serum vitamin D concentration than to those without diabetes[24-25], in addition to literature examining the association of vitamin D with type 2 diabetes, a number of studies have also investigated the role of vitamin D in the primary pathophysiological disorders underlying type 2diabetes, specifically insulin resistance and β-cell dysfunction. Most studies reported a significant inverse association of serum vitamin D with insulin resistance[18-26]. Regarding β-cell function, several studies reported the positive relationship between vitamin D and β-cell function[27-28-29].

High prevalence of VDD has been seen in the present study in diabetic patients and healthy controls at different age subgroups (table 3,4) that may be explained by shortage sunlight exposure, decreased vitamin D intake, the deep color of skin, deceased awareness about fortification food with vitamin D, and indoor residence due to increased temperature level in Iraq.

The difference in VDD in female than in male of type 2 DM could be explained by the difference in sun exposure between both gender because males spend a lot of time outdoors and the fact that females used sunscreen and wears clothes that cover most of their body so a higher sun index score was shown in males. In addition, an earlier growth spurt during puberty in females and the need for more vitamin D requirement for bones might be a further reason for the higher prevalence of vitamin D deficiency among females than males[30].

CONCLUSION:

The results of the present study revealed significant deficiency of serum vitamin D in type 2 diabetic patients. Supplementation of vitamin D may improves the control of this syndrome or even delay its incidence and complication.

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