Study of Serum 25-hydroxyvitamin D Status and Glycemic Control in Diabetic Patients

Abeer A. AL-Refai [1], Ahmed R. Tawfeeq [2]

Medical Biochemistry Department [1], Internal Medicine Department[2], Faculty of Medicine, al-Menoufyia University, Egypt.
drabeer512@hotmail.com

Abstract: Objectives: Low Vitamin D levels have been suspected to be a risk factor for glucose intolerance, and several studies suggest an active role for vitamin D in functional regulation of the pancreatic beta cells. This study was conducted to evaluate the vitamin D status in type 1 and type 2 diabetic patients compared to control and to study its relation to glycemic control and other DM- related metabolic parameters. **Subjects& methods:** A total of 50 subjects, with established diabetes mellitus T1DM (n=20), T2DM (n=30), and 20 age matched healthy subjects as a control were recruited for this study. Demographic data were collected, serum 25-hydroxyvitamin D (25-OHD) levels using, **Electrochemiluminescence immunoassay "ECLIA"** were measured. Also, calcium, phosphorous, alkaline phoasphatase, lipid profile and renal function were determined in diabetic patients and control group. **Results:** both cases and controls had vitamin D deficiency or insufficiency. Median (IR) 25- hydroxyvitamin D [25(OH) D] was significantly low, in diabetic patients [9.05 (5-13) ng/mL] against in controls [14.95 (12.23-22) ng/mL] (p=0.001). 14% of diabetic subjects were vitamin D insufficient compared to 35% in the control subject, while 86% of diabetes group were deficient {60.5% of them had severe vitamin deficiency (< 10 ng/ml)}, compared to 65% of control group (*p*<0.05). There was a significant negative correlation between serum 25(OH) D and both blood glucose and alkaline phosphatase in diabetic patients, a significant negative correlation between serum 25(OH) D and glycosylated hemoglobin was determined among diabetic patients with severe vitamin D deficiency (< 10 ng/ml) and patients with increased BMI. A significant positive correlation among serum vitamin D levels and both serum calcium and phosphorus was determined. **Conclusion:** These results indicate that vitamin D deficiency is common in diabetic patients and low 25 (OH) D level is associated with worse glycemic control. We recommend further study in large sample size of diabetic patients to assess vitamin D status and effect of vitamin D replacement on glycemic control.
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1. Introduction

About one billion persons worldwide have been reported to have vitamin D deficiency or insufficiency as deduced from several studies [1, 2]. The prevalence of Vitamin D deficiency in the general population is considerable and varies by ethnic background, sunlight exposure, and the presence of risk factors such as age, obesity, diabetes mellitus and other co morbidities [1]. Middle Eastern populations showed a high rate of hypovitaminosis D due to limited sun exposure based on cultural practices [3, 4]. In addition to well-known role of vitamin D in calcium/phosphorus homeostasis and bone physiology [5], furthermore, vitamin D deficiency plays an important role in many other diseases such as diabetes [6], hypertension [7], cardiovascular disease [8], immune disorders, osteoporosis and cancers [9]. In particular vitamin D potential effects on inflammatory and autoimmune conditions as well as on insulin secretion and possibly also on insulin resistance had increased the interest in its potential role in prevention and control

of the diabetic condition, both type-1[10, 11] and type-2 diabetes[12]. Also many reports proved that vitamin D deficiency causes reduced insulin secretion in rats and humans, and its replenishment improves β-cell function and glucose tolerance [13, 14]. There are increasing evidences about the relationship between serum level 25 hydroxy -vitamin D [25(OH) D] and the control of diabetes [12,13]. The majority of these studies on vitamin D status in diabetes mellitus are from developed countries. Considering the above facts, the present study was conducted with an objective to explore vitamin D status among population with diabetes mellitus, compare them with age and gender matched controls and to correlate vitamin D status, with glycaemic profiles and other DM- related parameters.

2.Materials and Methods:-
Study population:

This study was approved by the Ethical Committee of Faculty of Applied Medical Sciences of Umm Al-Qura University, according to the latest

revision of Declaration of Helsinki and informed consent was obtained from all participants. This study compromised 50 diabetic patients recruited at an outpatient diabetes clinic of King Abd-AL Aziz hospital. 20 patients were insulin-dependent (type 1 diabetes) and 30 were non-insulin dependent (type 2 diabetes). The duration of diabetes for patients with T1DM ranged from 1 to 31 years with a mean range of 12.35 ±9.35 years. 65% were females, while 35% were males with a mean age of 45.6± 1.6 years. The duration of diabetes for patients with T2DM ranged from 4 to 25 years with a mean range of 10.7 ±5.7 years. 60% were females, while 40% were males with a mean age of 52.36± 1.35 years. The presence of diabetes mellitus (DM) was diagnosed on the basis of revised American Diabetes Association criteria [15] (symptoms plus RPG ≥200 mg/dL or FPG ≥126 mg/dL or 2-h P.P G ≥200 mg/dL during OGTT). Twenty age and sex- matched healthy subjects were selected as the control group. History taking and complete clinical examination were done and recorded. Patients with major medical illness including congestive heart failure, malignancy, stage 5 kidney diseases, and chronic liver disease were excluded, also pregnant female were excluded from our study.

Sampling:

After an overnight fasting, 10 ml of peripheral blood was taken under complete aseptic precautions and divided into 3 portions, 3ml in fluoride tube, centrifuged and plasma separated for determination of fasting blood glucose, 3 ml in a clot activator tubes for glycosylated hemoglobin (HbA1c) and 4 ml in a plain test tubes, allowed to clot and centrifuged (at 1500 xg for 15 minutes), serum was then collected into separate plastic tubes for analysis of calcium, inorganic phosphorus, alkaline phosphatase, lipid profile and renal function. Serum for estimation of 25(OH) D was stored at -20ºC until subsequent assay.

Methods:

Both cases and controls were subjected to

a) Detailed physical examination, weight and height were recorded. Body mass index (BMI) was calculated using the standard formula (weight in kilograms /height in meter-square: kg/m^2) [16]. b) Circulating levels of fasting plasma glucose were analysed using hexokinase-glucose-6-phosphate dehygrogenase method[17], urea was assayed by urease/glutamate dehydrogenase coupled enzymatic technique[18], and creatinine determination employes a modification of kinetic Jaffe reaction [19]. Total alkaline phosphatase (ALP) activity was measured using a bichromatic rate techniques [20]. Triglycerides (TG) [21], total cholesterol (TC)[22] and high-density lipoprotein cholesterol (HDL-C)[23] were assayed by

colorimetric method, all kits were supplied by Dade Behring clinical chemistry system for dimesion autoanalyser (Siemens Dimension RXL, Dade Behring, Newark, DE). **Low density lipoprotein cholesterol (**LDL-C) value was calculated according to "Friedewald equation": LDL-C = Total cholesterol-(HDLc+TG/5) **[24]**. Circulating levels of glycosylated hemoglobin (HbA1c %), as a clinical indicator of blood glucose control, was measured in hemolyzed whole blood with using pressure cation exchange principle. Normal values of HbA1c ranged from 4.0 to 6.0% [25]. c) Serum calcium and phosphorus were analyzed using automated (cobas c111, applying Schwarzenbach with o-cresolphthalein complexone method [26] and UV Molybdate (End point assay) [27]. The color intensity of the complex formed is directly proportional to the concentrations and is measured photometrically with sample blanking). d) Serum total vitamin D (25-Hydroxyvitamin D) was measureeasured using **commercially available Electrochemiluminescence immunoassay "ECLIA"** kit[28] supplied by **(**Roch diagnostic GmbH, sandhofer strasse). **Electrochemiluminescence immunoassay "ECLIA"** intended for use on cobas e immunoassay analyzers. According to the manufacture instructions, (15 uL) of serum were incubated with pretreatment reagents, releasing bound vitamin D (25-0H) from the vitamin D binding protein. By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the vitamin D (25-OH) and the ruthenylated vitamin D binding protein is formed. After addition of streptavidin-coated microparticles and vitamin D (25-OH) labeled with biotin, unbound ruthenium labeled vitamin D binding proteins become occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D (25-0H) is formed and becomes bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier, and the light produced is indirectly proportional with concentration in patients sample. Results were determined via a calibration curve. The criteria used to define vitamin D status were: Serum 25(OH)D levels (ng/ml) were defined as: severe deficiency: <10; deficiency: 10- to <20 ng/ml; insufficiency: 20–29 and sufficiency: ≥30 [29].

Statistical analysis:-

Statistical analysis was done using SPSS software, version 16, Echosoft Corporation, USA.

Categorical data are presented as percentages and continuous variables as means± standard deviation (normally distributed variables) or as medians with interquartile ranges (skewed variables). The Kolmogorov-Smirnov test was done to determine the distribution of data. Comparisons between groups were calculated by Chi-Square test with P for categorical variables and by t-test for continuous variables or by Mann-Whitney-U test for skewed data. The correlation between the variables were analysed using pearson 's correlation (for normally distributed data), Sperrrman's rank correlation (not-normally distributed data). *P* values <0.05 were considered significant, whereas *p* values <0.01 were considered highly significant.

3. Results:

Fifty diabetic patients were studied with a mean ±SD age of 49.76±15.06 years. There were 19 males (38%) and 31 females (62%). 40% (n=20) of diabetics patients (7 male and 13 females) had T1DM and 60% (n=30) (12 males and 18 females) had T2DM. Twenty age and sex matched apparently healthy subjects as a control involved wit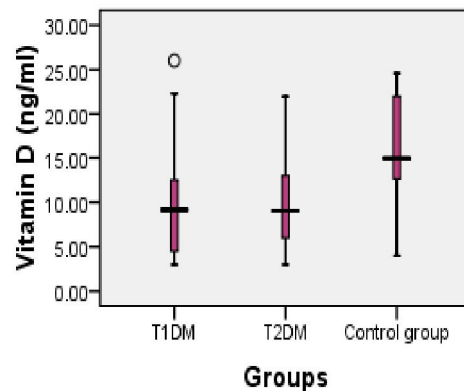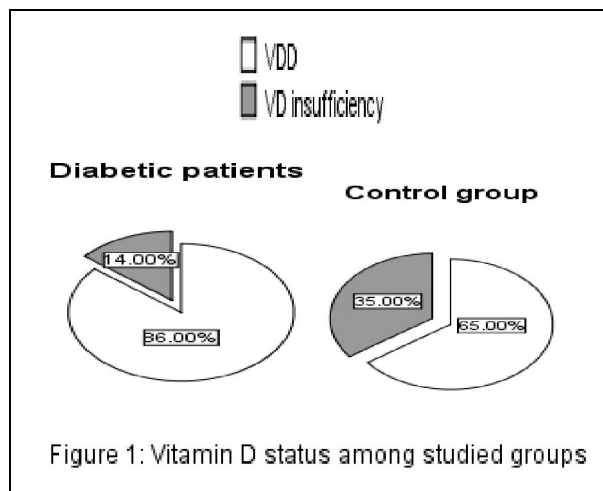h a mean ±SD age of 42.75±21.44. Out of 20 controls 11 were males and 9 were females, BMI was significantly higher in diabetics (28.16±5.62) than controls (24.81±3.94) (*p* =0.02) (Table1).

Median (IR) of serum 25(OH) D levels were significantly lower in diabetic patients [9.05 (5-13) ng/ml] than in the control subjects [14.95 (12.23-22) ng/ml; (*p* = 0.001)] (Table2). 43 of the 50 diabetic patients (86%) were vitamin D deficient (<20 ng/ml) [60.5% of them had severe vitamin D deficiency (< 10 ng/ml)], the remaining 7 diabetic patients (14%) were vitamin D insufficiency (20-29 ng/ml). Among healthy subjects, 13 (65%) subjects were Vitamin D deficient and 7 (35%) subjects were Vitamin D insufficient, the distribution of vitamin D status was significantly differ between diabetic (86%) and control (65%) groups regarding vitamin D deficiency (*P*<0.05) (figure1). As expected serum 25(OH) D levels were significantly lower in both T1DM [9.15(4.25-12.75) ng/ml] and T2DM [9.05 (5.75-13.25) ng/ml] compared to control group [14.95 (12.23-22) ng/ml] (*p* = 0.001). However, a non-significant difference regarding serum 25(OH) D levels were observed in T1DM vs. T2DM (*p* = 0.86) (Figure2).

Table 1: Demographic data among studied groups

	GROUP 1 (DM) X±SD		GROUP 2 (Control) X±SD	*p* -value
Numbers	50		20	--
	T1DM (20)	T2DM (30)		
Age (years)	49.76±15.06		42.75±21.44	0.19
M/F	19/31		11/9	---
	T1DM (7/13)	T2DM (12/18)		
Sex	1.62±.49		1.45±.51	0.2
BMI (kg/m2)	28.16±5.62		24.81±3.94	0.02

M: Male F: Female BMI: body mass index

Figure 1: Vitamin D status among studied groups

Figure 2:Serum 25 (OH) vitamin D in studied groups

Table 2: Biochemical parameters in diabetics and healthy controls

	GROUP 1 (diabetic group) X±SD/ Median (IR)*	GROUP 2 (control group) X±SD/Median (IR)*	P value
FPG(mg/dl)	172.5(122.5-246.5)*	87(79.25-94)*	0.001
HbA1C %	7.8 (5.85-10.1)% *	4.5 (2.4-5)% *	0.001
Vitamin D (ng/ml)	9.05(5-13)*	14.95(12.25-22)*	0.001
Ca (mg/dl)	8.76±0.5	9.01±0.34	0.02
Inorganic P (mg/dl)	3.9±0.7	3.95±0.5	0.9
ALP (U/l)	107.5(93.75-140)*	80(78-89)*	0.001
TC (mg/dl)	197(166.5-226.75)*	165(163-187.25)*	0.003
TG(mg/dl)	142.5(81.5-231.5)*	53(50-88)*	0.001
HDLc(mg/dl)	40(36.5-52.3) *	52.5(38-61.9) *	0.2
LDLC(mg/dl)	119.86±39	102.23±21.64	0.02
Urea(mg/dl)	27(20-41)*	26(22.25-28)*	0.4
Creatinine (mg/dl)	0.85(0.7-1.1)*	0.8(0.67-0.9)*	0.12

FPG: fasting plasma glucose HbA1C: glycated hemoglobin VDD: vitamin D deficiency Ca+: calcium ALP: alkaline phosphatase TC: total cholesterol TG: triglycerides HDLc: high density lipoprotein cholesterol LDLC: low density lipoprotein cholesterol.

A significantly lower serum calcium levels and higher total alkaline phosphatase levels were observed in diabetic patients (8.76±0.5, 107.5) vs. control subjects (9.01±0.34, 80; p =0.02, 0.001). By contrast a non-significant difference between diabetics and control groups regarding serum phosphorus (3.9±0.7 vs.3.95±0.53; p = 0.9) was reported (Table 2). For lipid profile, serum total Cholesterol (TC), Triglycerides (TG) and LDLc were significantly increased in diabetics patients (197, 142.5 and 119.86±39) compared to controls (165, 53 and 102.23±21.64; p =0.003, 0.001 and 0.019) (Table2). However, HDLc reveled a non-significant difference between diabetics and control [40(36.5-52.3) vs.; 52.5(38-61.9) p=0.2). Renal function parameters including blood urea and serum creatinine were increased in diabetics (27, 0.85) vs. control (26, 0.8) but this difference was statistically insignificant (p =0.4, 0.12 respectively) (table 2). Table 3 classified diabetic patients with respect to their vitamin D status, into three categories severe deficiency: <10; deficiency: 10- to < 20; and insufficiency: 20–29 (Table 3). For T1DM 17 patients (85%) had vitamin D deficiency (VDD) (< 20 ng/ml), including 10 patients (50 %) with (vitamin D < 10 ng/ml) and 7 patients (35%) with (vitamin D 10-to < 20 ng/ml) while 26 T2DM patients (86.6%) had VDD included 16 patients (53.3%) with vitamin D (< 10 ng/ml) and 10 patients (33.3%) with vitamin D (10-to < 20 ng/ml), while 3 patients (15%) in T1DM and 4 patients (13.3%) in T2DM had vitamin D levels in the insufficient range (Table 3). Vitamin D deficiency was significantly prevalent in diabetics (T1DM&T2DM) vs insufficiency (p = 0.003, 0.001 respectively).

The prevalence of vitamin D deficiency was 84.2% in males [including 7 males (36.8%) with Vitamin D (<10ng/ml) and 9 patients (47.4%) with

Vitamin D 10-to < 20 ng/ml] vs. 87.1% in females [including 19 females (61.3%) with vitamin D(<10ng/ml) and 8 females (25.8%) with vitamin D 10-to < 20 ng/ml]. vitamin D insufficiency was present in 3 diabetic males (15.8%) and in 4 diabetic female (12.9%) (Table3). Although, gender showed non-significant difference regarding both vitamin D deficiency and insufficiency, but percentage of below 10 ng/ml of 25(OH) D was more prevalent among female gender (p=0.003). Diabetes duration of patients with vitamin D deficiency was comparable to patients with vitamin D insufficiency (11.09±7.02 vs. 13.14±9.7; p =0.5). HbA1C and blood glucose were significantly increased in diabetics (7.8, 172.5) compared to control subjects (4.5, 87) (p = 0.001) regardless vitamin D level (Table 2). The average HbA1c was higher in diabetic patients with severe vitamin D deficiency (8.5%) compared to those with vitamin D insufficiency (7.7%), however this difference was not statistically significant (p =0.5) (Table 3). Our statistical analysis also reveled a significant increase of HbA1C (10.10%) in diabetic patients with vitamin D level <5ng/ml compared to diabetic with Vitamin D (5-to < 20 ng/ml) (7.79%) (p =0.026) (Figure3). A non-significant difference regarding calcium, phosphorus and alkaline phosphatase was detected in diabetics with vitamin D deficiency compared to diabetics with vitamin D insufficiency (p > 0.05), however, a significant decrease of serum calcium and phosphorus were observed in vitamin D deficient sub-groups [diabetics with **VD <** 10ng/ml (8.6±**0.85,** 3.7±0.7**)** vs diabetics with VD (10-to < 20) ng/ml (9±0.2, 4.3±**0.59;** p =0.001, 0.004] (Table 3). Serum TC, TG, LDLc and HDLc reveled a non-significant difference across diabetic individuals categorized by vitamin D status (p >0.05) (Table 3).

Table 3: Baseline characteristics of diabetic patients grouped according to vitamin D status.

		Vitamin D deficiency(< 20ng/ml) (43) (87.76%) x± SD/ median (IR)*		Vitamin D insufficient (20-29 ng/ml) x± SD	P value
		VD < 10ng/ml	VD (10-to < 20 ng/ml)		
No		26	17	**7(14%)**	0.002
Age		46.4±14.3	53.4±15.8	53.3±15.27	*p* > 0.05
Sex	**Male**	36.8% (7)	47.4% (9)	15.8% (3)	0.27
	Female	61.3% (19)	25.8% (8)	12.9% (4)	0.003
DM	**T1DM**	85% (17)		15% (3)	0.003
	T2DM	86.6% (26)		13.3% (4)	0.001
DM Duration		11.09±7.02		13.14±9.7	0.5
BMI		27.4±4.8	29.6±6.6	27.5±5.6	*p* 1=0.9 *p* 2=0.45 *p* 3=0.2
FPG		167(125-284)*	174(118-207)*	150(106-226)*	p1=0.3 p2=0.57 p3=0.48
HbA1C		8.5±2.7	8±2.9	7.7±2.6	p1=0.5 p2=0.8 p3=0.6
Vitamin D		5.5(4-7.4)*	12.5(10.25-13.5)*	21(21-22.3)*	p1=0.001 p2=0.001 p3=0.001
Ca		8.6±**0.85**	9±0.2	8.82±0.4	p1=0.2 p2=0.24 p3=0.001
Inorganic P		3.7±0.7	4.3±**0.59**	3.95±0.65	P1=0.4 P2=0.18 P3=0.004
ALP		117(98.5-140.2)*	100(92-134.5)*	103(52-125)*	p1-0.2 p2=0.53 p3=0.3
TCHOL		197(161.5-222)*	189(162-252)*	201(195-232)*	p1=0.3 p2=0.4 p3=0.9
TG		106(78.5-231.5)*	153(88-217)*	196(113-267)*	p1=0.24 p2=0.4 p3=0.3
LDLc		117.8±37.6	120.6±41.9	125.8±41.8	p1=0.6 p2=0.7 p3=0.8
HDLc		40(37-52.75) *	43.4(34.5-49.5) *	40(30-45) *	P1=0.5 P2=0.4 P3=0.9

FPG: fasting plasma glucose HbA1C: glycated hemoglobin VDD: vitamin D deficiency Ca+: calcium ALP: alkaline phosphatase TC: total cholesterol TG: triglycerides HDLc: high density lipoprotein cholesterol LDLC: low density lipoprotein cholesterol.

Bivariate analysis in diabetics, reveled that serum vitamin 25 (OH) D levels had significant positive correlation with both serum calcium and inorganic phosphate (r= 0.45, *p* = 0.001 & r=0.3, *p* =0.03) (Table 4), and strong negative correlation with blood glucose (r= -0.3, *p* =0.03) and alkaline phosphatase (r= -0.3, *p* = 0.03) (Table 4). A significant negative correlation between serum 25 (OH) D and HbA1C was observed in diabetics

with severe VDD (r= -0.45, *p* =0.018) (Table4). In addition, a border line negative significant correlation in diabetics with BMI > 25 (r= -0.3, *p* = 0.05) was observed between serum 25(OH) D and HbA1C (Figure 4). However, serum levels of vitamin D did not reveal any significant correlation with age, gender, diabetes duration, BMI, lipid profiles and renal function (Table 4).

Table 4: Correlation between vitamin D levels and various anthropometric and metabolic parameters in diabetic patients

|	Bivariate analysis			
	Dependant variable vitamin D			
	Diabetic patients (n=50)		Diabetic patients with severe VDD < 10 ng/ml (n=26)	
Variables	r	*p*	r	*p*
Age	0.1	0.35	-0.05	0.7
Sex	-0.2	0.2	-0.11	0.6
BMI	0.04	0.7	-0.09	0.6
DM duration	0.2	0.13	-0.22	0.26
Blood Glucose	-0.3	0.03	-0.26	0.08
HbA1C	-0.23	0.1	-0.45	0.018
TC	0.02	0.8	-0.15	0.3
TGs	0.08	0.5	-0.3	0.12
HDLc	-0.1	0.4	-0.1	0.6
LDLc	0.003	0.9	-0.06	0.7
Inorganic P	0.3	0.03	0.59	0.02
Ca	0.45	0.001	0.87	0.001
ALP	-0.3	0.03	-0.35	0.07
Urea	0.13	0.3	-0.27	0.18
Creatinine	0.25	0.07	-0.27	0.18
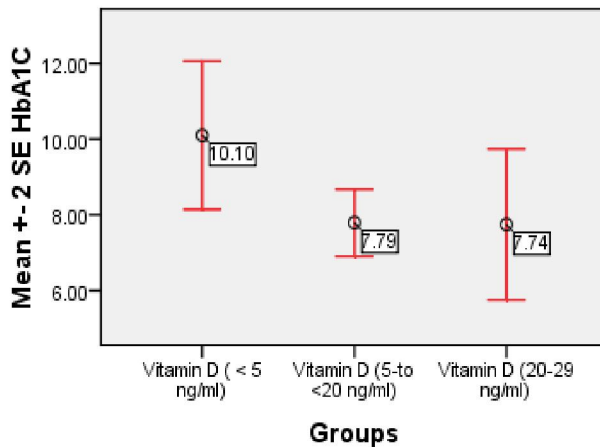
Figure 3: comparison of HbA1C among diabetic patients grouped according to vitamin D status
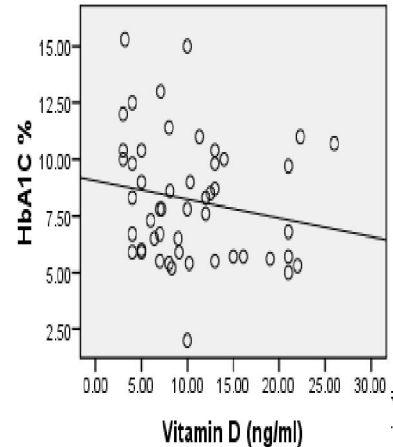

Figure 4: Correlation between 25(OH) and HbA1C in diabetics with BMI ≥25

4.Discussion:

Diabetes is a chronic condition associated with serious morbidity, increased mortality, increased financial costs and rapidly becoming a global epidemic and health care problem. The growing incidence and prevalence of diabetes highlights the need for innovative approaches in research for the management and prevention of the disease [30]. Vitamin D deficiency is newly recognized as a common condition of increasing prevalence worldwide. Clinically vitamin D has an established role in calcium and bone metabolism and has been recently shown to be associated with increased risk of developing type 1 and type 2 diabetes mellitus [31]. The aim of our study was to evaluate the vitamin D status in subjects with diabetes mellitus compared with apparently healthy subjects, and to examine the association of vitamin D level with HbA1c in diabetic patients. The results of the present study demonstrated that serum 25(OH) vitamin D was significantly reduced in patients with diabetes mellitus (T1DM & T2DM) as compared to the control group. These results were in consistent with various studies reported increased incidence of vitamin D deficiency and/or insufficiency in patients with either type 1 diabetes (T1D) or type 2 diabetes (T2D) [32-35] and inverse association between vitamin D status and diabetes [36-39]. Several cross-sectional and cohort studies have suggested an association between low serum 25(OH) D level and the occurrence of type 2 diabetes [31,39,40, 41]. **Lee *et al*.** [42] reported that the mean concentration in Korean patients with T2DM of 25(OH) D was (x±SD) (11.2 ±6.1) ng/ml. In contrast, the serum 25(OH) D levels of patients with T2DM in the United

States revealed higher mean values, 22.9 ng/ml [43] these results suggest that the 25(OH) D level in patients with T2DM varies widely according to ethnic, nutritional or other backgrounds. The mechanisms underlying the potential association between type 2 diabetes and 25(OH) D are unclear and could be explained by impact of 25(OH) D level on many factors, including pancreatic beta cell function, insulin action, and systemic inflammation. Associations between low 25(OH) D level and decreased insulin sensitivity have been reported in various cross sectional studies [40,42,44]. Evidence reported over the past decade shows a strong correlation between vitamin D and diabetes especially T1D [10]. **The nationwide Diabetes Incidence Study in Sweden (DISS)** found that the 25 (OH) D levels in the new onset T1D (n = 459) were low compared to controls (p-value < 0.001) [35]. **Pozzili et al**. also found that the 25OH (D) levels in new onset T1D (n = 88) were low compared to healthy controls (p-value < 0.01) [45]. The lower levels found at diagnosis compared with control subjects support the idea that vitamin D deficiency may be an important factor behind the development of type 1diabetes, perhaps with an immunological background [46]. The direct clinical evidence of the association between hypovitaminosis D and diabetes has come from interventional studies; these studies are more robust for T1DM, a 33% reduction (OR 0.67) in the subsequent risk of developing type 1 diabetes was seen by vitamin D supplementation during the first year of life [47]. In **Nurses' Health Study** which followed a large cohort of women over 20 years, a combined intake of > 800 U of vitamin D was associated with a decrease of 33% in the risk of T2DM compared with the intake of < 600 mgs of calcium and 400 U of vitamin D [38, 39]. Regarding serum 25 (OH) vitamin D among T1DM and T2DM, **Di Caser et al**. [48] reported that vitamin D deficiency has been found to be more prevalent among T2DM patients (63.5%, mean value 17.1 ng/ml) than patients with Type 1 diabetes mellitus (36%, mean value 23.6 ng/ml, $p < 0.05$) , with BMI > 25. However, our study reveled a non significant difference regarding serum 25 (OH) vitamin D between T1DM and T2DM, this controversy to our result may be due to small sample size and BMI variation. Regarding vitamin D status among diabetic patients, vitamin D deficiency is more in diabetics (T1DM&T2DM) than insufficiency ($p = 0.003$& 0.001), our study also revealed that 86% of diabetics had vitamin D deficiency [60.5% of them had Vitamin D (<10/ng/ml) and the remaining 39.5% had VD level (10- to < 20 ng/ml)] compared with 65% of control. In line with our finding regarding vitamin D status in DM, **Jung Re Yu et al**. [49] reported that up to 98% of the patients had Vitamin D insufficiency or deficiency,

in line with previous study both **Taheri et al**. and **Lee et al**. showed that the prevalence of vitamin D deficiency was 82.1%&85.9% in type 2 diabetic patients [50, 42]. On the other hand frequency of vitamin D deficiency in type 1 diabetes mellitus was found to be highly variable and may be as low as 15% or as high as 90.6% [32,33,51]. This could be explained by variation in sun exposure and nutritional status. **Devaraj et al**. [52] reported that levels of vitamin D were significantly reduced in T1D patients with or without microvascular complications. Lower rates of vitamin D sufficiency in adolescents with uncomplicated type 1diabetes support the hypothesis that diabetes per se may have a negative impact on vitamin D levels. **Thrailkill et al**. found increased urinary excretion of vitamin D binding protein (DBP) in type 1 diabetes patients presenting with albuminuria, this finding suggests that reduced levels of 25-(OH) D might be explained in part by urinary loss of DBP or increased catabolism of vitamin D [53]. An association between vitamin D receptor (VDR) polymorphism and T1DM was reported in Indian, German, and Taiwan populations [54,55]. **Batieha et al**. [56] revealed a high prevalence of low vitamin D status in Jordanian women >18 years old, **Lee et al**. [42] showed that the association of low serum 25(OH) D concentration with type 2 diabetes showed an age and gender-specific pattern: low serum 25(OH) D level was associated with type 2 diabetes in women <50 years. In consistent with previous result our study showed that, percentage of below 10 ng/ml of 25(OH) D was more prevalent among female (p =0.003) and 73.7% of them aged between18-49 years. **Hamd et al**. reported that serum level of calcium was significantly lower in diabetic patients compared with controls [57], these results go hand in hand with our result which demonstrated that serum level of calcium was significantly lower, in diabetic patients (14 cases **<**8.4) compared with control. Lower serum calcium levels may be resulted from decrease in duodenal calcium absorption and an increase in its urinary excretion in diabetic patients [57]. Modulation of calcium levels by vitamin D may also be important in this regard, since appropriate cytosolic calcium concentrations are crucial for the transport of glucose in muscle cells [58]. An indirect effect of vitamin D on beta cell insulin secretion is also postulated by means of increased intracellular calcium in the islet cells [31,38]. Raised value of serum ALP in diabetic patients has been reported in our study, this result was in agreement with **Iwamoto et al**. and **Siddiqui et al**. [59,60] Despite the highest value of alkaline phosphatase was seen in our diabetic group with severe vitamin D deficiency, the difference between ALP levels among patients with VDD or insufficiency were non-significant, this result was in accordance with Alam *et*

al. they reported that despite deficiency in vitamin D there were no significant alterations in alkaline phosphatase (ALP) [61]. The present study determined that the average HbA1c was higher in patients with severe vitamin D deficiency compared to those with vitamin D insufficiency (8.5% vs7.7%), in agreement with **Kant et al**. [62] who found that mean HbA1c was higher in patients with severe vitamin D deficiency when compared with patients with normal Vit D (8.18 %, vs 7.1%), however this difference was not statistically significant (*p* =0.5). Our statistical analysis also reveled that there was significant increase in HbA1C 10.10% in diabetic patients with vitamin D level <5ng/ml When compared to diabetic with vitamin D $\geq$ 5 ng/ml 7.79% (*p* =0.026). In support of such assumption, there was a significant negative correlation between serum levels of 25 (OH) D and blood glucose. In line with our study, low 25(OH) D levels are associated with higher fasting glucose and higher levels of glycosylated haemoglobin in patients with established diabetes mellitus [63]. Moreover, a significant inverse relation between 25 (OH) D and HbA1C was observed especially in severe vitamin D deficiency. Also, in diabetics with increased BMI (BMI > 25) a border line negative significant correlation was detected between serum 25(OH) D and HbA1C. The previous results were supported by **Suzuki et al**. reported that the mean level of 25 (OH) D in Japanese patients with type 2 diabetes, was inversely related to HBA1c [64]. In an analysis of data from the 2005 **HSE**, the association between low vitamin D levels and hyperglycemia, using HbA1c levels of 6.5% or greater, was investigated. Associations were shown for vitamin D levels at levels less than 25.0 nmol/L, but not for milder levels of vitamin D deficiency (50.0–74.9 nmol/ L). Other potential confounding factors did not account for the association (apart from alcohol consumption for vitamin D levels <25.0 nmol/L), and the time of the year at which the examination was performed did not substantially modify the association [65]. **Hypponnen et al**. also reported that serum 25 (OH) was inversely associated with HbA1C especially in concentration less than 65nmol/l and in participants with increased BMI [66]. Two other conflicting studies were reported about effect of vitamin D replacement on HBA1C as a marker of glycemic control, **Luo et al**. can't detect any relationship between hypovitaminosis D and glycemic control in well-established Chinese type 2 diabetes on the other hand **Ali et al**. reported that short term oral vitamin D replacement can provide more effective glycemic control in newly diagnosed diabetics. This controversy perhaps due to mean age (65 years versus 45.2), diabetes duration and ethnic variation between the previous two studies [67,68]. Exploring lipid profile, our study reveled a non significant association between (25) OH D and lipid profiles parameters, although there was a significant association of dyslipidemia and DM regardless type of diabetes (T1DM& T2DM) and level of glycemic control, in agree with our results regarding vitamin D relation to lipid profile **Lee et al**. [42], reported no association between the serum 25(OH) D level and lipid levels in Korean diabetic patients. **Heart Protection Study** reported that Patients with type 1 diabetes will have reduced HDL levels and increased triglycerides when glycemic control is poor. However, in patients with type 2 diabetes, HDL concentrations tend to be reduced and triglyceride concentrations elevated even in patients with good glycemic control. Patients with diabetes have more LDL particles, which tend to be small and dense [69]. This difference between our result and the previous study regarding T1DM could be related to small ample size in our study group. In summary our study showed that serum 25(OH) D was significantly low in people with diabetes compared with controls, Furthermore, Vitamin D deficiency predicts higher fasting blood glucose and HbA1C. Our findings suggest a path-physiological mechanism that at least in part may explain the inverse association between vitamin D status and DM. However, it is not apparent either DM is a direct consequence of vitamin D insufficiency or may result in vitamin D deficiency. Clinical intervention studies are needed to clarify whether treatment with vitamin D could improve glycemic control in diabetic patients

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