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Association of Glycosylated Hemoglobin Levels with Vitamin D Level among Patients with Type 2 diabetes Health Care Centers, Makkah city, Saudi Arabia

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Abstract:

Purpose: This study aimed to examine whether 25-hydroxyvitamin D (25OHD) levels (an indicator of vitamin D status) are independently associated with Glycosylated Hemoglobin Levels in patients with type 2 diabetes mellitus (T2DM). **Patients and Methods:** This was a cross-sectional study. Participants with T2DM were recruited from the health care centers ,Makkah city, Saudi Arabiaaccording to inclusion and exclusion criteria (219 patients). Data was collected using a checklist including items of age, gender, BMI, vitamin D level (mg/mL) and glycatedhaemoglobin percentage. Anthropometric measurements included height and weight was obtained. **Results:** The present study revealed that majority of diabetic patients included in the study had abnormal vitamin D level with a significant positive association with abnormal level of hemoglobin A1c, age, fasting blood glucose, triglycerides, total cholesterol and VLDL. **Conclusion:** Vitamin D is independently associated with elevated Glycosylated Hemoglobin Levels in patients with T2DM.

Keywords: Glycosylated Hemoglobin Levels, Vitamin D Level, Type 2 diabetes

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Introduction:

Diabetes is a complex; chronic disease occurs when there is raised level of blood glucose because of insulin deficiency and peripheral insulin resistance. It needs continuous medical care and multidisciplinary management. ADA 2018 Type 2 diabetes also known as "noninsulin-dependent diabetes" accounts for 90% of all diabetes (American Diabetes Association Standards of medical care in diabetes, 2018; International Diabetes Federation IDF diabetes atlas, 2017).

According to IDF the prevalence of diabetes in 2017 is 327 million 20-64 years' world wild and expected to be 438 million 20-64 years in 2045 (International Diabetes Federation IDF diabetes atlas., 2017).The overall prevalence of DM in Kingdom of Saudi Arabia is 23.7%. The prevalence in males was 26.2% while in females 21.5 %. The prevalence of diabetes mellitus among Saudis who are living in urban areas is 25.5% which is higher than whom residing in rural area {19.5%} ($p<0.00001$) (Al-Nozha et al., 2004).

Vitamin D3 is a prohormone produced in the skin from 7-dehydrocholesterol by exposure to sun ultraviolet rays. Then in the liver, it is metabolized to 25-hydroxyvitamin and then to 1alpha,25-dihydroxyvitamin D3 in the kidney (DeLuca., 2004).

Nearly in every tissue of the body, there are vitamin D receptors (VDR) which are the binding sites for vitamin D (Bikle., 2014). Through these receptors vitamin D can carry out many functions, including calcium absorption, phosphate absorption in the intestine, calcium mobilization in bone, and calcium reabsorption in the kidney (DeLuca.,2004). The pancreatic β cell expresses the VDR, and $1,25(OH)_2D$ promotes insulin secretion (Bikle., 2014). Thus, vitamin D deficiency can exhibit a reduction in insulin secretion (Norman et al., 1980; Selvarajan et al., 2021).

Vitamin D has effects on pathophysiological mechanisms of both types of diabetes, including pancreatic beta cell dysfunction or impaired insulin action by vitamin D receptors activation directly or indirect by calcium homeostasis regulation(Mitri& Pittas., 2014). So, low level of vitamin D is associated with increased risk of hyperglycemia both in diabetic and non-diabetic (Rafiq&Jeppesen., 2018). Therefore the researchers found it necessary to investigate the association between hemoglobin A1C as a potent and clear parameter for diabetic patient clinical condition and vitamin D level.

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Aim of the study

To investigate the relation of vitamin D and glycosylated hemoglobin in patients with diabetes attending diabetic clinics, at the health care centers, Makkah city, Saudi Arabia

METHODOLOGY

Study design:

Cross-sectional study design will be adopted

Study setting:

This study was conducted at the health care centers, Makkah city, Saudi Arabia

Target population:

All type 2 diabetic patients attending diabetic clinics at the health care centers, Makkah city, Saudi Arabiafrom 1st April, 2021 will be invited to participate in the study till the minimum requires sample size reached (219 patients).

Inclusion criteria

- Saudi adults (18 years or above) diabetic patient attending at the health care centers, Makkah city, Saudi Arabia
- Both sexes
- Diagnosed with type 2 diabetes for at least one year.
- Have recent (within three months) measures of glycatedhaemoglobin and vitamin D

Exclusion criteria

- Patients <18 years of age.
- Non-Saudi patients
- Gestational diabetes since they are followed up out of the center at obstetrics and Gynecology clinics.

Data collection tools:

Data was collected using a checklist including items of age, gender, BMI, vitamin D level (mg/mL) and glycatedhaemoglobin percentage (Kositsawat et al., 2010).

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Anthropometric measurements included height and weight was carried out with the help of trained nurse using a calibrated balance beam scale. Normal weight was defined as BMI<25 kg/m^2, overweight as 25≤BMI<30 kg/m^2 and obesity as BMI≥30 kg/m^2 (WHO Expert Consultation., 2004).

Administrative and ethical considerations

- Permission from the regional Research and Ethics Committee; health care centers directors were obtained.
- A verbal consent will be obtained from each patient.
- All collected data will be kept confidential and will not used except for the purpose of the scientific research.
- Ethical considerations will be observed throughout the research.

Data entry and statistical analysis

Collected data will entered into an own computer and will be analyzed using the SPSS version 23 with a significance of p-value < 0.05. Data will be presented in the form of frequency and percentage. Continuous variables will be presented by mean and standard deviation. Pearson`s correlation test will be used to test for the correlation between vitamin D and glycatedhaemoglobin variables. Other statistical tests will be applied whenever appropriate

Results:

Table I: Frequency distribution of patients' socio biodemographic characteristics:

This table sowed that approximately two thirds of patients were aged from 50-70 years of age. Majority were female and married (71.2%, 86.3%). Two thirds of patients were illiterate and primary education (24.7%, 38.8%). Regarding employment, it was noticed that more than half of patients were unemployed (69.4%). Majority of patients were nonsmokers (95.4%), overweight (28.8%) and obese (61.7%). Less than half of patients (40.6%) suffering from diabetic complications in a form of neuropathy (56.2%), retinopathy (32.6%), nephropathy (27.0%), and CVS (22.5%).

	N	%
Age group		
<50	42	19.2
50-60	68	31.1

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60-70	64	29.2
>70	45	20.5
Range	24-88	
Mean±SD	59.082±12.461	
gender		
Female	156	71.2
Male	63	28.8
marital status		
Married	189	86.3
Unmarried	30	13.7
educational level		
not educated	54	24.7
primary	85	38.8
intermediate	16	7.3
secondary	40	18.3
university	24	11.0
occupation		
unempolyment	152	69.4
governmental	35	16.0
retired	32	14.6
smoking status		
not smoker	209	95.4
smoker	10	4.6
BMI		
Normal weight	21	9.6
Overweight	63	28.8
Obese Class I	61	27.9
Obese Class II	53	24.2
Obese Class III	21	9.6
Range	18.98-64.2	
Mean±SD	32.460±6.467	
complications		

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Negative	130	59.4
Positive	89	40.6
neuropathy	50	56.2
nephropathy	24	27.0
retinopathy	29	32.6
CVS	20	22.5

Table II: Frequency distribution by mean and standard deviation for clinical data:

This table clarified that heart rate was 83.507±11.939, duration of diabetes in years was 12.822±7.978, hemoglobin A1C level8.622±1.995 and fasting glucose mmol/L. for LDL, hDL, Tg and total cholesterol, There were 2.734±0.854, 1.705±8.534, 1.605±0.760 and 4.481±1.065 respectively. Regarding VLDL mmol/L, it was 0.728±0.370.

heart rate	Range	58-117
	Mean±SD	83.507±11.939
duration of diabetes in years	Range	1-50
	Mean±SD	12.822±7.978
hemoglobin A1C level	Range	4.7-15.1
	Mean±SD	8.622±1.995
fasting glucose mmol/L	Range	0.5-27.8
	Mean±SD	9.524±4.041
LDL mmol/L	Range	0.7-5.9
	Mean±SD	2.734±0.854
HDL mmol/L	Range	0.097-127
	Mean±SD	1.705±8.534
TG mmol/L	Range	0.51-6.21
	Mean±SD	1.605±0.760
total cholesterol mmol/L	Range	1.1-8
	Mean±SD	4.481±1.065
VLDL mmol/L	Range	0.2-2.9
	Mean±SD	0.728±0.370

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Table III: frequency distribution of the type of insulin and oral hypoglycemic drug used:

It was noticed that majority of patient used Glargine (94.6%) and metformin (78.5%)

	N	%
insulin 1		
Glargine	106	94.6
Glulisine	1	.9
aspart	1	.9
humalog mix 25	1	.9
humalog mix 50	3	2.7
oral hypoglycemic drug 1		
Gliclazide	15	7.9
Glimepiride	4	2.1
Liraglutide	3	1.6
metformin	150	78.5
pioglitazone	2	1.0
sitagliptin	15	7.9
Vildagliptin	2	1.0

Table IV, figure I: frequency distribution of the level of vitamin D:

It was observed that the majority of patients (77.6%) were abnormal regarding the level of vitamin D wit mean of22.120±9.542

	N	%
vitamin d level mg/ml		
Group I (Normal)	49	22.4
Group II (Abnormal)	170	77.6
Range	6-65	
Mean±SD	22.120±9.542	

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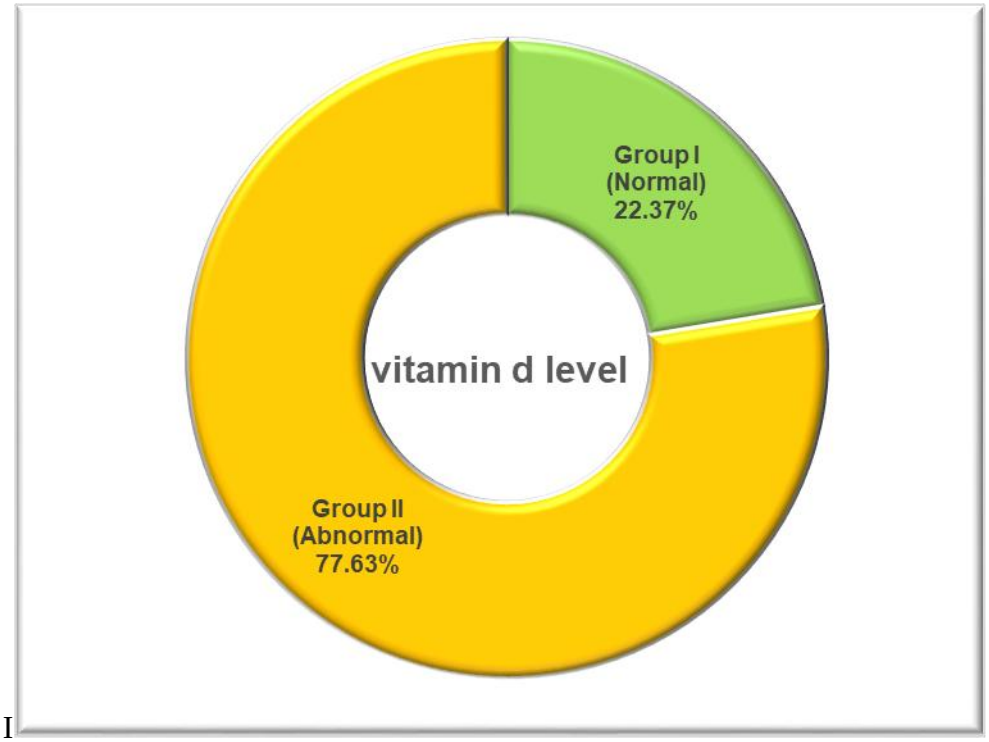

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Table V: significance difference between level of vitamin D and bio demographic data:

It was noticed that there was no statistical significance differences between patient in relation to socio demographic characteristics and level of vitamin D.

		vitamin d level mg/ml						Chi-square	
		Group I Normal		Group II Abnormal		Total			
		N	%	N	%	N	%	X^2	P-value
Age group	<50	5	10.2 %	37	21.8 %	42	19.2%	3.833	0.280
	50-60	16	32.7 %	52	30.6 %	68	31.1%		
	60-70	17	34.7 %	47	27.6 %	64	29.2%		
	>70	11	22.4 %	34	20.0 %	45	20.5%		
BMI	Normal weight	4	8.2%	17	10.0 %	21	9.6%	0.798	0.939

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	Overweight	14	28.6 %	49	28.8 %	63	28.8%		
	Obese Class I	13	26.5 %	48	28.2 %	61	27.9%		
	Obese Class II	14	28.6 %	39	22.9 %	53	24.2%		
	Obese Class III	4	8.2%	17	10.0 %	21	9.6%		
gender	**Female**	38	77.6 %	118	69.4 %	156	71.2%	1.275	0.259
	Male	11	22.4 %	52	30.6 %	63	28.8%		
marital status	**Married**	41	83.7 %	148	87.1 %	189	86.3%	0.356	0.551
	Unmarried	8	16.3 %	22	12.9 %	30	13.7%		
educational level	**not educated**	11	22.4 %	43	25.3 %	54	24.7%	6.486	0.166
	primary	22	44.9 %	63	37.1 %	85	38.8%		
	intermediate	4	8.2%	12	7.1%	16	7.3%		
	secondary	4	8.2%	36	21.2 %	40	18.3%		
	university	8	16.3 %	16	9.4%	24	11.0%		
occupation	**unempolyment**	37	75.5 %	115	67.6 %	152	69.4%	1.360	0.506
	governmental	7	14.3 %	28	16.5 %	35	16.0%		
	retired	5	10.2 %	27	15.9 %	32	14.6%		
smoking status	**not smoker**	47	95.9 %	162	95.3 %	209	95.4%	0.035	0.852

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	smoker	2	4.1%	8	4.7%	10	4.6%		
complications	**Negative**	27	55.1 %	103	60.6 %	130	59.4%	0.471	0.492
	Positive	22	44.9 %	67	39.4 %	89	40.6%		
neuropathy	**Negative**	11	50.0 %	28	41.8 %	39	43.8%	0.451	0.502
	Positive	11	50.0 %	39	58.2 %	50	56.2%		
nephropathy	**Negative**	15	68.2 %	50	74.6 %	65	73.0%	0.341	0.559
	Positive	7	31.8 %	17	25.4 %	24	27.0%		
retinopathy	**Negative**	14	63.6 %	46	68.7 %	60	67.4%	0.188	0.665
	Positive	8	36.4 %	21	31.3 %	29	32.6%		
CVS	**Negative**	17	77.3 %	52	77.6 %	69	77.5%	0.001	0.974
	Positive	5	22.7 %	15	22.4 %	20	22.5%		

Table VI: significance difference between level of vitamin D and line of treatment:

It was clear that there were no statistical significance differences between and level of vitamin D and line of treatment (insulin or oral hypoglycemic)

	vitamin d level ng/ml						**Chi-square**	
	Group I Normal		**Group II Abnormal**		**Total**			
	N	**%**	**N**	**%**	**N**	**%**	**X^2**	**P-value**

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insulin 1	**Glargine**	22	95.7%	84	94.4%	106	94.6 %	5.468	0.243
	Glulisine	0	0.0%	1	1.1%	1	.9%		
	aspart	1	4.3%	0	0.0%	1	.9%		
	humalog mix 25	0	0.0%	1	1.1%	1	.9%		
	humalog mix 50	0	0.0%	3	3.4%	3	2.7%		
insulin 1 frequency	**OD**	22	95.7%	85	95.5%	107	95.5 %	1.186	0.553
	TID	1	4.3%	2	2.2%	3	2.7%		
	BID	0	0.0%	2	2.2%	2	1.8%		
insulin 2	**Glulisine**	10	71.4%	39	60.9%	49	62.8 %		
	humalog	1	7.1%	8	12.5%	9	11.5 %		
	novomix	0	0.0%	1	1.6%	1	1.3%		
	apidra	0	0.0%	1	1.6%	1	1.3%		
	Aspart	2	14.3%	11	17.2%	13	16.7 %		
	liraglutide	0	0.0%	1	1.6%	1	1.3%		
	mixtard	0	0.0%	3	4.7%	3	3.8%		
	glargin	1	7.1%	0	0.0%	1	1.3%		
oral hypoglycemic drug 1	**Gliclazide**	6	13.6%	9	6.1%	15	7.9%	10.847	0.093
	Glimepiride	1	2.3%	3	2.0%	4	2.1%		
	Liraglutide	0	0.0%	3	2.0%	3	1.6%		
	metformin	31	70.5%	119	81.0%	150	78.5 %		
	pioglitazone	1	2.3%	1	.7%	2	1.0%		
	sitagliptin	3	6.8%	12	8.2%	15	7.9%		
	Vildagliptin	2	4.5%	0	0.0%	2	1.0%		
oral hypoglycemic drug 1 frequency	**OD**	19	43.2%	58	39.5%	77	40.3 %	1.344	0.511
	TID	17	38.6%	50	34.0%	67	35.1 %		

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	BID	8	18.2%	39	26.5%	47	24.6 %		
oral hypoglycemic drug 2	**gliclazide**	2	6.7%	22	22.2%	24	18.6 %	8.634	0.195
	glimepiride	5	16.7%	6	6.1%	11	8.5%		
	Liraglutide	1	3.3%	2	2.0%	3	2.3%		
	Metformin	2	6.7%	9	9.1%	11	8.5%		
	pioglitazone	4	13.3%	5	5.1%	9	7.0%		
	sitagliptin	15	50.0%	51	51.5%	66	51.2 %		
	vildagliptin	1	3.3%	4	4.0%	5	3.9%		
oral hypoglycemic drug 2 frequency	**OD**	0	0.0%	9	9.2%	9	7.0%		
	TID	29	96.7%	87	88.8%	116	90.6 %		
	BID	1	3.3%	2	2.0%	3	2.3%		
oral hypoglycemic drug 3	**Gliclizide**	5	41.7%	18	36.0%	23	37.1 %	4.824	0.567
	Glimepiride	1	8.3%	6	12.0%	7	11.3 %		
	liraglutide	0	0.0%	4	8.0%	4	6.5%		
	Metformin	1	8.3%	4	8.0%	5	8.1%		
	pioglitazone	0	0.0%	2	4.0%	2	3.2%		
	sitagliptin	5	41.7%	13	26.0%	18	29.0 %		
	vildagliptin	0	0.0%	3	6.0%	3	4.8%		
oral hypoglycemic drug 3 frequency	**OD**	0	0.0%	3	6.0%	3	4.8%	1.786	0.409
	TID	12	100.0 %	46	92.0%	58	93.5 %		
	BID	0	0.0%	1	2.0%	1	1.6%		
Other conditions	**Nill**	10	20.4%	53	31.2%	63	28.8 %	5.081	0.166
	dyslipidemia	13	26.5%	35	20.6%	48	21.9 %		

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	HTN	8	16.3%	40	23.5%	48	21.9 %		
	HTN, dyslipidemia	18	36.7%	42	24.7%	60	27.4 %		
Treatment used	**Insulin**	5	10.2%	23	13.5%	28	12.8 %	1.544	0.462
	oral hypoglycemic	25	51.0%	70	41.2%	95	43.4 %		
	Both	19	38.8%	77	45.3%	96	43.8 %		

Table VII, figure II-VII: correlation between level of vit D and patient biosocio demographic characteristics:

This table sowed a positive correlation between the level of vit D and age (0.002), hemoglobin A1C level (<0.001), fasting glucose mmol/L (<0.001), TG mmol/L (<0.001), and VLDL mmol/L (0.001)

Correlations	**vitamin d level ng/ml**	
	r	**P-value**
Age	0.204	0.002*
height in CM	-0.060	0.375
weight in KG	0.013	0.851
BMI	0.075	0.272
heart rate	-0.036	0.592
SBP	0.020	0.769
DBP	-0.056	0.414
duration of diabetes in years	-0.030	0.654
insulin 1 dose in unit	0.129	0.175
insulin 2 dose morning	-0.155	0.182
insulin 2 dose afteroon	-0.007	0.956
insulin 2 dose evening	-0.128	0.319
oral hypoglycemic drug 1 dose in MG	-0.054	0.459

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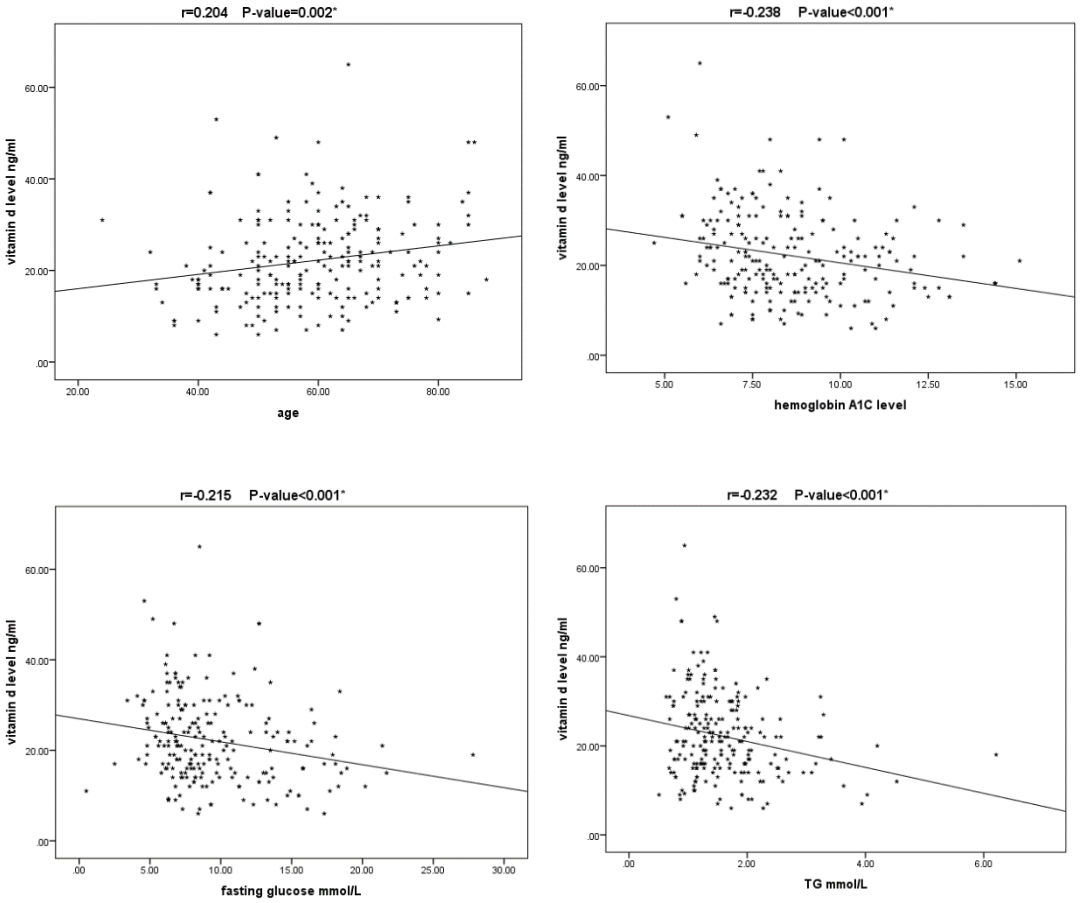
oral hypoglycemic drug 2 dose in MG	-0.139	0.116
oral hypoglycemic drug 3 dose in MG	0.041	0.751
hemoglobin A1C level	-0.238	<0.001*
fasting glucose mmol/L	-0.215	<0.001*
LDL mmol/L	-0.110	0.105
HDL mmol/L	-0.062	0.361
TG mmol/L	-0.232	<0.001*
total cholesterol mmol/L	-0.175	0.009*
VLDL mmol/L	-0.226	0.001*

Figure II-VII: correlation between level of vit D and patient biosocio demographic characteristics:



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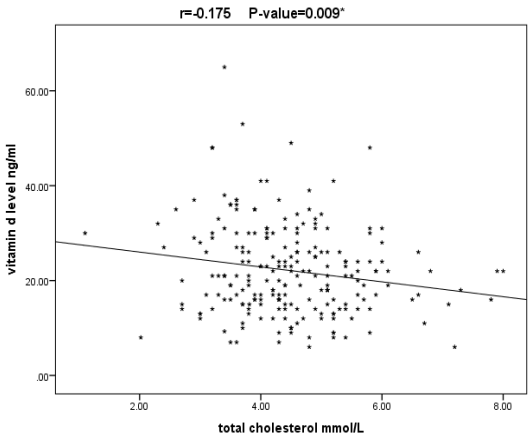

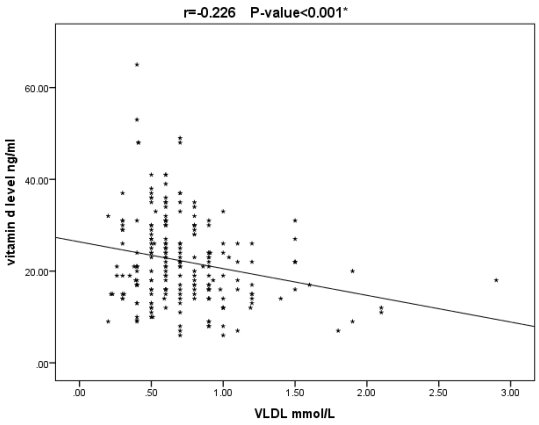

Multiple regressions between vitamin d level mg/ml and most significant characters:

This table showed that the most significant correlation and effect on vitamin d level mg/ml was for age wit P=0.019

Dependent Variable: vitamin d level ng/ml	**Unstandardized Coefficients**		**Standardized Coefficients**	**t**	**P-value**	**ANOVA**		**R^2**
	B	**Std. Error**	**Beta**			**F**	**P-value**	
(Constant)	28.367	4.797		5.913	0.000	5.369	<0.0001*	13.20%
age	0.119	0.050	0.155	2.358	0.019			
hemoglobin A1C level	-0.712	0.410	-0.149	-1.738	0.084			
fasting glucose mmol/L	-0.187	0.202	-0.079	-0.925	0.356			
TG mmol/L	-1.48	2.393	-0.118	-0.61	0.537			

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	1			9				
total cholesterol mmol/L	-0.445	0.627	-0.050	-0.710	0.479			
VLDL mmol/L	-1.350	4.902	-0.052	-0.275	0.783			

Discussion:

An increasing number of people have been diagnosed with type 2 diabetes mellitus (T2DM) with social and economic development. Diabetes affects all systems of the body and is followed by many complications (**Hossain et al., 2021**). It is proposed that VitD can influence the progression and control of T2D either directly by binding to its own receptor (VitD receptor) on β-cells of the pancreas or indirectly by regulating extracellular Ca or Ca influx to pancreatic β-cells (**Alharazy et al., 2021**)

The present study revealed that majority of diabetic patients included in the study had abnormal vitamin D level with a significant positive association with abnormal level of hemoglobin A1c, age, fasting blood glucose, triglycerides, total cholesterol and VLDL.

In this context, a study done by **Zhao et al., 2021**, **Albalawi et al., 2021** aimed to explore the relationship between vitamin D level and diabetic microvascular complications in general, including diabetic retinopathy (DR), diabetic nephropathy (DN), and diabetic peripheral neuropathy (DPN). It is necessary to spot that the results of this study was in harmony with our study findings as they found that Vitamin D deficiency is independently associated with higher risk of DPN and DN, but not DR, in T2DM patients. Further, it may be a potential predictor for both the occurrence and severity of DPN and DN. Moreover, **Nadri et al., 2021** found that Low serum vitamin D levels correlate with the presence of DRIL, EZ disruption and RPE alterations and increasedseverity of DR.

Many researches concerned with explore the correlation between diabetes mellitus parameters as hemoglobin A1C, diabetic complication s as retinopathy, nephropathy and neuropathy with certain vitamins and consequences. In this context, **Zhaoet al., 2021** investigate the relation between diabetic retinopathy with bone metabolism indices by comparing the changes in bone metabolism indices and bone mineral density between DR

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and no diabetic retinopathy (NDR), providing new evidence for preventing and treating DR and related clinical manifestations. They found a close association was observed between 25(OH) D level and DR in the elderly male patients and postmenopausal women with type 2 diabetes mellitus.

A study done by **Alharazy et al., 2021** support that Insulin resistance and obesity are associated with VitD status in T2D in this cohort. They also recommended that VitD supplements are able to improve degree of obesity and insulin sensitivity should be considered in further investigation. **Wang et al., 2021** study clarified that ensuring adequate vitamin D levels could reduce the prevalence of impaired fasting glucose IFG and T2DM, especially in females with high levels of testosterone

Additionally, in a wide population **hong et al., 2021** evaluated this relationship between vitamin D status and metabolic parameters and complications of T2DM for 1,392 Korean patients with T2DM and found Vitamin D deficiency was common among Korean T2DM patients; it was independently associated with microalbuminuria and HDL level, and positively related to diabetic nephropathy.

Furthermore, in a unique study **Buhary et al., 2021** performed a prospective observational cohort study of patients with type 1 and type 2 diabetes. HbA1c and vitamin D levels were recorded prior to supplementation and after 9 months of supplementation with vitamin D. the study results revealed lowering of HbA1c after vitamin D supplementation

Conclusion:

It is beneficial to advise patients with higher HbA1c to test their vitaminD level and correct any deficiency will result in better blood glucose control and benefit the patient's overall health.

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