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THE EFFECT OF GLYCEMIC CONTROL ON SOME HEMATOLOGICAL AND BIOCHEMICAL PARAMETERS IN TYPE 2 DIABETES MELLITUS PATIENTS

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Abstract:

Objectives: In type 2 diabetes mellitus (T2DM) patients, Vitamin D deficiency, increased C-reactive protein indices and abnormal lipid profile have a high association with cardiovascular(CDV) complications. This research was done to search relationship between glycemic control in T2DM patients and some parameters, included vitamin D, C-reactive protein (CRP), some complete blood count (CBC) and lipid status.

Materials and Methods: This study enrolled 100 subjects were divided into three groups. Group (I) (good glycemic control, n=35 patients with HbA1c levels <7%), group (II) (poor glycemic control, n=45 patients with HbA1c levels>7%),and control participants (non-diabetic individuals , n=20). We analyzed CBC variables, serum lipid profile, CRP and vitamin D levels.

Results: In group (II), Cholesterol (Cho), Triglyceride(TG), (CRP), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were higher, and the high density lipoprotein (HDL) levels, hemoglobin (Hb) concentration were lower than in the Control group. Other blood parameters and V D did not differ between 3 groups. Vitamin D levels inversely correlated with HemoglobinA1c (HbA1c), Fasting blood sugar (FBS), lipid profile, Platelets, WBC (lymphocyte, monocyte, granulocyt e), and CRP, and positively correlated with Hb, RBC and PCV.

Keywords: T2DM, HbA1c, CRP, CBC, Vitamin D, Lipid profile.

INTRODUCTION: Type 2 diabetes is a heterogeneous metabolic disorder characterized by chronic hyperglycemia [1]. It differs from the first type in resistance of insulin action and low secretion; Also, not properly respond of insulin receptors to insulin in the cell membranes of tissues [2], lead to high levels of insulin in the blood. The disease progresses lead to reduce insulin secretion and insulin injections are required. Many factors that can detect the cause and mechanism of T2DM, including the concentration of visceral fat in the abdominal area, which leads to insulin resistance. 55% of patients with type 2 diabetes suffer from obesity [3]. Obesity is an independent factor in increasing the risk of progresses disease, which is largely inherited [4]. Insulin resistance also leads to dyslipidemia and hypertension. The lipid profile associated with insulin resistance is characterized by high T.G, low HDL levels And the increased delivery of free fatty acids to the liver leads to an increase in the liver's production of fats in the form of VLDL In addition, cholesterol protein transferase increases insulin resistance and this promotes the conversion of lipids from HDL to VLDL, thus lowering HDL levels [5].

Vitamin D is a unique 'vitamin' that can be obtained through the diet and is also produced endogenously in response to exposure to ultraviolet (B-UV) sunlight. In addition to its classical function in bone mineralization and skeletal muscle, has functions elsewhere in the body and is associated with the disease through multiple mechanisms, vit D may help in reducing the risk of T2DM by reducing resistance of insulin and enhance secretion of it, reduce inflammation, and β-cell survival [6]. The CYP27B1 enzyme is very important and is responsible for converting the inactive vit D (25 (OH) D) to the biologically active form of vitD (1,25) (OH) 2 D) as well as the vit D receptor (VDR) in pancreatic cells. vit D has been shown a key to reduce inflammatory cytokines involved in insulin resistance and beta-cell dysfunction, specifically CRP, IL-6, and TNFα, VitD interferes with cytokine transcription by binding to the vitamin D receptor in genes that encode for inflammatory cytokines [7]. In addition, activated vit D (1,25(OH)2 D) has down regulates the nuclear factor B pathway that controls genes encoding for inflammatory

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cytokines including interleukin 6, CRP, and tumor necrosis factor α [8]. High serum levels of (25 (OH) D) are significantly associated with reduced all-causes CVD, that maintaining adequate vit D status may reduce mortality risk in diabetes patients [9]

MATERIALS AND METHOD: 80 blood samples were collected from patients with type 2 diabetes, and 20 blood samples were collected from normal healthy people in Ibn Al-Haytham laboratories for pathological and hormone analyzes Iraq/ Kirkuk. for the period from September 2020 to February 2021. Patients' ages ranged from 35 to 65 years, and patient samples were divided according to patients' control of diabetes, as they were distributed as follows:aaaaaa

1- The first group (I) : Good glycemic control, n=35 patients with HbA1c levels <7%.
2- Group (II): Poor glycemic control, n=45 patients with HbA1c levels>7%.
3-The third group: Control participants healthy people (non-diabetic individuals, n=20).

Demographic characteristics were collected from participants included (age, sex, duration of disease, presence of associated disease such as coronary artery disease, dyslipidemia, hypertension), and laboratory results including (FBS, HbA1c levels, lipid status, CBC, CRP and vitamin D) of the subjects there were done in Ibn Al-Haytham's lab. CBC analyses were carried out in tubes containing K2 ethylene diamine tetra acetic acid (EDTA) on a Horiba ABX Micros instrument (Horiba, France) within 2 hours. lipid status(T-cho, HDL, LDL, triglyceride, and VLDL) were run by an Automated device a Lipidocare system (SD Biosensor, Gyeonggi, Korea). Vitamin D levels were analyzed on a mini Vidas device (Enzyme-Linked Fluorescent Assay) System (Biomeriuex, Marcy-l'Etoile, France). HbA1c values and CRP levels of Participants were measured by Fluorescence-based Lateral Flow Immunoassay in a ichroma II system (Boditech, Korea).

STATISTICAL ANALYZES: Statistical analyzes were carried out using the prepared statistical program (SPSS) (Version 20, Chicago IL, USA). A t-test was used to measure the difference in means between two groups. A value $P < 0.05$ was considered statistically significant.

RESULTS: The current study found that most of the cases are those who do not control diabetes, the ages ranged from (35-65) years, they had kidney disease, heart disease and blood pressure, and the number of females was (28) more than males (17).

As shown in Table 1. The result not recorded significant differences between groups in the levels of Platelets, RBD cells, White Blood cells and packed cell volume percentage.

The lipid profile consisting of (triglyceride, cholesterol, and VLDL) were significantly higher in the group (II) than group (I) and control. significantly lower HDL and Hemoglobin levels recorded in group (I) and (II). While there were no significant differences among all groups in LDL levels. CRP level was significantly higher in the group (II) than group (I) and control.

In patients with T2DM the prevalence of hypovitaminosis was 83.7 % (n=67), and the vitamin D sufficiency was 16.3 % (n=13). There were no significant differences in vitamin D levels among groups ($p > 0.05$). The correlations coifeitiont between vit D and others (HbA1c, FBS, lipid status, total, differential WBC, RBC, Hb, PCV, platelet, and CRP) in group (II) are showed in table 2. The positive correlation was found between vitamin D and RBC, PCV, Hb levels. and inversely with HbA1c, FBS, lipid profile, Platelets, WBC (lymph, mono, gran), In addition to CRP ($r = -0.295$, $p = 0.049$*).

Table 1: Diabetes mellitus patients, demographic and laboratory data.

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	Group (I) HbA1c ≤ 7	Group (II) HbA1c >7	Control Non-diabetic	P-value
N	35	45	20	---
age	35-45: 7; 46-55: 20; 56-65: 8	35-45: 11; 46-55: 16; 56-65: 18	35-45: 5; 46-55: 9; 56-65: 6	---
gender	19 F, 16 M	28 F, 17 M	12 F, 8 M	---
HbA1c %	6.234±0.663 b	8.878±1.598 a	4.787 ± 0.537 c	≤0.01**
FBS (mmol/l)	11.74±2.241 b	14.43±2.772 a	5.47±1.450 c	≤0.01**
Vitamin D (ng/ml)	18.27±2.94 a	16.39±2.93 a	19.75±3.59 a	0.221
RBC (million cell/µl)	4.931±0.624 a	4.888±0.4101 a	4.836±0.3452 a	0.782
Hemoglobin (gm/dl)	12.669 ±2.165 b	12.913±1.714 ab	13.995±1.887 a	≤ 0.05*
PCV %	40.860±6.370 a	40.796±4.121 a	41.105±4.166 a	0.974
WBC (Cell/Lx10^9)	7.460±2.109 a	7.789±2.811 a	6.860±1.544 a	0.348
Platelet (Plt/ml^{3}x10^3)	205.70±9.40 a	196.30±6.14 a	196.35±5.04 a	0.836
Cholesterol (mmol/l)	4.303±1.215 b	4.891±1.892 a	3.959±1.076 c	≤0.05*
Triglyceride (mmol/l)	2.268±0.992 a	2.376±1.031 a	1.557±0.825 b	≤0.01*
HDL (mmol/l)	1.0629±0.2390 b	1.0244±0.2442 b	1.2250±0.4940 a	≤0.05*
LDL (mmol/l)	2.288±1.022 ab	2.374±0.892 a	2.110±0.695 b	0.063
VLDL (mmol/l)	1.0326±0.4820 a	1.0684±0.5051 a	0.6910±0.3771 b	≤0.01**
CRP (mg/L)	8.72±1.58 b	16.94±1.90 a	6.06±1.75 b	≤0.01**
Different letters indicate a significant difference (a b c) - Similar letters indicate no significant differences -				

Table 2: Correlations between studied parameters in Group (II)

parameters	parameters	**r**	**p.value**
HbA1c	FBS	0.506	0.000
	CRP	0.448	0.002
V.D	LYM	-0.370	0.012
	MONO	-0.458	0.002
	PLT	-0.393	0.008
	CHO	-0.307	0.040
	B.UREA	0.380	0.010
	CRP	-0.295	0.049
WBC	LYM	0.441	0.002
	MONO	0.354	0.017
	GRAN	0.959	0.000
	Hb	-0.359	0.007
	CRP	0.295	0.035
	V.D	-0.370	0.012

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LYM	WBC	0.441	0.002
	MONO	0.673	0.000
	PLT	0.382	0.010
MONO	V.D	-0.458	0.002
	WBC	0.354	0.017
	LYM	0.673	0.000
	PLT	0.375	0.011
	T.G	0.315	0.035
	VLDL	0.304	0.042
	CRP	0.319	0.032
GRAN	WBC	0.959	0.000
	Hb	-0.433	0.003
RBC	Hb	0.316	0.034
	PCV	0.627	0.000
Hb	WBC	-0.359	0.007
	GRAN	-0.433	0.003
	RBC	0.316	0.034
	PCV	0.785	0.000
	CREA	0.300	0.045
PCV	RBC	0.627	0.000
	Hb	0.785	0.000
PLT	V.D	-0.393	0.008
	LYM	0.382	0.010
	MONO	0.375	0.011
CHO	V.D	-0.307	0.040
	HDL	0.388	0.009
	LDL	0.433	0.003
TG	MONO	0.315	0.035
	VLDL	0.998	0.000
HDL	CHO	0.388	0.009
	CREA	0.312	0.037
LDL	CHO	0.433	0.003
VLDL	MONO	0.304	0.042
	T.G	0.998	0.000
B.UREA	V.D	0.380	0.010
	CREA	0.322	0.031
CREA	Hb	0.300	0.045
	HDL	0.312	0.037
	B.UREA	0.322	0.031

DISCUSSION:

The study agreed with what was found [10] [11] those who indicated a significant increase in glycated hemoglobin in patients with uncontrolled diabetes compared with the controlled diabetes. The reason for this is due to the fact that hemoglobin (A1c) is formed from the linkage of glucose with various amino groups, including valine and lysine for each of the (alpha) chain. And the two chains (Beta) of normal adult hemoglobin A (hemoglobin A), where the rate of formation of HbA1c is directly proportional to the concentration of blood glucose.

there are nearly a billion people around the world classified as having insufficient levels of vitamin D. The deficiency in our vitamin D level is due to environmental conditions where temperatures are high most of the time. People stay indoors and exposure to sunlight is reduced, in addition to the nature of nutrition in Iraq, as most canned foodstuffs such as milk and yogurt are not sufficiently fortified with vitamin D, in addition to the fact that seafood rich in vitamin D is very few, which is a cause for concern because of its association with many Chronic diseases and negative health outcomes [12] [13]. a study concluded that seasonal differences

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have an impact on cardiovascular mortality, as deaths increased in the winter season and the reason is attributed to a decrease in ultraviolet (UV-B) rays, which is believed to affect the production of vitamin D [14]

In a study [15] conducted in 2020 to investigate the correlation between vit D status, lipid profile, and CRP in gestational diabetes women, it was found that high levels of vit D were associated with low lipid profile (Cho, TG, HDL, LDL, VLDL). And the results of our study were consistent with this study, where it found an inverse relationship between the levels of vitamin D, lipid profile, and C-reactive protein. Studies show that dyslipidemia is often associated with elevated levels of inflammatory markers (βTNF, IL-6, and hs-CRP) and is closely associated with metabolic diseases including obesity, type 2 diabetes, and cardiovascular disease [16], In the study [17], which was conducted on patients with T2D in 2016, it was found that vitamin D was inversely associated with C-reactive protein (CRP), and the association was significant ($p = < 0.05$), and our results agreed ($r = -0.295$) and ($p < 0.05$) with this study as well.

Vitamin D supplementation has been found to reduce HbA1c and FBG in patients with vitamin D deficiency [18]. A significant decrease in HbA1c has also been observed with vitamin D supplementation in patients with type 2 diabetes. In addition, researcher Ali found an improvement in the level of HbA1c when vitamin D supplements were taken by diabetic patients, and it had positive effects on HbA1c by reducing insulin resistance [19]. Some studies also showed that supplementation with this vitamin significantly reduced LDL [20].

Vitamin D helps protect against CVD by reducing systemic inflammation including inflammatory C-reactive protein (CRP), which is an independent predictor of Cardiovascular disease risk [9].

Despite all these plausible mechanisms and evidence from epidemiological studies that support a role for vitamin D in improving blood lipids, some trials do not support these findings [21]. On the other hand, some studies show an improvement in blood lipids in proportion to vitamin D levels and a reduction in its harmful effects [22], [23].

And study agreed with [24] Who indicated that there are significant differences in the rate of hemoglobin in the blood between diabetic patients and non, this is due to the high percentage of inflammatory factors, such as (IL-3, IL-11, and IL-6) caused by type 2 diabetes, which can reduce Synthesis of erythropoietin or its ineffectiveness, which leads to poor production of hemoglobin and red blood cells.

These results agreed with previous study [25] who indicated that the high level of cholesterol is associated with a high level of glucose, obesity and high blood pressure, and this rise is due to several factors, including heredity and the pattern of nutrition used, as it was noticed that patients did not adhere to the diet in the group of non-dominant patients, which leads to an increase in fat levels and the development of atherosclerosis in the future, Several researches revealed that insulin affects the production of apolipoprotein in the liver, and controls the enzymatic activity of the lipoprotein lipase and the cholesterol ester transport protein, which causes dyslipidemia. In addition, insulin deficiency reduces the activity of the lipase enzyme and slows the steps of producing the active lipoproteinlipase enzyme. Biologically, this explains the cause of high cholesterol in patients with diabetes who are dominant [26] [27].

The results came same with the findings of [28], where they indicated an increase in the level of triglycerides (T.G) in diabetic patients, due to the enzyme lipoprotein lipase analyzes the triglycerides found in VLDL and the chylomicron, as the latter contains large amounts of triglycerides, which produce fatty acids that are taken up by the tissues of the body and oxidized in the tissues either for storage in the form of triglycerides or for energy, in addition to the failure of many patients to adhere to a correct diet with a low percentage of fats and sugars.

These results are consistent with the findings of the research [29], who indicated a decrease in high-density lipoproteins in patients with diabetes, and this decrease came as a result of changes in liver functions, which caused inhibition In the production of (Apo-A1), which is a major protein involved in the synthesis of HDL-Cho, and thus decreased the concentration of HDL-Cho (which has an important role in transporting cholesterol from the various cells of the body to the liver) and this poses a risk and predisposes to atherosclerosis.

In diabetic patients, a dysfunction occurs in the hepatic cell receptor Apo B-l00, which plays an important role in transporting of LDL-C fatty compound into the liver cells, so it becomes unable to transport it into the liver, which increases its undesirable concentrations in the blood serum. This is what was reached [30] [31]. Also, the high level of glucose in the blood leads to the association of glucose molecules with the amino groups of LDL by the process of

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glycosylation.

The results of the study agreed with the findings of [25] [32], where the strong relationship between inflammation and blood sugar control in diabetic patients was indicated. The second type, and it was found that inflammation plays an important role in causing diabetes and the development of its complications [33]. The reason for the increase in patients who do not control the disease is attributed to the fact that more than half of the people included in the study were suffering from chronic diseases such as heart disease, pressure and blood vessels.

CONCLUSION: There is a close, inverse association between vitamin D levels and inflammatory variable specially CRP, vitamin D is inversely related to glycated hemoglobin. In addition to controlling diabetes, blood lipid levels must be controlled.

RECOMMENDATIONS: Blood glucose, lipid status and CRP should be controlled to prevent or slow diabetic complications progression.

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