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Study of Some Biochemical Parameters in Patients with Coronary Artery Disease with and without Type 2 Diabetes

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Muntaha A. Arif [*1,2] **Manal K. Rasheed** [1] **Ammar A. Ismaeel** [2]
[1] Department of Clinical Biochemistry, College of Medicine, Baghdad University, Baghdad, Iraq.
[2] The Iraqi Center for Heart Disease, Medical City, MOH, Baghdad, Iraq.

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Abstract

Background: Impaired lipid metabolism and vitamin D deficiency are well-recognized risk factors for coronary artery disease (CAD) which is considered the major reason of mortality and morbidity in both high and low-income countries. The incidence of coronary artery disease is 2-8 folds higher in Type 2 diabetes patients than those without Type 2 diabetes.

Objective: The current study aimed to study some biochemical variables (vitamin D3, lipid profile, Insulin, Fasting blood glucose and glycated hemoglobin HbA1c) in patients with diabetes and coronary artery disease and compare them with healthy people.

Subjects and Methods: A case- control study included 190 male subjects divided into four groups: including 40 healthy controls, 40 diabetic patients, 55 patients with coronary artery disease and 55 patients with Type 2 diabetes and coronary artery disease were recruited from the catheterization unit in cardiologic clinics of Iraqi Center for Heart Disease and specialized center for endocrinology and diabetes, Baghdad Teaching Hospital/ Medical City, during the period from February 2022 to November 2022. Physicians diagnosed them, and they were evaluated by physical and full medical history. All the sample set parameters were measured in the fasting state for each group .The biochemical testes included fasting blood glucose, lipid profile measured by Colorimetric method, while vitamin D3, Insulin, glycated hemoglobin HbA1c measured by enzyme linked immunosorbent assay (ELISA). Various statistical analyses were applied to analyze the research data. The statistical analysis was performed using Student t-test was used for calculating the probability using the statistical analysis program (PAST version 3.09, 2004).Two-way ANOVA and Least significant differences (LSD) post hoc test were performed as well as paired t-test. P< 0.05 was considered statistically significant.

Results: About 65.3% of study subjects had Vit.D insufficiency and 16.8% had Vit.D deficiency. A statistical significant difference was found in mean (± SD) level of BMI, insulin level, vitamin D, HOMA-IR, Fasting blood glucose and HbA1c between the 4 groups. For lipid profile, Control group participants had significantly lower total cholesterol level in comparison to the other groups. Control group participants had significantly lower triglyceride levels in comparison to the other groups. Control group participants had significantly higher high-density lipoprotein (HDL) in comparison in coronary artery disease group and coronary artery disease with Type 2 diabetes group. Control group participants had significantly lower low-density lipoprotein (LDL) in comparison in coronary artery disease group and coronary artery disease with Type 2 diabetes group.

Conclusions: Depending on the results of the current study, it was found that there is a strong association between low vitamin D and coronary artery disease, and it was found that insulin resistance, which mainly causes type 2diabetes, has increased health problems in coronary artery disease patients.

Keywords: Coronary Heart Disease; Diabetes Mellitus; Insulin; Lipid Profile; Vitamin D.

Introduction

Vitamin D is the only vitamin that the body can synthesize; it is often referred to as a hormone and is produced in the skin when exposed to ultraviolet B radiation from the sun (1). Chronic hyperglycemia and problems of the metabolism of carbohydrates, fats, and proteins as a result of defects in insulin secretion, insulin action, or both are characteristics of diabetes mellitus (DM), one of the chronic metabolic disorders that is marked by hyperglycemia (2–3). The danger of T2DM is significantly reduced by vitamin D, a benefit that is probably mediated by the vitamin's effects on beta cell activity, insulin sensitivity, and inflammation throughout the body (4-6). Its development appears to be influenced by a number of variables, including genetic, dietary, environmental, and lifestyle factors. Vitamin D is

**Corresponding Author: Muntaha A. Arif*
montaha.abdulmajeed1109f@comed.uobaghdad.edu.iq

anticipated to play a significant role among nutritional variables, either in glycemic management or in reducing diabetic complications (7). The vitamin D receptor (VDR), a potential gene for T2DM (1, 8), is how vitamin D functions. Independent of body mass, vitamin D deficiency is linked to increased fat infiltration in skeletal muscle, which is thought to cause systemic corresponding Author: inflammation, which is known to play a significant role in the etiology of T2DM (9), and may potentially be reduced by vitamin D. Finally, calcium is required for both the processes of insulin secretion and sensitivity (10). Due to changes in the amount of calcium and flow via the cell membranes within the pancreas and insulin-responsive tissues, vitamin D may consequently indirectly affect both pathways (11). The rationale for conducting the study summarized in diabetes mellitus increases the risk of CAD through increasing atherosclerosis and adversely affects the lipid profile and promotes the formation of atherosclerotic plaque in the coronary artery vessels and Vitamin D deficiency has a link with an increased risk of coronary artery disease. Hence, it has been previously shown that CAD is more fatal and severe in diabetic patients as compared to CAD in patients without diabetes disease. This study aims to correlate the Vit.D levels and other biochemical variables in prediction and early identification in patients with coronary artery disease with and without Type 2 diabetes and compare them with healthy people in Iraqi patients. To examine the utility of serum vitamin D as a predictor coronary artery disease.

Subjects, Materials, and Methods

This case-control study was carried out at the Department of biochemistry, College of Medicine, University of Baghdad, during the period from February 2022 to November 2022. It included one hundred ninety male participants, aged between (41-75 years), body mass index (22 - 37 Kg/m^2), Subjects were divided into four groups: including 40 healthy controls, 40 diabetic patients, 55 patients with CAD and 55 patients had DM and CAD. The phenotype analysis included the measurements of serum insulin, vitamin D, HOMA-IR, HbA1c, fasting blood sugar and lipid profiles. All the sample et parameters were measured in the fasting state for each group (ELISA and Colorimetric method). The formula for calculating insulin resistance is HOMA-IR (homeostatic model assessment for insulin resistance) = Glucose X insulin / 405 (glucose in mg/dl). The continuous variable data were expressed as mean (±SD),where the statistical analysis was performed using Student t-test was used for calculating the probability using the statistical analysis program (PAST version 3.09, 2004).The ANOVA test for difference among more than two independent means.

In all statistical analysis, the level of significance was P-value ≤ 0.05.

Sample Collection: After a 12-hour overnight fast, patients and healthy controls had their blood drawn. Peripheral vein blood sampling of (5ml) at 08:00 - 10:00 AM was done using disposable syringes.

Collected samples were divided into two disposable plastic tubes, for all healthy controls and patients.

Statistical Analysis: The statistical analysis of the data was performed using the computer programs SPSS version 21 and GraphPad Prism version 8. Bar graphs and the results of statistical tests were expressed as Mean±SE. The means of between the patient and healthy groups were compared using the unpaired of t-test (Man-Whitney U-test).

Results:

Demographic data: The current study included 190 male participants divided into 4 groups, including 40-health control, 40 diabetic patients, 55 patients with CAD and 55 patients had DM and CAD, as presented in figure 1.

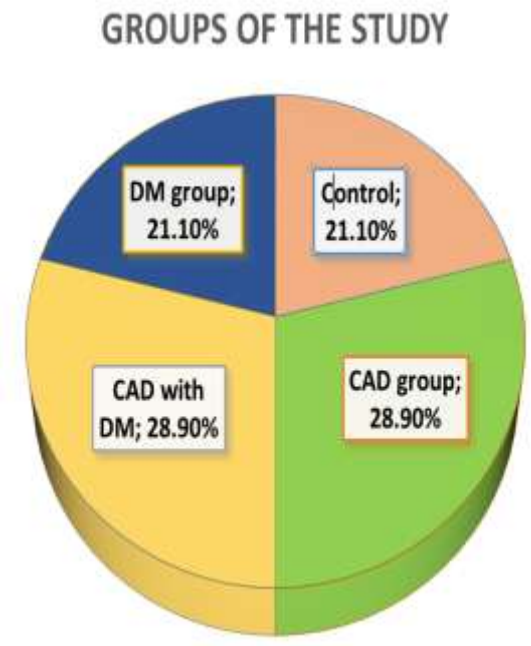

Figure 1: Proportion of studies groups.

Subgroups of BMI, Vitamin D, glycemic control and insulin sensitivity in all participants. Evaluating the BMI of participants revealed that 16.3% had normal BMI, 55.8% were overweight and 27.9% were obese. 65.3% had vitamin D. insufficiency and 16.8% had vitamin D deficiency. The glycemic control evaluation showed that 37.4% had good glycemic control and 16.8% had poor control and 45.8% had excellent control. All participants had insulin resistance in which 31.6% had early resistance, as presented in table 1.

Table (1): Numbers and percentages of BMI, Vitamin D, Glycemic control and Insulin sensitivity in all participants

		No.	%
BMI group Kg/m2	normal BMI	31	16.3
	overweight BMI	106	55.8
	Obese	53	27.9
Vitamin D state ng/ml	deficiency	32	16.8
	insufficiency	124	65.3
	Normal level	32	16.8
Glycemic control	excellent	87	45.8
	good	71	37.4
	poor	32	16.8
Insulin sensitivity	early resistance	60	31.6
	insulin resistance	130	68.4
	Total	190	100.0

Mean differences of studied biomarkers between the 4 groups a statistical significant differences in mean levels of BMI, vitamin D, insulin level, HOMA-IR, FBS and HbA1c among the 4 groups, as presented in table 2.

Table 2: Mean levels of BMI, vitamin D, insulin level, HOMA-IR, FBS and HbA1c among the 4 groups

Marker	Group	Mean ± SD	SE	*P*-value
BMI Kg/m2	Control [a, b]	25.82 ± 1.74	0.30	< 0.0001*
	CAD group [a, e]	29.88 ± 4.11	0.55	
	CAD with DM [b, f]	29.16 ± 3.23	0.43	
	DM group [e, f]	26.97 ±1.94	0.30	
Vitamin D ng/ml	Control [a, b, c]	37.02 ± 6.97	1.10	< 0.0001*
	CAD group [a, e]	15.47 ± 5.68	0.76	
	CAD with DM [b]	12.77 ± 5.78	0.77	
	DM group [c, e]	21.86 ± 3.73	0.59	
Insulin µIU/ml	control [a, b, c]	9.18 ± 1.70	0.26	< 0.0001*
	CAD group [a, d, e]	15.88 ± 5.91	0.79	
	CAD with DM [b, d, f]	32.76 ± 9.39	1.26	
	DM group [c, e, f]	22.77 ± 3.35	0.53	
HOMA-IR	control [b, c]	2.16 ± 0.43	0.06	< 0.0001*
	CAD group [d, e]	3.77 ± 1.55	0.20	
	CAD with DM [b, d, f]	15.03 ± 7.05	0.95	
	DM group [c, e, f]	9.15 ± 1.88	0.29	
FBS mg/dl	control [b, c]	97.85 ± 11.10	1.75	< 0.0001*
	CAD group [d, e]	95.09 ± 9.32	1.25	
	CAD with DM [b, d, f]	183.29 ± 46.17	6.22	
	DM group [c, f]	164.20 ± 28.03	4.43	
HbA1c %	control [b, c]	5.17 ± 0.64	0.10	< 0.0001*
	CAD group [d, e]	5.87 ± 6.40	0.86	
	CAD with DM [d]	8.85 ± 2.02	0.27	
	DM group [c, e]	8.05 ± 1.03	0.16	

a: control and CAD group
b: control and CAD with DM group
c: control and DM group
d: CAD group and CAD with DM group
e: CAD group and DM group
f: CAD with DM group and DM group

Lipid profile: Control group participants had significantly lower total cholesterol levels in comparison to the other groups. Control group participants had significantly lower TG levels compared to the other groups. Control group participants had significantly higher HDL in comparison in CAD group and CAD with DM group. Control group participants had significant lower LDL compared to in CAD group and CAD with DM group, as presented in Table 3.

Levels of total cholesterol in all studied group, where Total cholesterol demonstrated significant (P ≤0.0001) elevated in CAD (210.36±32.83mg/dl) and CAD with DM (225.32 ± 26.06 mg/dl) groups compared to control group (146.48 ± 30.39 mg/dl). Levels of Total cholesterol demonstrated significant (P ≤0.05) elevated in DM groups (168.65 ± 17.81 mg/dl) compared to control group.

Control group participant had significant lower TG level in compare to the other groups, *P*<0.001, < 0.001 and 0.002. Patients in CAD group had significant lower TG in compare to patients in CAD with DM group, p-value ≤ 0.001. Patients in CAD with DM group had significant higher mean of TG in compare to patients in DM group, *P*-value ≤ 0.00.

Levels of triglycerides in all studied group, where triglyceride demonstrated significant (*P* ≤0.0001) elevated in CAD (170.72 ± 57.54 mg/dl) and CAD with DM (213.07 ± 70.26 mg/dl) groups compared to control group (103.82 ± 26.61 mg/dl). Levels of triglyceride demonstrated non-significant (P <0.05) differences in DM groups (149.10 ± 54.08 mg/dl) compared to control group.

However, HDL demonstrated significant (P ≤0.0001) reduced in CAD (39.02 ±24.75 mg/dl) and CAD with DM, (31.33 ± 11.52 mg/dl) groups compared to control group (49.37 ± 7.89 mg/dl). Levels of HDL demonstrated non-significant (P ≤0.05) differences in DM groups (41.12 ± 5.94 mg/dl) compared to control group, where LDL demonstrated significant (P ≤0.0001) elevated in CAD (131.91 ± 32.19 mg/dl) and CAD with DM

(151.45 ± 24.02 mg/dl) groups compared to control group (81.06 ± 34.77 mg/dl). Levels of LDL demonstrated non-significant (P ≤0.05) differences in DM groups (96.72 ± 19.81 mg/dl) compared to control group.

Table 3: Mean differences of lipid profile among the groups

Total cholesterol mg/dl	control [a, b, c]	146.48 ± 30.39	4.80	< 0.0001*
	CAD group [a, d, e]	210.36 ± 32.83	4.42	
	CAD with DM [b, d, f]	225.32 ± 26.06	3.51	
	DM group [c, e, f]	168.65 ± 17.81	2.81	
TG mg/dl	control [a, b, c]	103.82 ± 26.61	4.20	< 0.0001*
	CAD group [a, d]	170.72 ± 57.54	7.76	
	CAD with DM [b, d, f]	213.07 ± 70.26	9.47	
	DM group [c, e, f]	149.10 ± 54.08	8.55	
HDL mg/dl	control [a, b]	49.37 ± 7.89	1.24	0.003
	CAD group [a, d]	39.02 ±24.75	3.33	
	CAD with DM [b, d, f]	31.33 ± 11.52	1.55	
	DM group [c, f]	41.12 ± 5.94	0.93	
LDL mg/dl	control [a, b]	81.06 ± 34.77	5.49	< 0.0001*
	CAD group [a, d, e]	131.91 ± 32.19	4.34	
	CAD with DM [b, d, f]	151.45 ± 24.02	3.23	
	DM group [e, f]	96.72 ± 19.81	3.13	
VLDL mg/dl	control [a, b, c]	21.28 ± 5.37	0.84	< 0.0001*
	CAD group [a, d]	34.93 ± 13.52	1.82	
	CAD with DM [b, d, f]	42.63 ± 14.42	1.94	
	DM group [c, f]	29.96 ± 11.03	1.74	

a: control and CAD group **d: CAD group and CAD with DM group**
b: control and CAD with DM group **e: CAD group and DM group**
c: control and DM group **f: CAD with DM group and DM group**

ϖ **Some data have high SD because the sample was chosen randomly**

Discussion

Numerous authors have looked into vitamin D levels as a cardiovascular risk factor in recent years (15). However, conflicting information has been found about the relationship between vitamin D levels and some pathologies, such as coronary artery disorders (CAD) (16). In the current study, there were 190 male participants; 40 were healthy controls, and the rest were either CAD patients, CAD plus DM patients, or diabetic only patients. The average participant age was (56.06±7.22years), and those between the ages of 51 and 60 made up the largest percentage. Age was on average lower in the control group than in the other groups and greater in the CAD with DM group. Similar to current data,The American Heart Association (AHA) reports that the incidence of CVD in men and women is ~40% from 40–59 years, ~75% from 60–79 years, and ~86% in those above the age of 80 (17). This confirms the results of the current study in that the increase in the incidence of cardiovascular disease increases with age. The prevalence of coronary heart disease increased dramatically as people aged, particularly the intima of artery walls, which expanded as people aged. More than half of participants (55.8%) were overweight and 27.9% were obese which was near the proportion reported in previous studies carried in Baghdad (66.9% overweight or obesity) (18), in Kuwait (37% overweight and 40.3% obesity) (19), Jordan (>75% overweight or obesity) (20), and Iran (59.3%) (21). Differences between studies could be related to socio-cultural differences and eating habits differences between the communities. The prevalence of vitamin D insufficiency was found in 65.3% of participants and 16.8% had vitamin D deficiency. The prevalence of vitamin D deficiency was lower than reported in previous studies 37.8% in Duhok, 24.1% in Erbil (22), 76.1% in KSA (23), 41.6% in US (24), the disparity can be attributed to the fact that the current study only included men, in contrast to all comparable studies that included both sexes. This is because after menopause, women were more likely to experience vitamin D deficiency. BMI included participants who were statistically different among the groups, being lower in the control group and higher in CAD and CAD with DM groups. Similar findings were observed in Eweida et al. (13) study that had the same current study design. In a previous study which evaluated risk factors in symptomatic CAD patients, those with BMI > 25 had higher prevalence of DM compared to patients with BMI < 25 (24.5% vs. 13.6%, respectively) (25). Lowest mean (± SD) of vitamin D was reported in CAD with DM followed by CAD group, similar to Eweida et al. (13) study finding. In previous studies carried out by Dziedzic EA to assess vitamin D in diabetic and non-diabetic CAD patients in 2017 and 2019, those who presented with stable CAD (diabetic or non-diabetic) had significantly higher mean of vitamin D compared to those who presented with ACS. Additionally, the study observed a decrease in mean of vitamin D level as severity of CAD increase (26). Both serum insulin and HOMA-IR were higher in CAD with DM group of patients compared to others, similar to Srinivasan et al. (27) study, diabetic patients who had major adverse cardiac event (MACE) as ACS had significant

higher insulin level compared to patients without MACE (25.09 ± 7.2, 18.52 ± 6.08, p-value <0.001). An increase of 1% in HbA1c is associated with a 2.8-fold increase in CAD and in the severity of coronary artery lesions; it is noteworthy that even HbA1c value in the normal range is associated with the presence and severity of CAD (28). Similar to Bhutto et al (29) study, those with CAD with DM had lower HDL and higher lipid profile markers (total cholesterol, TG, LDL, and VLDL) compared to other groups. The mean of these markers in CAD without DM was likewise lower than that in CAD with DM but above the normal upper limit. A lack of vitamin D impairs the activity of the pancreatic beta cells, which leads to insulin resistance, abnormalities in the metabolism of lipoproteins, and eventually higher TG and lower HDL-C levels. The management of the risk of dyslipidemia in T2DM is a key component of CAD prevention. These results revealed that elevated atherogenic lipoproteins are simply predicted by low 25-OH D levels, and that vitamin D administration may help avoid cardiovascular disease.

Limitation: The small sample size was a limitation point in the current study; a larger number is needed to generalize the current finding on the Iraqi population.

Conclusions

Based on the results of the current study, it was found that there is a strong association between low vitamin D and CAD, and it was found that insulin resistance, which mainly causes type 2 diabetes, has increased health problems in CAD patients. On the other hand, current study demonstrated that high levels of LDL and cholesterol were closely associated with events and raised the risk of CAD.

Recommendations:

- Future studies need to increase the number of patients to ensure better results with larger samples size in order for the results to be more accurate for the Iraqi people.
- Maintaining an ideal weight to avoid insulin resistance and thus type two diabetes and an increased risk of coronary artery disease.
- Hyperlipidemia should be treated aggressively.
- Smoking should be avoided and discourage.
- Intake vitamin D as supplement for new cases of coronary artery disease. In addition, it should be recommended in the routine screening of vitamin D status for patients with type 2 diabetes mellitus and healthy controls.

Authors' declaration

Conflicts of Interest: The authors declare no conflict of interest.

We confirm that all the Figures and Tables in the manuscript belong to the current study. Besides, the Figures and images, which do not belong to the current study, have been given permission for re-publication attached to the manuscript. Authors sign on ethical consideration's approval-Ethical Clearance: The project was approved by the local ethical committee inapproved by the local ethical committee Iraqi Ministry of Health, - Medical City / National Center for Educational Laboratories. Medical City / Iraqi Center for Heart Disease according to the code number (ISU.134 –Date 23.7.2023).

Author Contributions

Study conception & design: (Dr.Muntaha Abdulmajeed Arif). Literature search: (Prof.Dr. Manal Kamal Rasheed). Data acquisition: (Dr.Muntaha Abdulmajeed Arif & Dr.Ammar Adil Ismaeel). Data analysis & interpretation: (Dr.Muntaha Abdulmajeed Arif & Prof.Dr. Manal Kamal Rasheed). Manuscript preparation: (Dr.Muntaha Abdulmajeed Arif). Manuscript editing & review: (Dr.Muntaha Abdulmajeed Arif, Prof.Dr. Manal Kamal Rasheed).

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دراسة بعض المتغيرات البيوكيميائية في مرضى الشريان التاجي المصابين والغير مصابين بداء السكري من النوع الثاني

د. منتهى عبد المجيد عارف / جامعة بغداد، كلية الطب، دكتوراه-كيمياء الحياتية السريرية، بغداد، العراق
أ.م.د. منال كمال رشيد/ قسم الكيمياء الحياتية السريرية، كلية الطب، جامعة بغداد، بغداد، العراق
د. عمار عادل أسماعيل / وزارة الصحة، دائرة مدينة الطب -المركز العراقي لأمراض القلب، بغداد، العراق

الخلاصة
الخلفية: يعد ضعف التمثيل الغذائي للدهون ونقص فيتامين (د) من العوامل المعروفة لمرض الشريان التاجي (**CAD**) والتي تعتبر السبب الرئيسي للوفيات والمراضة في كل من البلدان المرتفعة والمنخفضة الدخل. معدل حدوث مرض الشريان التاجي هو 2-8 أضعاف في مرضى داء السكري النوع الثاني من أولئك الغير مصابين بداء السكر النوع الثاني
الهدف: تهدف الدراسة الحالية إلى دراسة بعض المتغيرات البيوكيميائية (فيتامين **D3**، الدهون، الأنسولين والسكر التراكمي) في مرضى السكري وأمراض القلب التاجية ومقارنتها مع الأشخاص الأصحاء.
الموضوعات والطرق: تعتبر الدراسة إحصائيًا [دراسة حالة وضبط]، حيث تم تقسيم المتطوعين (190 ذكر) إلى أربع مجموعات: بما في ذلك 40 مريضًا أصحاء، و40 مريضًا مصابا بالسكري من النوع الثاني، و55 مريضًا يعاني من أمراض القلب التاجية و55 مريضًا يعانون من مرض القلب التاجي ومصابين بداء السكر النوع الثاني.
النتائج: قيمت النتائج أن 65.3٪ يعانون من نقص فيتامين (د) و16.8٪ يعانون من نقص فيتامين (د). هناك فرق ذو دلالة إحصائية في متوسط مستوى مؤشر كتلة الجسم وفيتامين (د) ومستوى الأنسولين ومقاومة الأنسولين ونسبة السكر الصائم بالدم والسكر التراكمي بين المجموعات الأربعة. بالنسبة لنتائج الدهون، كان لدى المشاركين في مجموعة الاصحاء مستوى منخفض من الكوليسترول الكلي مقارنة بالمجموعات الأخرى. كان لدى المشاركين الاصحاء مستوى الدهون الثلاثية أقل بكثير مقارنة بالمجموعات الأخرى. وكان لدى المشاركين في مجموعة الاصحاء الدهون العالية الكثافة أعلى بكثير مقارنة بمجموعة مرضى انسداد الشرايين التاجية للقلب المصابين والغير مصابين بداء السكري النوع الثاني. وكان لديهم مستوى الدهون المنخفضة الكثافة أقل بكثير مقارنة بمجموعة مرضى انسداد الشرايين التاجية للقلب المصابين والغير مصابين بداء السكري النوع الثاني.
الاستنتاجات: اعتمادا على نتائج الدراسة الحالية، وجد أن هناك ارتباطًا قويًا بين انخفاض فيتامين (د) وأمراض القلب والأوعية الدموية، كما وجد أيضًا أن مقاومة الأنسولين، والتي تسبب بشكل رئيسي مرض السكري من النوع 2، قد زادت من المشاكل الصحية في أمراض القلب والأوعية الدموية. من ناحية أخرى، أظهرت الدراسة الحالية أن المستويات العالية من الدهون المنخفضة الكثافة **LDL** والكوليسترول كانت مرتبطة ارتباطًا وثيقًا بالأحداث وزيادة خطر الإصابة بأمراض القلب التاجية.
الكلمات المفتاحية: أمراض القلب التاجية، السكري، الدهون، فيتامين (د) ، الأنسولين.