in vivo *36*: 2481-2489 (2022)
doi:10.21873/invivo.12984

Relation of Hypoxia Inducible Factor, Dyslipidemia and CAD Saudi Patients With Type 2 Diabetes

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Abstract. *Background/Aim: Type 2 diabetes (T2DM) is a metabolic condition associated with an increased risk of death, morbidity, and vascular problems. This study investigated the association of HIF-1α and dyslipidemia with the incidence of coronary artery disease in Saudi patients with T2DM. Patients and Methods: This study included 100 Saudi patients aged 40-60 years who were attending King Abdulaziz Specialist Hospital in Taif, as well as 50 healthy controls. All were divided into three groups of 50 subjects each: control, patients with T2DM, and patients with T2DM with coronary complications. Serum levels of HIF-1α, fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c %), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were estimated. Results: Serum HIF-1α, FBG, HbA1c %, TC, TG, and LDL-C levels were significantly increased in both groups of patients with diabetes (p<0.001) relative to the control group. Among patient groups, their levels were significantly increased in patients with coronary complications as compared to patients with diabetes (p<0.001). Serum HDL-C levels in both groups of patients with diabetes were significantly lower (p<0.001) than those in the control group. When HDL-C levels were compared between the two patient groups, its levels in patients with diabetes with coronary complications were significantly lower (p<0.001). Significant positive correlations were observed between serum HIF-1α and each of FBG, HbA1c %, TC, TG, and LDL-C levels, whereas negative correlations were observed with HDL-C in both groups of patients with diabetes. Conclusion: Increased serum HIF-1α levels are linked to dyslipidaemia in Saudi patients with T2DM, particularly those with coronary artery disease.*

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Key Words: Lipid profiles, diabetes mellitus, dyslipidemia, coronary artery diseases.

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder caused by the pancreas' failure to produce sufficient insulin or the body's inability to use the insulin it produces properly. Its prevalence has increased dramatically in developing countries. The number of people with T2DM is projected to more than double by 2030, reaching 366 million people worldwide (1). The prevalence of diabetes in Saudi Arabia is the second highest in all of the Middle Eastern countries and ranks seventh worldwide (2).

T2DM is a multifactorial disease involving genetic and environmental factors (3). T2DM's macro-vascular complications include coronary artery disease, cardiomyopathy, arrhythmias, and cerebrovascular and peripheral artery diseases. Coronary artery disease (CAD) is a disorder in which the heart muscle does not receive enough blood and oxygen. It is caused by blockage of the coronary arteries, which leads to a mismatch of supply and demand for oxygen. The most common symptom is the growth of plaques in the lumen of the coronary arteries, which obstruct blood flow (4).

Multiple risk factors are linked to the acceleration of atherosclerosis, which can lead to cardiovascular disease, including insulin resistance, hyperinsulinemia, dyslipidaemia (5), hypertension, endothelial dysfunction, visceral obesity, and non-alcoholic fatty liver disease (3).

For all living cell entities, oxygen homeostasis is critical. Hypoxia is a condition in which the oxygen demand exceeds the oxygen supply. Complex adaptive systems have evolved to help cells survive under hypoxic conditions. Transcription factors, such as the hypoxia-inducible factor (HIF), play a vital role in maintaining cellular oxygen homeostasis by directly activating hundreds of target genes (6). HIFs-induced adaptive responses to hypoxia are protective in

many diseases but can be detrimental, such as when they promote cancer progression (7, 8).

The oxygen-dependent prolyl hydroxylase domain (PHD) enzyme family targets HIF-1α for degradation in normoxia by hydroxylating proline residues. During myocardial ischemia, the PHDs are inhibited, HIF1α is stabilized and then translocated to the nucleus, where it dimerises with HIF-1β and binds to conserved hypoxia response element (HRE) to induce transcription of HIF target genes (9).

The tissue or organism can survive hypoxia because HIF controls hundreds of genes involved in metabolism, cell proliferation, angiogenesis, and erythropoiesis (10). It is critical for the heart to have optimal HIF activation in order to keep its contractile function after myocardial infarction (MI) and pressure-overload hypertrophy (11, 12).

The promotion of glycolysis and suppression of fatty acid oxidation is a critical component of HIF activation. It is interesting to note that this HIF-mediated metabolic shift goes in the opposite direction to that caused by diabetes, which decreases glucose metabolism and increases fatty acid oxidation. As a result of this, HIF-1α and diabetes have conflicting impacts on heart metabolism (13).

HIF-1α has been linked to diabetes as a risk factor for cardiovascular disease. All diabetic problems are affected by hypoxia (14). Hyperglycaemia affects the HIF-1α protein's stability and activity, making cells and tissues more vulnerable to hypoxia (15, 16). Diabetes and its consequences can be exacerbated by hypoxia and hyperglycaemia (17).

T2DM is characterized by hyperglycemia and hyperlipidemia in addition to other symptoms (18). Under conditions of insulin-resistance, cardiomyocytes lose their ability to activate the adaptive hypoxic response when HIF-1 stability is impaired by diabetes (19).

The increase in fatty acids in the myocardium has a direct effect on the activation of HIF-1α. Fatty acids decrease the build-up of succinate and fumarate, which are essential for proper HIF stabilization *via* inhibition of PHD enzymes in hypoxic conditions (20). Methylglyoxal, a by-product of hyperglycaemia, triggers HIF-1α instability in a PHD- or VHL-independent way (21), and diabetic hearts are less capable of coping with hypoxia and adapting in the long run (22). Cardiovascular disease is the leading cause of mortality in diabetes, and even with optimal managed risk factors (glucose, blood pressure, cholesterol), people with T2DM still have 21% increased risk of cardiovascular disease (23).

The aim of the current study was to evaluate the association of HIF-1α and dyslipidaemia with the occurrence of CAD in T2DM Saudi patients in Taif city.

Patients and Methods

Study design and population. The present case control research was carried out between October 2021 and April 2022. The research was carried out in female section of Department of Laboratory Sciences, Collage of Applied Medical Sciences, Taif University, Saudi Arabia. A total of 100 Saudi adult patients, aged between 40-60 years, of both sexes, attending King Abdulaziz Specialist Hospital, Taif were recruited for the study as well as 50 healthy controls. All were divided into three groups of 50 subjects each: control, type 2 diabetic, and type 2 coronary diabetic. The following inclusion and exclusion principles were used to categorize all subjects into the studied groups:

Inclusion criteria:

- Controls who appear to be healthy individuals, with no clinical or laboratory indication of T2DM, CAD, or any other illness, matched according to age and sex with the patient groups.

- For diabetic patients: The American Diabetes Association criteria, (24) were used to detect T2DM patients.

- For diabetic coronary patients, the following criteria were used to diagnose CAD: Electrocardiogram (ECG), Echocardiogram using sound waves to produce images of the heart, cardiac catheterization, angiogram, and cardiac computed tomography (CT) scan.

Exclusion criteria:

Individuals with any of the following diseases were excluded from the study: renal diseases, hepatic diseases, chronic illness, malignancy, or any blood diseases. Furthermore, patients with type 1 diabetes, gestational diabetes, and malabsorptive conditions were excluded.

Ethical approval for this work (No 43- 152) was obtained from the ethics committee at Taif University. After explaining the purpose of the study, each participant provided informed consent to participate in the study.

Blood samples. Five ml fasting blood specimens were obtained from all participants under complete sterile conditions, then allocated into two tubes: EDTA-containing tubes for fasting blood glucose and glycosylated hemoglobin (HbA1c %) assessment and ordinary tubes for serum extraction for HIF-1α and lipid profile measurement, which were then stored at –20˚C until use.

Estimation of fasting blood glucose, lipid profile and glycosylated hemoglobin. Fasting blood glucose (FBG), total cholesterol (TC), high density lipoprotein- cholesterol (HDL-C) and triglycerides (TG), levels were analyzed using enzymatic colorimetric techniques according to Trinder (25), Allain *et al*. (26), Lopes-Virella *et al*. (27), and Glick *et al*. (28), respectively utilizing kits purchased from ELITech Group (Puteaux, France). Low density lipoprotein-cholesterol (LDL-C) was estimated based on Friedewald *et al*. (29) as follows:

$$\text{LDL-C (mg/dl)} = \text{TC} - (\text{HDL-C} + \text{TG}/5)$$

Table I. *Clinical and demographic characteristics of the control and diabetic patients' groups.*

Parameters	Control group (n=50)	Diabetic patients (n=50)	Diabetic coronary patients (n=50)
Age (years)	51.9±3.8[c]	49.7±5.7[c]	53.9±3.6[c]
Sex			
Male	24 (48%)	23 (46%)	28 (56%)
Female	26 (52%)	27 (54%)	22(44%)
Body mass index (BMI) (kg/m^2)	21.86±8.5	26.79±7.8a	28.53±8.4a
Fasting blood glucose (mg/dl)	91.77±21.47	165.64±12.55a	236.50±38.72[ab]
Systolic blood pressure (mmHg)	128±14.5	134±16.3[c]	150±27.5[a]
Diastolic blood pressure (mmHg)	85.7±9.5	87.4±8.6[c]	96±14.3[a]

Data are means±Standard Deviation (SD). [a]significant difference (p<0.001) in comparison to control group by Tukey's HSD post-hoc test. [b]significant difference (p<0.001) in comparison between the diabetic groups by Tukey's HSD post-hoc test. [c]non-significant (p>0.005) in comparison to control group by Tukey's HSD post-hoc test.

Table II. *Biochemical characteristics of the control and diabetic patients' groups.*

Parameters	Control group (n=50)	Type 2 diabetic patients		p-Value
		Diabetic patients (n=50)	Diabetic coronary patients (n=50)	
HbA1C %	5.29±1.01	7.63±1.017a	9.76±1.13[ab]	>0.001*
Triglycerides (mg/dl)	128.93±10.97	197.03±23.18[a]	238.98±26.96[ab]	>0.001*
Total cholesterol (mg/dl)	144.49±15.25	226.36±14.84[a]	259.98±36.17[ab]	>0.001*
LDL-C (mg/dl)	58.13±7.64	143.24±9.73[a]	232.27±22.99[ab]	>0.001*
HDL-C (mg/dl)	64.35±7.34	51.48±9.196[a]	41.50±6.72[ab]	>0.001*
HIF-1α (pg/ml)	48.57±12.61	71.39±20.54[a]	96.95±18.58[ab]	>0.001*

Data are means±standard deviation (SD). *Significant p<0.0001 between studied groups by ANOVA. [a]Significant difference p<0.001 from control group by Tukey's HSD post-hoc test. [b]Significant difference p<0.001 in comparison between diabetic groups by Tukey's HSD post-hoc test.

HbA1c was analyzed using the automated glycosylated hemoglobin analyser (Bio-Rad, Hercules, CA, USA).

Estimation of serum hypoxia inducible factor-1 (HIF-1α) by ELISA technique. Highly sensitive human quantitative sandwich ELISA Kit was utilized for the estimation of serum HIF- 1α (MyBioSource, San Diego, CA, USA). The detection range was 31.2-2,000 pg/ml with a sensitivity of <13.9 pg/ml.

Statistical analysis. GraphPad Prism version 8 was used to analyze the data. To assess the statistical significance of different variables, a one-way analysis of variance (ANOVA) was used, followed by a post-hoc test (Tukey's multiple comparison) for group comparison. The Pearson correlation coefficient has been used to evaluate the relationship between HIF-1α with other analyzed variables. Moreover, a receiver operating characteristics (ROC) curve was designed to analyze HIF-1α serum levels in diabetes and diabetic coronary complications, evaluating HIF-1α's ability to identify the severity of CAD. The ROC curve's area under the curve and 95% confidence intervals were computed. p-Values of 0.05 were considered statistically significant.

Results

Table I shows the clinical and demographic characteristics of age, sex distribution, body mass index (BMI), fasting blood glucose (FBG), and systolic and diastolic blood pressure in the studied groups. Our results showed that means±SD of age of the diabetic groups was not significantly varied as compared to control group. Regarding sex distribution, our results demonstrated that in the diabetic group, the male patients were 23 (46%) and females were 27 (54%). In the diabetic coronary group, the percentage of male patients was 28 (56%) and of female was 22 (44%). In comparison to the control group, the means±SD of BMI in all diabetic patients were significantly higher (p<0.001). In contrast, BMI did not significantly vary when comparing diabetic and diabetic coronary groups. In all diabetic patients' groups, when compared to the control group, the

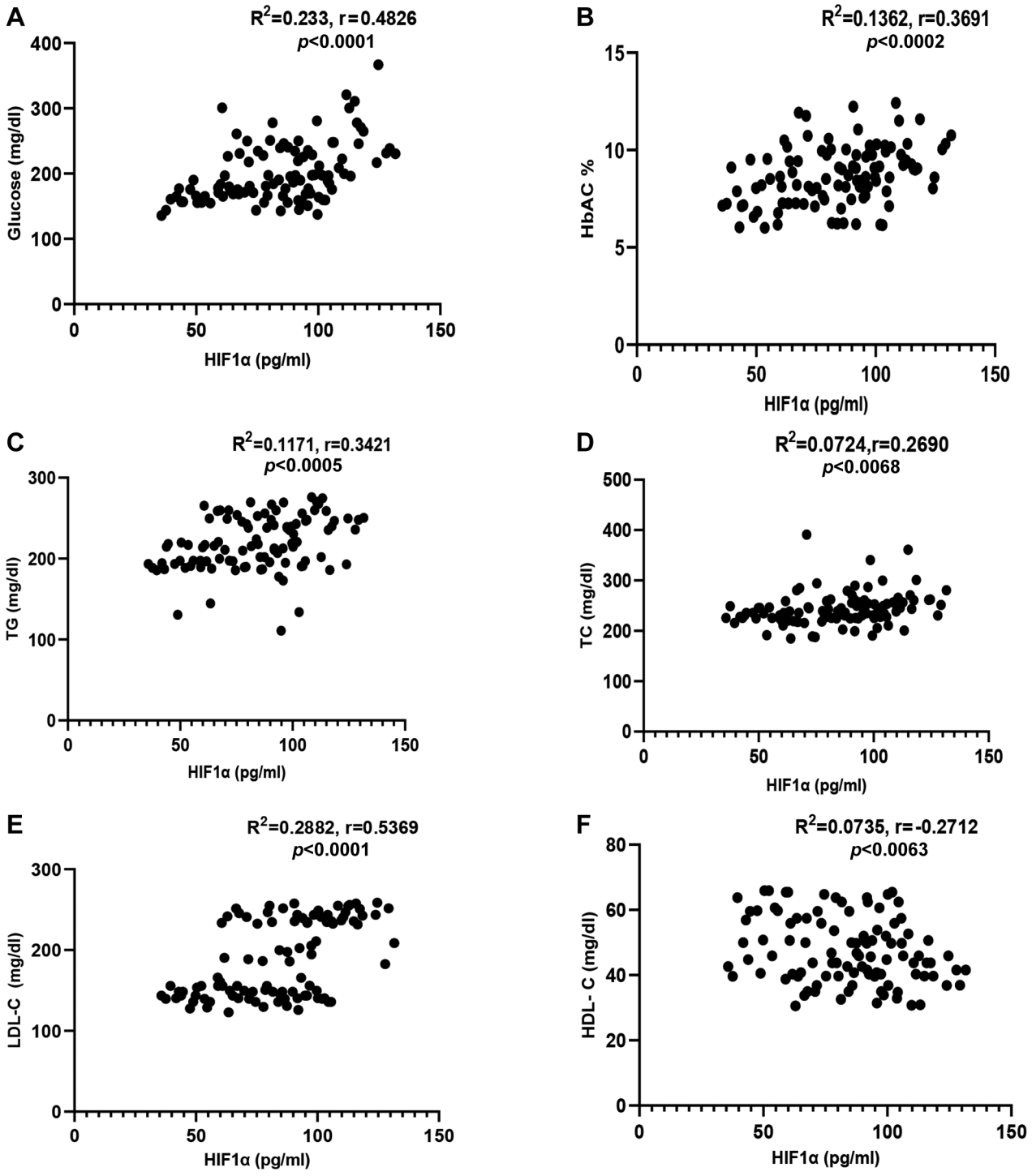

Figure 1. *Pearson's correlation between serum HIF-1α levels and fasting blood glucose levels (A), and glycosylated hemoglobin (HbA1C %) (B), serum triglycerides (TG) (C), total cholesterol (TC) (D), low density lipoprotein total cholesterol (LDL-C) (E), and high-density lipoprotein cholesterol (HDL-C) (F) levels in all diabetic patients.*

means±SD of FBG levels were significantly higher (p<0.001). Furthermore, there were significantly increased FBG levels in coronary diabetic patients compared to diabetics without complications. Regarding blood pressure, in diabetic coronary patients, systolic and diastolic blood pressure was significantly higher (p<0.001) compared to

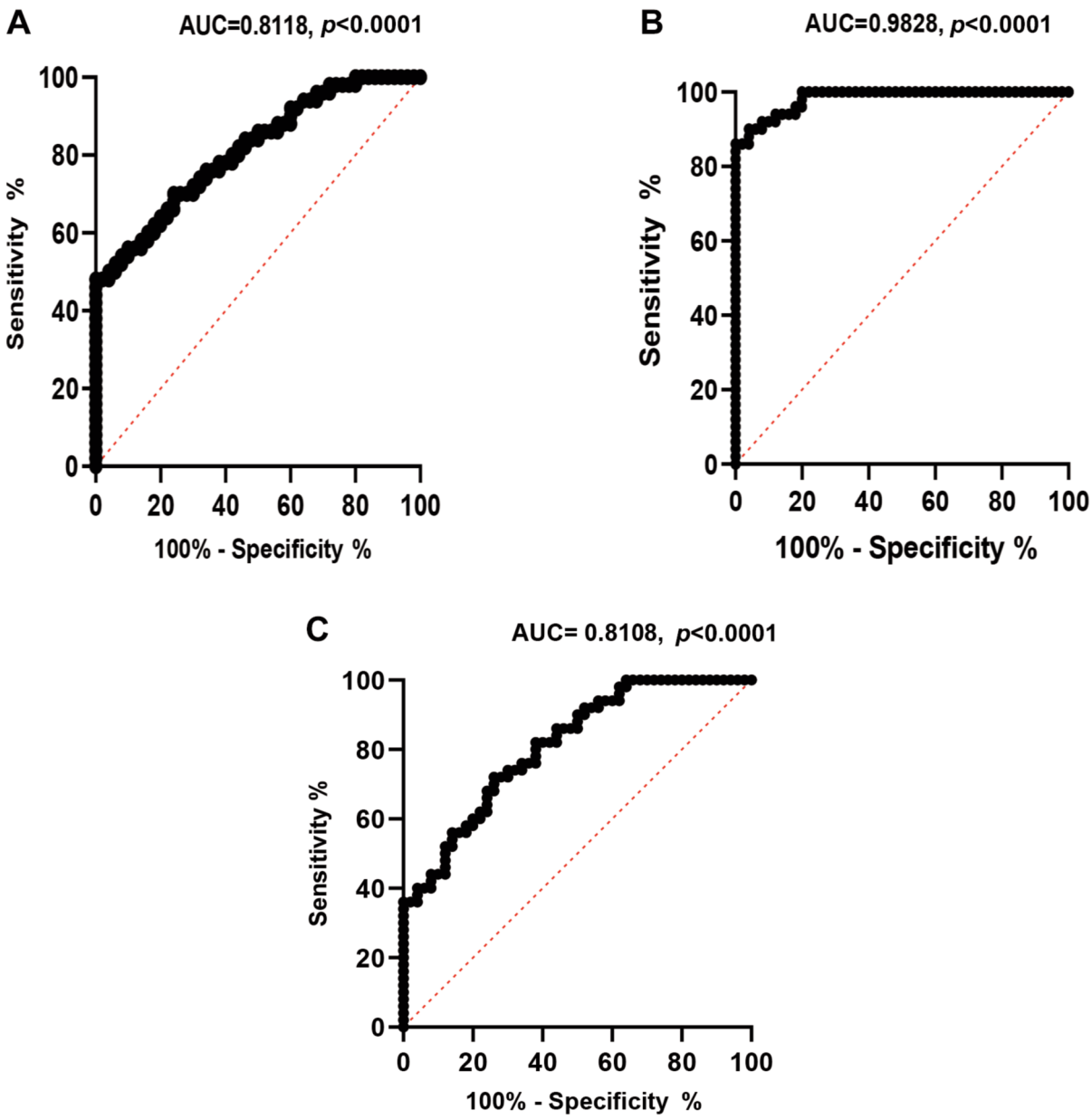

Figure 2. *Receiver operating characteristic (ROC) curve analysis of serum HIF1α in diabetic and diabetic coronary patients versus the controls (A, B) and ROC curve analysis of serum HIF1α in both diabetic and diabetic coronary patients (C).*

controls, but the blood pressure was of non-significant value when comparing the diabetic groups.

Table II shows the levels of biochemical parameters and their significant differences in the control and diabetic patients' groups. Our results showed significantly increased (p>0.001) levels of blood HbA1C %, serum TG, TC, LDL-C, and HIF-1α and significantly decreased (p>0.001) levels of serum HDL-C in both diabetic groups compared to control. In addition, significant differences were observed (p>0.001) when we compared both diabetic groups.

Figure 1 shows the correlation coefficient between HIF-1α and each of serum FBG, HbA1c % and serum lipid profile parameters among all diabetic patients' groups. Significant positive correlations were observed between serum HIF-1α and each of serum FBG, HbA1c %, TC, TG, and LDL-C, whereas negative correlations were obtained with HDL-C in all diabetic patients' groups.

Figure 2 shows the receiver operating characteristic (ROC) curve analysis of serum HIF1α in diabetic and diabetic coronary patients *versus* the controls. The area under

the curve (AUC) of both ROC curves were as follows: 0.8118 and 0.9828 with a sensitivity of 96% and 98%, respectively. AUC of ROC curve analysis of serum HIF1α in both diabetic and diabetic coronary patients was 0.8108 with a sensitivity of 98%.

Discussion

Untreated T2DM can lead to several comorbidities, including micro- and macro-vascular complications. One of the major risk factors for CAD is diabetes mellitus (30, 1). HIFs have a role in the progression of β cell dysfunction and diabetes. HIF-1α protein is destabilized by hyperglycemia, resulting in poor hypoxia responses. Deregulation of HIF-1α is involved in both diabetes-associated macro-vascular and micro-vascular complications. The majority of problems are linked to decreased HIF-1 activity (31).

The pathophysiology of diabetes is multifactorial, and glycemic control is a challenge. In order to improve treatment efficiency and avoid diabetic complications, the possibly modifiable factors influencing glycemic control such as hypertension, obesity, and dyslipidemia must be taken into consideration (32).

The goal of this study was to explore the association between HIF-1α and dyslipidemia with the occurrence of CAD in Saudi patients with type 2 diabetes. This study included three groups: control, non-complicated patients with diabetes, and patients with diabetes with coronary complications.

The current research revealed a substantial increase (p<0.001) in blood HIF-1α levels in both groups of patients with diabetes compared to controls, as well as a significant increase in patients with diabetes with coronary complications in comparison to patients with diabetes. According to recent findings, hypoxia, and improper responses to it caused by dysfunctional HIF-1α signaling appear to be major pathogenic contributors in both tissues central to the development of diabetes (β cells of pancreas) and in tissues prone to diabetes comorbidities such as heart, kidney, nervous and vascular tissues (33).

Previous studies yielded conflicting results, regarding serum HIF-1α levels in patients with diabetes. Since the majority of problems are linked to decreased HIF-1α activity; it seems logical to consider whether increasing it is beneficial (31). HIF-1α down-regulation in pancreatic β cells has been shown to suppress glucose-stimulated insulin production, indicating that suppression of pancreatic β cells HIF-1α could be a pathogenic factor of diabetes (34).

However, other studies suggested that HIF-1α may have a harmful impact on islet function. For example, deletion of the *VHL* gene causes beta cell malfunction, which can be corrected by inhibition of the *HIF-1* gene. Furthermore, rats with pancreatic beta VHL deletion, have glucose intolerance with insulin secretion problems due to higher levels of HIF-1α (35). These findings are consistent with a study showing that rats with pancreatic β cells specified HIF-1α deletion are protected from high-fat diet-induced diabetes, indicating that HIF-1α activation is deleterious to beta cell function (36).

Catrina and Zheng, (33) suggested that elevation of HIF-1α in response to hypoxia in normal conditions in the absence of diabetes or other metabolic syndromes, plays a significant role in β cell activity regulation and glucose metabolism. They further stressed on the need of HIF-1α signaling balance for appropriate beta cell activity. Extremely low levels of HIF-1α following its deletion or extremely high levels, as in homozygous VHL deletion, or excessive hypoxia, are detrimental.

Rusdiana *et al*., (37) revealed that patients with T2DM who have increased HIF-1α levels have a worse prognosis than those who have lower concentrations because high HIF-1α increases the risk of cancer and diabetic complications. Higher blood HIF-1α concentrations may play a role in vascular calcification in T2DM. In patients with diabetes, vascular calcification comprises both intimal and medial calcification, and intimal calcification of coronary arteries is most commonly associated with atherosclerotic lesions (38).

Regarding FBG, HbA1C % and serum lipid profiles, our findings show a substantial elevation (p<0.001) in FBG, HbA1C % and serum TC, TG, and LDL-C, as well as substantial reduction (p<0.001) in serum HDL-C in both groups of patients with diabetes compared to controls with a much higher increase in FBG, HbA1C % and dyslipidemia in patients with diabetes with coronary complications compared to patients with non-complicated diabetes, indicating disturbed metabolism in these patients.

In line with our findings, Bobby and Vinodha, (39) found that FBG, TC, LDL-C, and TG levels increased while HDL-C levels decreased in patients with T2DM compared to controls. Patients with T2DM had a significantly greater risk of dyslipidemia than non-diabetics, according to Schofield *et al*., (40) and Ibrahim *et al.,* (41), and dyslipidemia is a risk factor for CAD.

According to Wang *et al.,* (42) and Sharif-Askari *et al*., (43), the detrimental lipid profile, notably HDL-C dyslipidemia, is associated with a significant increase in CAD risk factors such as obesity, smoking, and sedentary lifestyle in insulin resistant patients, so defining and managing dyslipidemia risk factors in T2DM is a central component for CAD avoidance.

According to a study by Rattarasarn *et al*. (44), plaque development and atherosclerosis have an independent relationship with fasting blood glucose concentrations. Hyperglycemia causes changes in several metabolic and cellular functions, including dyslipidemia, endothelial dysfunction, and cardiac metabolism disturbances. Variations in blood lipoprotein levels in individuals with diabetes might be caused by insulin insufficiency and hyperglycemia.

According to Ferrannini and Cushman (45), uncontrolled diabetes is related to hypertension and dyslipidemia. Furthermore, these three disorders have been linked to an increased risk for negative outcomes and death from cardiac diseases. The patients included in this study with hypertension or dyslipidemia are more likely to have uncontrolled diabetes.

Several studies have found a higher incidence of dyslipidemia in patients with diabetes with poor glycemic control, rising plasma levels of glycated hemoglobin, and hypercholesterolemia (46, 47).

Insulin resistance has been linked to the development of dyslipidemia in diabetic patients, which is characterized by increased production of small dense particles of LDL and impaired glucose tolerance (48).

Zhao *et al*. (49), confirmed that HbA1c % is increased in patients with T2DM. Long-term unregulated hyperglycemia enhances the productions of glycated components, as well as the activation of macrophages and the generation of inflammatory cytokines, which promote plaque formation and atherosclerosis progression.

The assessed HIF-1α levels in all studied patients with diabetes were correlated with FBG, HbA1c %, and lipid profiles in an effort to discover the possible risk factors related to the CAD.

In agreement with our findings, Liang *et al*. (50) found that serum HIF-1α levels in type 2 diabetes were correlated significantly with FBG and HbA1c percent and hyperglycemia was associated with greater HIF-1α serum levels. Furthermore, Ece *et al*. (51) revealed that HIF-1α blood levels were substantially greater in patients with diabetes with breast cancer than in normal individuals. This study revealed a link between diabetes and HIF-1α blood levels.

Wang *et al*. (52), revealed that adipocyte HIF-1α modulates lipid metabolism in atherosclerosis and adipocyte HIF-1α activation increased SMPD3-mediated ceramide synthesis, which exacerbated atherosclerosis by blocking cholesterol clearance and increasing local and systemic inflammation levels. This supports the use of PX-478, a HIF-1α specific inhibitor, in combination with new medications to reduce the risk of atherosclerosis.

In the current study, ROC curve analysis of serum HIF-1α levels showed high AUC with high sensitivity and specificity particularly in patients with diabetes with coronary complications relative to controls (AUC=0.9828, 95%CI=0.965-1.000, $p<0.0001$), and when comparing both groups of patients with diabetes (AUC=0.8108, 95%CI=0.7297-0.8919, $p<0.0001$). Therefore, serum HIF-1α levels can be used as a predictor for the extent of diabetic coronary complication. Li *et al*. (38) have reported that HIF-1α can predict the incidence and severity of coronary artery calcification, according to ROC curves analysis (AUC=0.775, 95%CI=0.686-0.863, $p<0.001$).

Conclusion

Our results showed a significant increase in serum HIF-1α with significant differences in dyslipidemia in patients with diabetes with coronary complications relative to other groups. In addition, the results of the ROC curve analysis showed high sensitivity of serum HIF-1α particularly in the coronary diabetic group. Therefore, we concluded that there was a strong association of serum HIF- 1α and dyslipidemia with coronary artery complications in Saudi patients with type 2 diabetes. In addition, HIF- 1α can be used as predictor for the occurrence and severity of coronary artery complications in patients with type 2 diabetes.

Conflicts of Interest

The Authors declare that they have no competing interests in relation to this study.

Authors' Contributions

A F Gharib: designed the study; performed the research; analyzed the data and wrote the article. Taisir Saber: performed the research and analyzed the data. Ahmad El Askary: performed the research and analyzed the data. Afaf Alharthi: performed the research, analyzed the data, and contributed essential reagents. Nouf Ali Alsalmi: performed the research and analyzed the data. Saja Talal Alhashmi: analyzed the data. Rana Fawaz Al-Asiri: analyzed the data and contributed essential reagents. Alaa Shafie: performed the research and analyzed the data.

Acknowledgements

The Authors acknowledge the support of Taif University Researchers Supported Project number (TURSP-2020/131), Taif University, Taif, Kingdom of Saudi Arabia.

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Received June 16, 2022
Revised July 23, 2022
Accepted July 25, 2022