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The Value of Hypoxia Inducible Factor 1 alpha as a Biomarker for Stable Coronary Artery Disease Diagnosis and Risk Stratification

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SOUHRN

Kontext: Stabilní ischemická choroba srdeční (ICHS) představuje celosvětový zdravotní problém; proto vědci neustále pokračují v pátrání po důvěryhodných biomarkerech pro stanovení diagnózy ICHS a stratifikaci rizika. Primární regulátor buněčné odpovědi na hypoxii, hypoxií indukovatelný faktor-1 alfa (hypoxia inducible factor 1 alpha, HIF-1α), sice již byl důkladně zkoumán v kontextu akutních kardiovaskulárních příhod; nicméně jeho potenciální využití jako biomarkeru při stabilní ICHS dosud nebylo spolehlivě dokumentováno a je třeba jej teprve ověřit.
Cíle: Zhodnotit diagnostický a prognostický význam HIF-1α při stabilní ICHS a jeho korelaci se závažností onemocnění.
Pacienti a metody: Do této prospektivní studie bylo zařazeno 73 pacientů (50 mužů, 23 žen) s podezřením na ICHS, kteří byli vyšetřeni koronarograficky ve fakultní nemocnici ve městě Kená (Egypt) v období mezi srpnem 2024 a lednem 2025. Skupinu I (kontroly, n = 22) tvořili jedinci s normálním výsledkem koronarografie; ve skupině II (ICHS, n = 51) odhalilo koronarografické vyšetření alespoň 70% stenózu koronární tepny. Při rozšířené klasifikaci v rámci skupiny II se rozlišovalo postižení současně několika tepen (multi-vessel disease, MVD) (n = 29) a postižení jedné tepny (single-vessel disease, 1VD) (n = 22). Pro stanovení hodnot HIF-1α v plazmě byla použita ELISA; hodnotily se i Gensiniho skóre a další klinické údaje.
Výsledky: Ve srovnání s kontrolní skupinou byly průměrné hodnoty HIF-1α při ICHS značně vyšší (4,594 ± 3,101 ng/ml vs. 3,563 ± 0,5957 ng/ml; $p = 0{,}0265$). Větší statisticky významný rozdíl byl zaznamenán mezi podskupinou 1VD a kontrolní skupinou ($p = 0{,}0457$), a větší rozdíl – ne však statisticky významný – byl pozorován mezi podskupinou MVD a kontrolní skupinou ($p = 0{,}2652$). Vyšší průměrné hodnoty HIF-1α byly naměřeny u pacientů s chronickým celkovým uzávěrem (chronic total occlusion, CTO) (5,36 ± 3,7 ng/ml); tento rozdíl byl ve srovnání s hodnotami v kontrolní skupině statisticky významný, $p = 0{,}0310$. Hodnoty HIF-1α však statisticky významně nekorelovaly s Gensiniho skóre.
Závěr: Hodnoty HIF-1α byly vyšší při stabilní ICHS, zvláště při postižení jedné tepny a CTO; po adjustaci na věk, pohlaví a klinické faktory však tento rozdíl přestal být statisticky významný a nebyl nezávisle spojen se závažností postižení. Není pravděpodobné, že by HIF-1α mohl sloužit jako samostatný biomarker při stabilní ICHS, může však mít jistou úlohu v kombinaci s jinými biomarkery.

Keywords:
Biomarkers
Cardiovascular risk factors
Hypoxia-inducible factor 1-alpha
Stable coronary artery disease.

ABSTRACT

Background: Stable coronary artery disease (CAD) is a worldwide health concern, and trustworthy biomarkers for CAD diagnosis and risk assessment are still being investigated. The primary regulator of the cellular response to hypoxia, hypoxia inducible factor 1 alpha (HIF-1α), has been thoroughly investigated in the context of acute cardiovascular events; however, its function as a biomarker in stable CAD is less well-established and requires validation.
Objectives: To evaluate the diagnostic and prognostic significance of HIF-1α in stable CAD and its correlation with disease severity.

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Patients and methods: This prospective study comprised 73 patients (50 males, 23 females) with suspected CAD who had coronary angiography at Qena University Hospital from August 2024 to January 2025. Group I (normal control, n = 22) consisted of patients with normal coronary angiography, while Group II (CAD, n = 51) had coronary artery stenosis of at least 70%. Multi-vessel disease (MVD) (n = 29) and single-vessel disease (1VD) (n = 22) were the additional classifications given to Group II. ELISA was used to assess plasma HIF-1α levels, and Gensini scores and other clinical data were examined.

Results: Compared to the control group, the mean HIF-1α levels in CAD were considerably higher (4.594 ± 3.101 ng/ml vs. 3.563 ± 0.5957 ng/ml, $p = 0.0265$). A higher significant difference was noted between 1VD subgroup and control ($p = 0.0457$), but higher insignificant difference between MVD subgroup and control ($p = 0.2652$). Higher mean HIF-1α levels were observed in chronic total occlusion (CTO) patients (5.36 ± 3.7 ng/ml) and compared to the control group, this increase was significant, $p = 0.0310$. However, HIF-1α levels had no significant correlation with the Gensini score.

Conclusion: HIF-1α levels were higher in stable CAD especially in single-vessel disease and CTO but lost significance after adjustment for age, sex, and clinical factors and showed no independent association with disease severity. HIF-1α is unlikely to serve as a standalone biomarker in stable CAD but may have a possible role within a multi-biomarker approach.

Introduction

Stable coronary artery disease (CAD) is a common disorder characterized by the presence of atheromatous plaques in the coronary arteries, impairing blood flow to the heart. This disorder is one of the leading causes of morbidity and death worldwide.[1] The ISCHEMIA Trials biorepository analysis showed that biomarkers like high-sensitivity troponin T and NT-proBNP, when combined with clinical factors, CAD severity, and inducible ischemia, improve cardiovascular event prediction over a median follow-up of 3.5 years.[2] These markers, however, need further prospective validation, which emphasizes the necessity of investigating other options such hypoxia-inducible factor-1α (HIF-1α).

Gregg L. Semenza and Guang Wang identified the HIF transcriptional complex in 1995, and it is crucial for cellular oxygen sensing and hypoxia tolerance. HIF-1α regulates genes involved in angiogenesis, glycolysis, and cell survival. Semenza, William Kaelin Jr., and Peter J. Ratcliffe received the Lasker Award in 2016 for their work on HIF-1's role in adapting to hypoxic conditions. In 2019, they were together awarded the Nobel Prize in Physiology or Medicine for their Insights that improve understanding how HIF regulates cellular reactions to variable oxygen levels.[3]

HIF-1α is structurally a subunit of the HIF-1 transcription factor, which is made up of the constitutively produced HIF-1β subunit and the oxygen-sensitive HIF-1α subunit.[4] A number of functional domains are present in the HIF-1α protein, such as two PAS (Per-Arnt-Sim) domains for dimerization stability, an oxygen-dependent degradation domain (ODDD) for oxygen-sensitive regulation, two transactivation domains (TAD-N and TAD-C) that stimulate target gene transcription, and an N-terminal basic helix-loop-helix (bHLH) domain for DNA binding. Under normoxic circumstances, prolyl hydroxylase domain (PHD) enzymes hydroxylate proline residues in the ODDD, triggering recognition by the von Hippel–Lindau (VHL) protein and subsequent degradation. This hydroxylation, however, is prevented in hypoxic environments, allowing HIF-1α to solidify, go into the nucleus, dimerize with HIF-1β, and initiate the transcription of genes related to angiogenesis, glycolysis, and cell survival.[5,6]

In cardiovascular diseases, HIF-1α signaling supports ischemic adaptation through angiogenesis and metabolic shifts, yet its dysregulation is implicated in CAD progression, heart failure, and other conditions like cancer and peripheral artery disease.[7,8]

Despite its biological significance, the clinical utility of HIF-1α in stable CAD remains underexplored. Prior studies have largely focused on acute coronary syndromes or advanced multi-vessel disease, with limited attention to its role in stable CAD phenotypes like single-vessel disease or chronic total occlusion (CTO). Furthermore, small-scale studies and a lack of standardized protocols have hindered its translation into practice, leaving gaps in understanding its diagnostic and prognostic value, including its potential influence on plaque stability.[9]

The purpose of this research is to investigate the relationship between stable CAD and HIF-1α levels in individuals experiencing coronary angiography. Additionally, we seek to explore the correlation of HIF-1α with CAD severity, utilizing the Gensini score, left ventricular ejection fraction (LVEF), and clinical risk factors for a comprehensive assessment of disease burden. To our knowledge, this is the first study to specifically explore the diagnostic and prognostic significance of HIF-1α in stable CAD, highlighting its association with distinct obstructive CAD subtypes, offering novel insights into its value as a targeted biomarker for better disease management.

Patients and methods

Study design and setting

This cross-sectional, observational prospective study was carried out at the Cardiology Department, Qena University Hospital, between August 2024 and January 2025.

Study population

This study comprised 73 individuals with presumed stable CAD who were scheduled for diagnostic coronary angiography due to chest pain. After receiving written informed consent and clearance from South Valley University's Ethics Committee on Research on Humans, patients were enrolled. Assuming a moderate to large effect size (Cohen's d = 0.7–0.8) based on previous biomarker studies in CAD, the sample size was calculated using a power

analysis aiming for 80% power and a significance level (α) of 0.05 to detect significant changes in HIF-1α levels between CAD patients and controls. A minimum of 64 participants was required, and after screening 96 patients and applying exclusion criteria, 73 were included in the final analysis. Patients were categorized into two main groups based on their angiographic findings:

Group I (Normal Control Group, n = 22): Subjects with normal or non-obstructive coronary arteries (<70% stenosis in any epicardial coronary artery).

Group II (CAD Group, n = 51): Patients with ≥70% stenosis in at least one epicardial coronary artery on coronary angiography. This group was further subdivided into:

- Subgroup A (Single-Vessel Disease, n = 22): Patients with ≥70% stenosis in one major epicardial coronary artery.
- Subgroup B (Multi-Vessel Disease, n = 29): Patients with ≥70% stenosis in two or more major epicardial coronary arteries.

Inclusion criteria: Patients who satisfied all of the following requirements were eligible to participate in the study:

- 18 years of age or older.
- Suspected stable CAD, requiring coronary angiography for evaluation of chest pain.
- No prior history of myocardial infarction, revascularization, or other acute cardiac events in the last six months.
- LVEF (left ventricular ejection fraction) ≥50% with a sinus rhythm.
- Availability of blood samples without hemolysis, ensuring accurate biomarker analysis.

Exclusion criteria: Patients were excluded if they had any of the following:

- Acute coronary syndrome (ACS) (unstable angina, NSTEMI, or STEMI) within the past six months.
- History of coronary revascularization (CABG or PCI) within the past six months.
- Hemolyzed blood samples that could interfere with biomarker analysis.
- Severe comorbidities or systemic diseases, including: Chronic inflammatory or autoimmune diseases (e.g., rheumatoid arthritis, lupus), active infections or malignancies, chest disorders, moderate to severe valvular heart disease, atrial fibrillation or other significant arrhythmias, heart failure with reduced ejection fraction (LVEF <50%), thyroid dysfunction (hypothyroidism or hyperthyroidism), hematological disorders (e.g., anemia, coagulopathies), chronic kidney disease (eGFR <30 mL/min/1.73 m²) or hepatic dysfunction (cirrhosis, significant liver enzyme elevation).

Blood sample collection and HIF-1α measurement

Each participant who fasted for eight hours had two milliliters of venous blood extracted and placed in tubes containing EDTA. After centrifuging the samples for ten minutes at 3500 rpm, the separated plasma was aliquoted into one milliliter cryotubes and kept at –80 °C until biochemical analysis. ELISA assay kits from Bioassay Technology Laboratory (BT LAB, China, Catalogue No. E0422Hu) were used to determine the levels of plasma HIF-1α. The measurement was performed using a microplate ELISA reader (EMR-500, USA), with a sensitivity of 0.01 ng/mL and a standard curve range of 0.05–15 ng/mL. To minimize potential biases, laboratory personnel who performed HIF-1α measurements were blinded to patient clinical and angiographic data. Both the intra-assay and inter-assay precision were less than 8% and 10%, respectively.[10]

Demographic, clinical, and echocardiographic data collection

Demographic data such as age, sex, and body mass index (BMI) were recorded. Additionally recorded were clinical risk factors such as diabetes, hypertension, and a history of smoking. Furthermore, each participant's echocardiographic left ventricular ejection fraction (LVEF%) was determined. Echocardiographic assessment was done by a GE Vivid E95 ultrasound system (GE Healthcare, Chicago, IL, USA) guided by the American Society of Echocardiography recommendations.[11]

Coronary angiography and Gensini score calculation

Coronary angiography was done after admission via standard techniques to assess coronary artery status and classify disease severity based on the 2018 ESC/EACTS Guidelines for Revascularization of the myocardium. An experienced cardiologist, blinded to clinical and laboratory data, analyzed the findings. CTO (chronic total occlusion) is defined as an epicardial coronary artery occlusion without antegrade flow across the lesion and with a duration of ≥3 months. Patients with CTO classically have coronary collaterals distally on coronary angiography, however, these collaterals may not deliver enough blood flow to the myocardium, causing ischemia and anginal pain. The Gensini score was used to quantify coronary artery disease severity. Each lesion was assigned a score based on stenosis severity: 25% is equal to one point, 50% to two, 75% to four, 90% to eight, 99% to sixteen, and 100% to thirty-two points. This score was then multiplied by a weighting factor reflecting the lesion's anatomical location: Left main = 5, proximal LAD = 2.5, mid LAD = 1.5, distal LAD = 1, proximal LCX = 2.5, mid LCX = 2, distal LCX = 1, RCA = 1, major first diagonal, obtuse marginal and posterior descending artery = 1. The final Gensini score was the sum of all weighted lesion scores, providing a comprehensive assessment of coronary atherosclerosis.[12,13]

Study endpoints

The primary endpoint is to assess the correlation between the presence of coronary artery disease and HIF-1α levels.

Secondary endpoint: To evaluate the relationship between HIF-1α levels and individual clinical risk factors (e.g., smoking, diabetes, and hypertension), left ventricular ejection fraction (LVEF), and the severity of CAD as determined by the Gensini score.

Ethical considerations

The Ethics Committee on Research on Humans at South Valley University gave approval to the study protocol (SVU-MED-MBC004-MED018-4-25-1-4). The study adhered to the ethical principles outlined in the Declaration of Helsinki, and all participants provided informed consent before participation.

Statistical analysis

GraphPad InStat 3.10 (GraphPad Software, San Diego, CA, USA) was used for all statistical analyses. Continuous variables were tested for normality using the Shapiro–Wilk and Kolmogorov–Smirnov tests. Normally distributed data are presented as mean ± standard deviation (SD), and non-normally distributed data as median (interquartile range, IQR). Categorical variables are expressed as frequencies and percentages. Between-group comparisons were performed using the unpaired t-test or Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables. Welch's adjustment was applied to the unpaired t-test when there were unequal variances. Correlations were assessed using Pearson or Spearman coefficients as appropriate. Multiple regression was used to evaluate the independent association of HIF-1α with CAD after adjustment for clinical factors. ROC analysis was performed to assess its diagnostic performance using the area under the curve. A *p*-value <0.05 was considered statistically significant.

Results

A summary of the baseline demographic, clinical, biochemical, angiographic, and echocardiographic features is provided in **Table 1**, where:

- The CAD group was significantly older and had a higher proportion of males compared to the control group.
- The prevalence of diabetes mellitus (DM), hypertension (HTN), and smoking, as well as the mean BMI, was higher in the CAD group, though not statistically significant. This suggests a potential association between these factors and an increased risk of CAD.

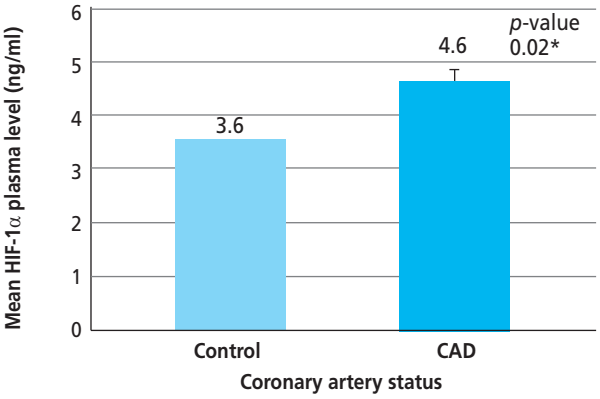

Fig. 1 – The difference between CAD and control groups in terms of baseline mean HIF-1α plasma levels. HIF-1α levels in CAD patients significantly increased compared to normal controls. CAD – coronary artery disease; HIF-1α – hypoxia inducible factor one alpha. * Significant p-value (<0.05). The two groups were statistically analyzed using the unpaired t-test with Welch's correction.

HIF-1α levels in study groups

- The CAD group's mean HIF-1α levels were considerably higher than those of the control group (4.594 ± 3.101 ng/mL vs. 3.563 ± 0.5957 ng/mL, $p = 0.0265$), indicating that the raised HIF-1α level may be linked to the presence of CAD (**Fig. 1**).
- HIF-1α levels were considerably higher in subgroup A (single vessel disease) than in the control group (5.169 ± 3.506 ng/mL vs. 3.563 ± 0.5957 ng/mL, $p = 0.0457$).
- The HIF-1α levels in subgroup B (multi-vessel disease) were greater than those in the control group, but they were not statistically significant (4.158 ± 2.737 ng/mL vs. 3.563 ± 0.5957 ng/mL, $p = 0.2652$).

Table 1 – Comparison between the study groups regarding demographics, clinical, and biochemical data

Variables	Normal control (n = 22)	All CAD (n = 51)	Single-vessel disease (1VD) (n = 22)	Multi-vessel disease (MVD) (n = 29)	*p*-value (control vs. CAD)	*p*-value (IVD vs. MVD)
Age (years)	53.1 ± 8.7	58.1 ± 9.2	56.1 ± **8.2**	59.6 ± 9.8	**0.03***	0.2
Male sex (n, %)	10 (45%)	40 (78.4%)	18 (81%)	22 (75.8%)	**0.01***	0.7
BMI (Kg/m^2)	26.6 ± 3.6	27.3 ± 2.0	26.8 ± 2.0	27.5 ± 1.9	0.5	0.2
HTN (n, %)	7 (32%)	27 (52.9 %)	13 (59%)	14 (48%)	0.1	0.6
DM (n, %)	6 (27%)	24 (47%)	8 (36%)	16 (55%)	0.1	0.3
Smoking (n, %)	6 (27%)	25 (49%)	13 (59%)	12 (41%)	0.1	0.3
LVEF (%)	57.3 ± 4.1	56.5 ± 5.7	57.1 ± 6.4	56.2 ± 5.1	0.5	0.5
Gensini score (median)	–	36.0	24.0	**72.0**	–	< 0.001**
HIF-1α (ng/mL)	3.6 ± 0.6	4.6 ± 3.1	5.2 ± 3.5	4.2 ± 2.7	**0.03***	0.3

BMI – body mass index; CAD – coronary artery disease; DM – diabetes mellitus; 1VD, single-vessel disease; HIF-1α – hypoxia inducible factor one alpha; HTN – hypertension; LVEF – left ventricular ejection fraction; MVD – multi-vessel disease; n – number.
Continuous data expressed as mean ± SD, categorical data in the form of n (%). Comparison between the controls and all CAD groups was performed using unpaired t-test and Fisher's exact test, while HIF-1α *p*-value was done by unpaired t-test with Welch's correction.
** *P*-value represent the difference between 1VD and MVD subgroups in the median Gensini score and was highly significant (<0.001) in **bold**, done by unpaired t-test with Welch's correction. * Significant *p*-values are in **bold** (<0.05).

- Among CAD patients, those with chronic total occlusion (CTO) and coronary collaterals (n = 14, from subgroup A or B) exhibited the highest mean HIF-1α levels (5.36 ± 3.7 ng/mL) and when compared to the control group, this increase was significant, $p = 0.0310$.

Comparison within CAD subgroups

- Median Gensini scores were significantly higher in subgroup B (multi-vessel disease) compared to subgroup A (single vessel disease) (72 vs. 24, $p < 0.001$).
- HIF-1α levels in subgroups A and B did not differ significantly (5.169 ± 3.506 ng/mL vs. 4.158 ± 2.737 ng/mL, $p = 0.2702$).

Correlation analyses

No significant correlation was found between HIF-1α levels and: Gensini score (r = –0.03338, $p = 0.816$), age (r = –0.02256, $p = 0.8498$), BMI (r = 0.03735, $p = 0.7537$), diabetes (r = –0.0283, $p = 0.81$), smoking (r = 0.05733, $p = 0.63$), hypertension (r = 0.1089, $p = 0.41$), LVEF % (r = 0.09701, $p = 0.35$).

Multivariate and logistic regression analysis with confounder adjustment

A multiple regression model was used to evaluate the independent association of HIF-1α with CAD while adjusting for confounders. The model included age, sex, LVEF, BMI, diabetes mellitus, hypertension, and smoking as independent variables. It explained 25.68% of the variance in CAD ($R^2 = 0.2568$, $p = 0.0109$).

Confounder adjustment was performed by including clinically relevant covariates known to influence CAD risk. Diabetes mellitus ($p = 0.04$) and male sex ($p = 0.0180$) were significant predictors, while HIF-1α showed a positive but non-significant association ($p = 0.1803$). Other factors, including age, LVEF, BMI, hypertension, and smoking, did not reach statistical significance. Variance inflation factors (VIF <1.64) confirmed no multicollinearity, ensuring independent effects of each variable.

To address potential bias, additional adjustments were made using logistic regression, treating CAD as a categorical outcome (≥70% stenosis), The odds ratio (1.2380) suggests a modest increase in the likelihood of CAD with higher HIF-1α levels, but the confidence interval includes 1.0, and the p-value is not significant ($p = 0.0883$). Sensitivity analyses stratified the sample by diabetes status and sex to assess potential effect modification. Subgroup analyses evaluated differences in HIF-1α levels across CAD severity (Gensini score) (**Table 2 and Fig. 2**).

Despite not being a significant independent predictor, HIF-1α's association with obstructive CAD and its inclusion alongside established risk factors suggest its potential role in CAD risk assessment. To confirm these results, more research with larger sample sizes is required.

The ROC curve analysis demonstrated poor overall diagnostic performance for CAD (AUC = 0.510), with low sensitivity (35.29%) despite high specificity (95.45%). While high specificity suggests HIF-1α could help rule out CAD in specific cases, its low sensitivity limits its clinical utility as a standalone diagnostic marker. Additional studies are

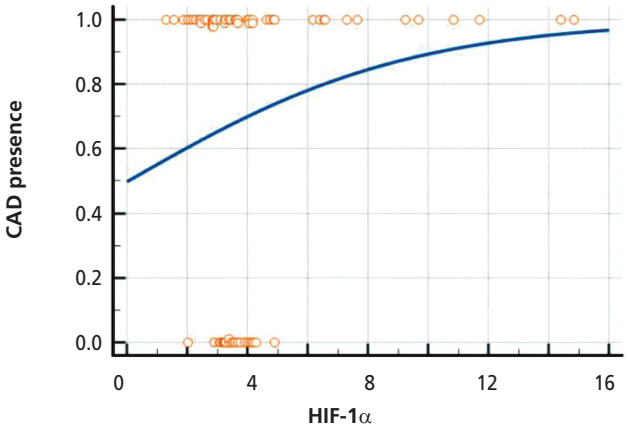

Fig. 2 – The relationship between HIF-1α levels and the existence of coronary artery disease (CAD) was assessed using logistic regression analysis. Although the *p*-value is not significant ($p = 0.09$), the odds ratio indicates a little increase in the risk of CAD with greater HIF-1α levels, and the confidence interval encompasses 1.0. CAD – coronary artery disease; HIF-1α – hypoxia inducible factor one alpha.

Table 2 – Multiple Regression Analysis of Predictors of CAD

Variable	Coefficient	Std. error	95% CI	t-ratio	*p*-value
Constant	–0.1	0.9	–1.8 to 1.7	0.1	1.0
HIF-1α	0.0	0.0	–0.0 to 0.1	1.4	0.2
Age	0.0	0.0	–0.0 to 0.0	1.1	0.3
LVEF	–0.0	0.0	–0.0 to 0.0	0.3	0.7
BMI	–0.0	0.0	–0.0 to 0.0	0.0	1.0
DM	0.2	0.1	0.0 to 0.5	**2.0**	*0.049
HTN	0.1	0.1	–0.1 to 0.4	1.3	0.2
Smoking	0.1	0.1	–0.2 to 0.4	0.8	0.5
Male sex	0.3	0.1	0.1 to 0.6	**2.4**	**0.02***

BMI – body mass index; CAD – coronary artery disease; CI – confidence interval; DM – diabetes mellitus; HIF-1α – hypoxia inducible factor one alpha; HTN – hypertension; LVEF – left ventricular ejection fraction.
* Significant *p*-values (<0.05) are in **bold**.

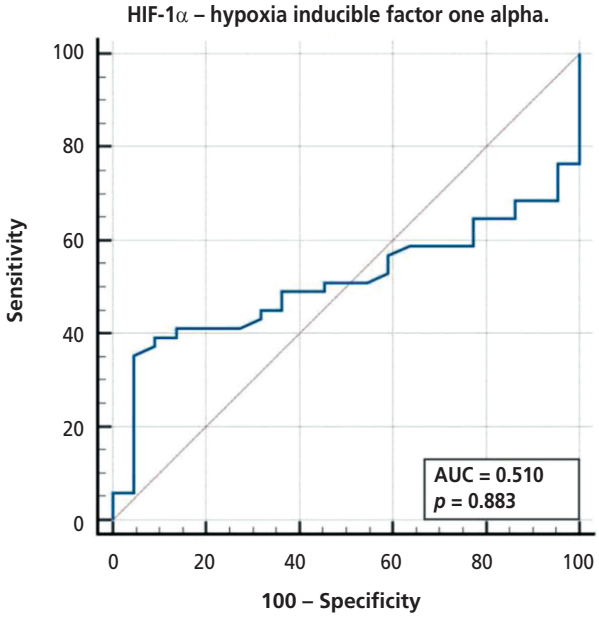

Fig. 3 – The ROC curve analysis demonstrated poor overall diagnostic performance for CAD (AUC = 0.510), with low sensitivity (35.29%) despite high specificity (95.45%).

necessary to decide whether HIF-1α could be integrated into multi-biomarker models to improve diagnostic accuracy (**Fig. 3**).

Discussion

Hypoxia-inducible factor 1-alpha (HIF-1α) is considered as a crucial modulator of cellular response to hypoxia, stimulates angiogenesis, vascular remodeling, and metabolic changes. Studies by **Akbaş et al. (2021)** and **Liu et al. (2013)** have shown that HIF-1α is upregulated in acute ischemic events and is associated with coronary collateral development, reinforcing its role in ischemic adaptation, aligning with our study.[14,15]

But it's still unknown how HIF-1α contributes to stable CAD. Our findings, consistent with those of **Czibik et al. (2008)**, suggest that in chronic ischemia, HIF-1α activation may be insufficient to sustain significant hypoxic signaling. This is supported by demonstrating lower transcriptional activity of HIF-1α in stable CAD compared to acute settings, likely due to differences in ischemic stress thresholds. Moreover, stable CAD may involve compensatory mechanisms such as collateral circulation and metabolic reprogramming, which reduce the extent of HIF-1α activation. These results could explain our observation of high but not statistically significant levels of HIF-1α in CAD patients with multivessel disease.[16]

Additional mechanistic insights suggest that HIF-1α exerts its effects in CAD through both protective and pathological pathways. **Vink et al. (2007)** found that HIF-1α is associated with an inflammatory plaque phenotype and is upregulated in activated macrophages, indicating its involvement in both protective angiogenesis and pathological inflammation in atherosclerosis.[17] Additionally, **DeNiro et al. (2013)** demonstrated that vascular endothelial growth factor (VEGF) can activate the NF–κB signaling pathway, linking HIF-1α-induced angiogenesis to inflammatory processes. These interactions suggest that elevated HIF-1α in chronic hypoxia may lead to maladaptive vascular changes, including endothelial dysfunction and plaque instability, complicating its role as a straightforward biomarker.[18]

The potential diagnostic value of HIF-1α in CAD remains debated. Although HIF-1α levels were higher among the included CAD patients, especially in those with single-vessel disease and chronic total occlusion (CTO), our research revealed that these levels did not correspond to the severity of the disease. HIF-1α levels may be higher in single-vessel CAD due to acute localized ischemia, which activates hypoxic signaling. Multi-vessel CAD often develops collateral circulation which may explain reducing ischemic damage and HIF-1α activation in stable multi-vessel CAD patients. Plaque instability and acute inflammation may be another explanation, further increasing HIF-1α levels. This aligns with **Akbaş et al. (2021)**, who reported elevated but statistically non-significant HIF-1α levels in acute coronary syndrome (ACS), possibly due to localized ischemia, immediate medical therapy, or sample size limitations, suggesting variability in its diagnostic reliability.[14]

The findings of **Qin et al. (2024)** further illustrate this variability. Their study identified a HIF-1α cutoff for diagnosing coronary artery calcification (CAC) in non-dialysis CKD patients, with high specificity and moderate sensitivity. In contrast, our study showed poor diagnostic performance of HIF-1α for CAD (AUC = 0.510), suggesting that its clinical utility may be context-dependent, influenced by disease mechanisms such as vascular calcification versus atherosclerotic plaque burden. Both studies confirmed the higher prevalence of males and older patients in disease groups.[19]

Similarly, while **Gharib et al. (2022)** linked elevated HIF-1α to coronary complications in diabetes, our findings did not establish it as an independent risk factor for CAD. **López-Reyes et al. (2014)** found that HIF1A polymorphisms modulate metabolic risk and the HIF1A rs2057482 polymorphism may account for population-level variations in HIF-1α expression and CAD susceptibility since it is linked to the risk of developing early coronary artery disease as well as certain metabolic and cardiovascular risk factors.[20,21]

In patients with notable coronary artery stenosis, **Song-Ming et al. (2008)** discovered a robust correlation between the presence and degree of coronary collaterals and greater HIF-1α expression at both the protein and mRNA levels. This in agreement with our research findings that elevated HIF-1α levels were observed in obstructive CAD, particularly CTO cases that often involve collateral development. However, no direct correlation was found between HIF-1α levels and disease severity or collateral grading.[22]

Stojanovic et al. (2021) identified renalase as a hypoxia-responsive gene linked to HIF-1α, promoting cardiac protection. These results lend credence to a wider function of hypoxia-related markers in the prediction of is-

chemia. However, our study suggests that HIF-1α alone lacks sufficient discriminatory power for stable CAD risk stratification.[23]

Limitations and future directions

This study has several limitations. Single center study and the relatively small sample size may restrict the generalizability of findings to broader CAD populations. The cross-sectional design precludes causal inferences between HIF-1α levels and CAD severity and limits our ability to assess dynamic changes in HIF-1α over time or in response to treatment. While we adjusted for age, sex, diabetes, hypertension, smoking, BMI, and LVEF, other potential confounders—such as genetic polymorphisms (e.g., HIF-1α gene variants), additional comorbidities (e.g., chronic kidney disease, dyslipidemia), and medications (e.g., statins, beta-blockers, or antiplatelets)—were not accounted for, despite their possible influence on HIF-1α expression and CAD severity. Furthermore, ethnic variability and genetic differences across populations were not fully explored, potentially limiting applicability to diverse groups. To confirm these results and clarify the clinical significance of HIF-1α in CAD, larger, long-term investigations with more diverse populations and balanced gender representation are required.

Future studies should use larger, multicenter cohorts and longitudinal follow-up to validate HIF-1α as a potential biomarker and to see how HIF-1α changes through different stages of CAD. It will also be important to look at how HIF-1α works alongside other biomarkers such as sST2, hsTnT, NT-proBNP, GDF-15, NF-κB, and YKL-40, which are already linked to CAD progression. Research on HIF-1α gene variations and how they affect disease severity in different populations is also needed. Our results suggest that HIF-1α may play a role in stable CAD, but it does not seem useful on its own as a diagnostic or prognostic marker. A combined multi-biomarker and mechanistic approach may help define its real value in CAD.

Conclusion

HIF-1α levels were higher in patients with stable CAD compared to controls, with numerically higher values in single-vessel disease and chronic total occlusion. These differences, however, were no longer significant after adjustment for age, sex, and other clinical factors. HIF-1α did not correlate with angiographic disease severity and did not independently predict the presence of CAD. The results indicate that HIF-1α is not suitable as a standalone diagnostic or prognostic biomarker in stable CAD, although it may still contribute as one element within a broader multi-biomarker strategy. Larger and better-controlled studies are needed to define its potential role.

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