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Research Article

Association of hsCRP and Serum Kalirin Levels with the Development and Severity of Premature Coronary Artery Disease in Iraqi Patients

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Abstract

Background: Coronary artery disease (CAD) is a major contributor to morbidity and mortality worldwide. Early-onset CAD, also known as PCAD, is a severe form of CAD associated with high mortality and a poor prognosis. Early diagnosis is crucial to reducing complications. While hsCRP is an established biomarker for CAD, kalirin is a potential novel biomarker due to its role in promoting smooth muscle proliferation and endothelial dysfunction. ***Objective***: To evaluate the relationship between serum kalirin and hsCRP levels with the presence and severity of PCAD and to compare the diagnostic value of both biomarkers. ***Method***: The study recruited 92 participants into two groups: the PCAD group (46) included patients with confirmed CAD by angiographic findings and the second group was the non-CAD group (46) with negative findings by coronary angiography. The levels of serum kalirin and hsCRP were measured for both groups using enzyme-linked immunosorbent assay (ELISA) kits. ***Results***: Serum levels of kalirin and hsCRP were strongly associated with the presence of PCAD (p<0.001), and both biomarkers were associated with disease severity (p=0.002, <0.001, respectively). ROC analysis showed that hsCRP possesses a slight advantage (AUC=0.796) over kalirin (ROC=0.717) as a diagnostic marker for PCAD. ***Conclusions***: Serum kalirin and hsCRP levels are associated with PCAD and with the severity of the disease, both markers possess moderate diagnostic capabilities for PCAD with a slight advantage for hsCRP.

Keywords: Coronary artery disease, hsCRP, Kalirin, Premature coronary artery disease.

ارتباط مستويات hsCRP و Kalirin في المصل مع تطور وشدة مرض الشريان التاجي المبكر لدى المرضى العراقيين

الخلاصة

الخلفية: مرض الشريان التاجي (CAD) هو مساهم رئيسي في المراضة والوفيات في جميع أنحاء العالم. CADالمبكر، المعروف أيضا باسم PCAD ، هو شكل حاد من CAD المرتبط بارتفاع معدل الوفيات وسوء التشخيص. التشخيص المبكر أمر بالغ الأهمية للحد من المضاعفات. في حين أن hsCRP هو علامة حيوية راسخة ل CAD، فإن kalirin هو علامة حيوية جديدة محتملة نظرا لدوره في تعزيز تكاثر العضلات الملساء والخلل البطاني. **الهدف**: تقييم العلاقة بين مستويات مصل كاليرين و hsCRP مع وجود وشدة PCAD ومقارنة القيمة التشخيصية لكل من المؤشرات الحيوية. **الطريقة**: شارك في الدراسة 92 مشاركا في مجموعتين: مجموعة PCAD (46)شملت المرضى الذين يعانون من CAD مؤكد من خلال نتائج تصوير الأوعية والمجموعة الثانية كانت المجموعة غير CAD (46) مع نتائج سلبية عن طريق تصوير الأوعية التاجية. تم قياس مستويات مصل كاليرين و hs-RP لكلا المجموعتين باستخدام مجموعات مقايسة الممتز المناعي المرتبط بالأنزيم (ELISA). **النتائج**: ارتبطت مستويات مصل الكليرين و hsCRP ارتباطا وثيقا بوجود PCAD ، وارتبطت كلتا المؤشرات الحيوية بشدة المرض. أظهر تحليل ROC أن hsCRP يمتلك ميزة طفيفة (AUC = 0.796) على kalirin (ROC = 0.717) كعلامة تشخيصية ل PCAD. **الخلاصة**: ترتبط مستويات مصل كاليرين و hsCRP ب PCAD ومع شدة المرض، تمتلك كلتا العلامتين قدرات تشخيصية معتدلة ل PCAD مع ميزة طفيفة لhsCRP .

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INTRODUCTION

Coronary artery disease (CAD) is a cardiovascular disease (CVD) that occurs due to atherosclerosis or atherosclerotic occlusion of the coronary artery [1] and is a major cause of mortality and morbidity worldwide [2]. In Iraq, cardiovascular disease (CVD) is a leading cause of mortality. According to the World Health Organization (WHO), CVD accounted for over 18.50% of total deaths in 2017, placing Iraq 19th in the world for CVD-related mortality [3]. Premature coronary artery disease (PCAD), which is the occurrence of CAD in men and women younger than 45 and 55, respectively, is rising rapidly in developing countries [4]. PCAD is an aggressive and chronic condition characterized by a high rate of ischemic recurrences; it often progresses rapidly

towards multi-vessel disease and is associated with an increased risk of premature death. [5,6]. Diagnosing PCAD early is of great importance in order to reduce its complications and mortality [7]. Protein markers of inflammation have been researched as noninvasive indicators of underlying atherosclerosis in individuals who appear healthy [8]. The most extensively studied biomarker of inflammation in cardiovascular diseases is serum C-reactive protein (CRP) [9]. The advantage of measuring CRP instead of other inflammatory cytokines is that protein levels are much higher and remain elevated for longer periods of time. The high-sensitivity C-reactive protein (hsCRP) test can detect lower concentrations of the protein more accurately, making it more sensitive than the standard CRP test. This increased sensitivity makes the hsCRP test more useful in predicting the risk of cardiovascular disease in apparently healthy individuals [10]. It also provides a prognostic value for patients with established CAD [11], which provides a much-needed risk stratification that could guide the use of interventions or therapies [12]. Genome-wide association studies (GWAS) have identified several genetic mutations linked to the development of CAD [13]. The kalirin (*KALRN*) gene was found to be strongly associated with the disease [14]. Kalirin is a protein encoded by the *KALRN* gene that functions as guanine nucleotide exchange factor (GEF) for the GTPases Rac1 and RhoaA. Kalirin's activity may contribute to atherogenesis by promoting vascular smooth muscle cell (SMC) proliferation and migration, as well as causing endothelial dysfunction, among other mechanisms [15]. Kalirin is linked to many atherosclerotic diseases [16], and its levels were shown to be elevated in patients with PCAD [17]. This study aims to find the association between kalirin and hsCRP with the presence and severity of PCAD in Iraqi patients and the plausibility of using kalirin as a novel serum biomarker for the disease.

METHODS

Study design and participants

This cross-sectional study was conducted between June 2022 and June 2023 at the Baghdad Heart Center, Baghdad Teaching Hospital, Medical City, Iraq. Ninety-two patients were divided into two groups: the PCAD group, consisting of 46 patients diagnosed with premature coronary artery disease (defined as having 50% or more blockage in one or more coronary arteries as shown by coronary angiography, with an age of less than 45 years for males and less than 55 years for females), disease severity was presented according to the number of occluded coronary vessels into [18]: single vessel disease (one vessel involved), do The non-CAD group also included 46 people of the same age who had no coronary artery blockage, as determined by coronary angiography. All patients underwent elective coronary angiography, and the diagnosis was performed under the supervision of a qualified cardiologist who followed the most recent guidelines from the American Heart Association and the American College of Cardiology [19,20].

Inclusion criteria

Patients undergoing coronary angiography who were males younger than 45 years old or females younger than 55 years old were considered for enrollment in the study. Group allocation was based on the angiographic results.

Exclusion criteria

Patients with Congenital heart disease, cardiomyopathy or valvular heart disease, were excluded from the study.

Ethical consideration

On January 12, 2022, the Scientific and Ethical Committee at the College of Pharmacy, University of Baghdad, granted ethical approval under the number (RECAUBCP1212022). All study participants provided informed consent, and this work complied with the Declaration of Helsinki and its amendments.

Data collection

A specially prepared data sheet was used to collect participants' demographic and clinical information during enrolling. Gender, age, weight, height, smoking status, relevant prior medical history, and CAD family history were all noted. In addition, the angiographic results and the number of blocked arteries were recorded. BMI was computed by dividing a person's weight in kilograms by the square of their height in meters (kg/m^2) [21]. Current smokers were classified as those who smoked one or more cigarettes per day. Past medical information, including hypertension, diabetes, dyslipidemia, and family history, was gathered from the patients' medical records and confirmed by asking the participants personally.

Sample collection

Two milliliters of venous blood were drawn from each participant in both groups and transferred into gel tubes. The blood samples were left at room temperature for 30 minutes to clot, then centrifuged for 10 minutes at 4000 rpm to obtain serum. The serum was stored in Eppendorf tubes and used to measure levels of kalirin and hsCRP using the enzyme-linked immunosorbent assay (ELISA) technique [22]. The samples were frozen at -20 °C until enough were collected for analysis. Kalirin and hsCRP serum levels were measured using ELISA kits supplied by [Bioassay Technology Laboratories, China], following the quantitative sandwich ELISA technique as per the manufacturer's instructions [23,24]. Absorbance was measured at 450 nm using a

microplate reader from BioTek, USA. The concentration of kalirin was expressed in ng/L and hsCRP in mg/L, calculated using a standard curve.

Statistical analysis

The results were statistically analyzed using SPSS software version 26 for Windows (SPSS, Illinois, USA). Categorical variables were provided as frequencies and percentages, and associations were checked using the chi-square test. The Shapiro-Wilk test was used to determine whether continuous variables had a normal distribution. The data were then presented as the mean and standard deviation. The student's t-test was performed to compare means. Non-normal distribution data was provided as median and interquartile range (IQR), and the Mann-Whitney U test was used to compare the median of two groups, while the Kruskal-Wallis test was used to compare the median of three groups for statistical significance. A receiver operating characteristics (ROC) analysis was done to assess the diagnostic performance of kalirin and hsCRP for PCAD. p-values < 0.05 were considered statistically significant.

RESULTS

Table 1 displays the characteristics of the enrolled participants according to the study groups. Age, BMI, and sex did not show significant differences between the two groups. Some clinical characteristics, such as the presence of hypertension and family history of CAD, were similar.

Table 1: Assessment of socio-demographic and disease characteristics

Parameters	Non-CAD (n=46)	PCAD (n=46)	*p*-value
Age (year)	42.28±5.82	42.46±5.12	0.879
BMI (kg/m^2)	27.13±5.37	28.07±4.31	0.362
Sex			
Female	17(37.0)	15(32.6)	0.662
Male	29(63.0)	31(67.4)	
Smoking	13(28.3)	26(56.5)	0.006
Hypertension	26(56.5)	30(65.2)	0.392
DM	13(28.3)	25(54.3)	0.011
Dyslipidaemia	17(36.9)	31(67.9)	0.004
Family history of CAD	22(47.8)	27(58.7)	0.296

Values are expressed as frequencies, percentages, and mean±SD.

However, other factors, including smoking status, diabetes, and dyslipidemia prevalence, were significantly different between the groups ($p< 0.05$). Kalirin levels were significantly higher ($p<0.001$) in the CAD group compared to the non-CAD group, as illustrated by Table 2. Serum hsCRP levels were significantly more elevated ($p<0.001$) in the CAD group when compared with the non-CAD group (Table 3). When comparing the levels of serum kalirin among the classes of disease severity, kalirin was statistically different ($p= 0.002$) among the classes of disease severity (Table 4).

Table 2: Levels of serum kalirin among the study groups

Parameters	Non-CAD (n=46)	PCAD (n=46)	*p*-value
Kalirin (ng/ml)	201.6 (122.6-312.0)	312.8 (219.7-447.6)	<0.001

Values are expressed as median (interquartile range)

Table 3: Levels of hsCRP among the study groups

Parameters	Non-CAD (n=46)	PCAD (n=46)	*p*-value
hsCRP (mg/L)	1.5 (1.003-2.33)	3.29 (2.463-4.59)	<0.001

Values are expressed as median (interquartile range)

Table 4: Association of serum kalirin levels with disease severity

No. of vessels involved	kalirin levels	*p*-value
Single vessel disease	287.42(153.25-384.08)	
Double vessel disease	294.08(220.75-324.08)	0.002
Triple vessel disease	566.59(393.25-761.79)	

Values are expressed as median (interquartile range)

The levels of serum hsCRP were also statistically different ($p< 0.001$) when comparing among classes of disease severity (Table 5).

Table 5: Association of serum hsCRP levels with disease severity

No. of vessels involved	hsCRP levels	*p*-value
Single vessel disease	2.95(2.35-4.14)	
Double vessel disease	4.02(2.67-4.39)	0.298
Triple vessel disease	4.69(1.59-5.53)	

Values are expressed as median (interquartile range)

A receiver operating characteristics (ROC) analysis was conducted to find the area under the curve (AUC) and cutoff points of serum kalirin and serum hsCRP when used as a predictor of CAD, as well as to determine their sensitivity and specificity (Figure 1 and Table 6).

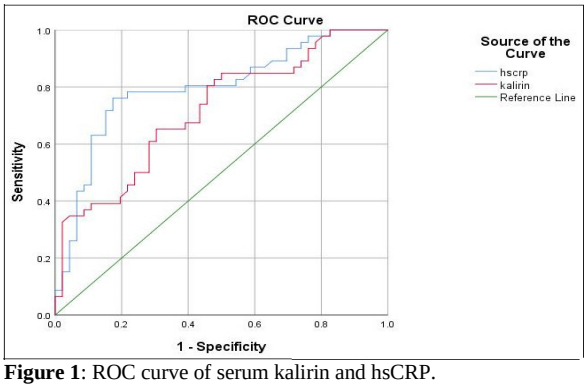

Figure 1: ROC curve of serum kalirin and hsCRP.

Table 6: ROC analysis of serum biomarkers

Parameter	State variable	AUC (mean±SE)	95% CI	Cutoff	Sensitivity (%)	Specificity (%)	*P*-value
Serum Kalirin	Presence of CAD	0.717±0.05	(0.613-0.820)	>283.25	0.652	0.696	<0.001
Serum hsCRP	Presence of CAD	0.796±0.05	(0.702-0.990)	>2.445	0.761	0.826	<0.001

DISCUSSION

This study aimed to explore the association between kalirin and hsCRP with the presence and severity of PCAD in Iraqi patients. Our main findings indicated that serum kalirin levels and serum hsCRP levels were both significantly increased in patients with PCAD, and both markers showed a significant association with the severity of PCAD presented as the number of coronary vessels occluded. The ROC analysis showed a slight advantage for hsCRP over kalirin as a serum predictor for PCAD, suggesting that kalirin could also potentially serve as a novel serum biomarker for PCAD. The PCAD and non-CAD groups show similarity in demographic and clinical characteristics; however, the PCAD group has significantly more smokers and more participants with diabetes, as well as participants with dyslipidemia. Several studies have demonstrated that traditional risk factors such as diabetes, hypertension, smoking, obesity, hypercholesterolemia, and a family history of coronary artery disease are associated with a high incidence of PCAD [6,25-27]. However, in the current study both groups showed a high incidence of hypertension, which could be explained by the finding that the prevalence of hypertension is relatively high among Iraqi young patients [28]. When comparing the kalirin serum levels for both groups, it was observed that serum kalirin levels were significantly higher in the PCAD group; moreover, it was also strongly associated with disease severity (Table 4). Only a single study directly explored the relationship of kalirin with CAD in a case-control study; although the levels of kalirin were elevated in patients with CAD in the mentioned study [17], it failed to indicate a statistically significant relationship. Kalirin plays a significant role in the pathogenesis of coronary artery disease (CAD) and atherosclerosis through various mechanisms. Kalirin's RhoGEF activity can enhance atherogenesis by promoting smooth muscle cell (SMC) proliferation and migration, as well as endothelial dysfunction [29]. Kalirin-9 activates Rac1 and PAK1 signaling, which are critical for SMC migration, and prevents NOS2 dimerization, thereby inhibiting NOS2 activity [30]. This inhibition of NOS2 activity leads to decreased nitric oxide (NO) production, promoting neointimal hyperplasia and SMC proliferation, which contribute to atherosclerosis [31]. Genetic variations in *KALRN* may lead to endothelial dysfunction and atherosclerosis through their influence on the Rac-1 signaling pathway [32]. Kalirin also mediates SMC migration through downstream signaling of receptor tyrosine kinases such as EphB2 and its agonist ephrinB2, further contributing to neointimal hyperplasia [17]. Collectively, these findings highlight the potential of kalirin as a modulator of atherogenic potential and a contributor to CAD susceptibility, and our current study provides additional evidence to support these findings. The serum levels of hsCRP in the current study were strongly associated with PCAD, as well as with disease severity. The relationship between hsCRP and CAD has been previously established in the literature [33–35]. However, the differences observed in different values for hsCRP in different studies could be attributed to the effects of other traditional risk factors such as obesity, diabetes and dyslipidemia [36], which could affect the levels of hsCRP. Also, the effects of some medications on hsCRP levels, such as aspirin and statins [37], make it difficult to determine normal values and cutoff values for hsCRP [38]. It was noted that hsCRP possessed a slight advantage over serum kalirin in terms of reliability as a diagnostic marker, as well as having better overall sensitivity and better specificity (Table 6), which indicate that hsCRP outperformed serum kalirin in that regard. The advantages of serum hsCRP as a biomarker for CAD include that it is a stable compound [39], and it can be measured at any time a day without regard to meal, unlike other markers for inflammation such as interlukin-6, which makes it suitable to be performed in clinical settings [40]. The disadvantages include variations in levels and cutoff points mentioned earlier; however, there is consensus supported by the American Heart Association that hsCRP levels > 3 mg/L are suggestive of high risk of cardiovascular disease [41]. This value is similar to the observed cutoff value in the current study of 2.44 mg/L, which supports our findings.

Study limitations

It is crucial to point out that the current study has some limitations, including the relatively small sample size, single-center protocol, the case-control study design, which can only measure association but not causality, and the inability to measure additional risk factors or serum biomarkers that may influence the results.

Conclusion

The study results indicated a correlation between the newly discovered serum marker kalirin and hsCRP, as well as the occurrence and intensity of PCAD in the Iraqi patients. The study also assessed the diagnostic efficacy of both markers and determined that hsCRP is marginally superior in terms of accuracy and specificity compared to kalirin as a serum marker for CAD. This underscores the possibility of kalirin serving as an alternate marker in future investigations.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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