

ORIGINAL ARTICLE

A ROLE FOR INFLAMMATORY IL-6 IN THE DEVELOPMENT OF CORONARY ARTERY DISEASE: A CASE CONTROL STUDY AT AL-QADISIYAH GOVERNORATE, IRAQ

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Summary

Background: Disruption of the IL-6 axis can result in the beginning or progression of a variety of disease states, including cardiovascular diseases. Because of IL-6's pathogenic function, pharmacologic manipulation of the IL-6 axis is a sensible therapeutic strategy; nevertheless, various expected, yet frequently overlooked, effects on tissues and organs other than blood vessels may also occur. The study's goal is as follows: The purpose of this study is to see how inflammatory Interleukin-6 affects the development of coronary artery disease.

Materials and Methods: Serum IL-6 level was estimated using Elabscience® kits. A case-control study included 30 CAD patients, 30 positive controls (PC) [(have family history for CAD)], and 30 negative controls (NG) [they have no family history for CAD]. Ranged from 30 to 81 years old. Patients with acute or chronic illnesses such as liver disease, renal disease, thyroid function disorders, COVID-19, and autoimmune disease were excluded from the study. During the period from December 2020 to May 2021. Our study was included other factors such as ages, genders, smoking, family history, antihypertensive drugs or drugs for other diseases, body mass index (BMI) which were assessed.

Results: The results of this study show increased levels of IL-6 in patients with CAD compared with the PC and NC. These results showed a highly significant difference ($p < 0.0001$) in the concentrations compared with PC and NC.

Conclusions: IL-6 could be one of the prognostic signs of CAD.

Key words: Coronary Artery Disease; IL-6; Inflammation; Cytokines

Introduction

Coronary artery disease (CAD) is a cardiovascular disease that is leading to death in developed-developing countries (1, 2). CAD is an atherosclerotic disease that is inflammatory (3). CAD is manifested by stable angina, unstable angina, myocardial infarction (MI), or sudden cardiac death (4). Although family history has long been recognized as a risk factor for CAD, genome-wide association studies have made significant progress in elucidating the genetic architecture of the disease only in the last decade (5).

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The first phase is the major underlying cause of CAD, inflammation in the blood vessel wall promotes endothelial dysfunction, has a tight relationship with obesity and inflammation (6). Inflammation and deregulation of lipoprotein metabolism promote the production of foam cells and plaques on the arterial wall. Adipokine, which impacts whole-body lipids, glucose metabolism, and inflammation, is secreted by adipose tissue. Obesity and adipose tissue dysfunction cause adipokine secretion dysregulation, including adiponectin, resistin, visfatin, TNF- α , and IL-6. Injection of IL-6 causes atherosclerosis, while TNF- α increases the expression of vascular adhesion molecules (7).

Cytokines have a role in leucocyte recruitment, endothelial adherence, and migration into inflamed vascular walls via activating adhesion molecules and chemokine expression (8). The balance of pro-and anti-inflammatory cytokines can also influence atherosclerotic plaque stability. Cytokines are tiny proteins released by the cells that make up the atherosclerotic plaque (9). Increased production of these cytokines activates a number of molecular/cellular pathways. Cytokines have critical roles in the immune system, hematopoiesis, and vascular function modification. Cytokines are involved in the function of smooth muscle cells, macrophages, T cells, and endothelial cells (ECs). They cause the development and progression of atherosclerosis by activating numerous signaling pathways (10, 11).

Interleukin (IL-6) is a pleiotropic cytokine that is produced by a variety of different cell types, including healthy cells, fibroblasts, endothelial cells, and tumor cells (12). Traditional signaling is thought to be engaged in the anti-inflammatory and regenerative effects of IL-6, whereas the trans-signaling is thought to be involved in the pro-inflammatory responses caused by this cytokine (13).

IL-6 is required for the initiation and maintenance of the inflammatory response in atherosclerosis. The IL-6 gene, which has 5 introns and 6 exons, is found on chromosome 7p21-24 (14). IL-6 is also important in the etiology of CAD, directly causing endothelial dysfunction, macrophage/monocyte activation, extracellular matrix breakdown, and indirectly increasing the manufacture of coagulation factors. In addition, IL-6 stimulates the production of additional inflammatory markers in the liver (15).

IL-6 stimulates autocrine and paracrine monocytes in the arterial wall, which adds to fibrinogen deposition. IL-6 also decreases lipoprotein lipase activity and monomeric lipoprotein lipase levels in plasma, which results in enhanced lipid absorption by macrophages and foam cell generation (16). Circulating IL-6 stimulates the hypothalamic-pituitary-adrenal axis, which is associated with central obesity, hypertension, and insulin resistance (17).

IL-6 is a critical mediator of atherosclerotic disease, and it has been associated with CAD and acute ischemia (18). IL-6, on the other hand, not only plays a role in the genesis of CAD but its levels may also be altered by CAD and its consequences. This cytokine is a promising noninvasive biomarker for monitoring CAD patients before and after a heart attack. Recent studies have shown associations between IL-6 levels and the severity of CAD (19, 20). Cardiovascular illness results in long-term cardiovascular mortality and progression to heart failure (18, 21, 22).

Methods

Sample collection: A case-control study was performed during the period from December 2020 to May 2021, the study was comprised of 30 patients diagnosed with CAD (17 males and 13 females), 30 positive controls (20 males and 10 females), and 30 negative controls (20 males and 10 females). In our study, the population's average age varied from 30 to 81 years old. Patients were visited the specialized center for cardiac catheterization and surgery in Al-Diwaniyah. Specialized doctors diagnosed all patients in this study, and the diagnosis was verified by clinical features, cardiac catheterization, history of patients, electrocardiogram (ECG), and biochemical investigations. These investigations include the lipid profile test. The record of patients consists of the following: Ages, genders, smoking, family history, antihypertensive drugs or drugs for other diseases, and body mass index. The practical of the current study was conducted in the Department of Medicinal Chemistry of College Medicine at Al-Qadisiyah University.

Exclusion criteria: The research excluded acute or chronic liver disease, renal disease, thyroid functions disorders, COVID-19, and autoimmune disease.

Blood collection: A 5 ml of blood was collected from each patient who participated in the study by vein puncture. After that, the blood was left 15 minutes stable at room temperature. The blood was separated by centrifuge at 11000 rpm for 5 minutes. Then, the serum was transferred into an Eppendorf tube, labeled the tube, and stored at - 80 °C till used. Body mass index (BMI) and waist to hip ratio (WHR) were measured using common ways (6, 24, 25).

Interleukin-6: Serum IL-6 level was estimated using Elabsience® kits as illustrated below:

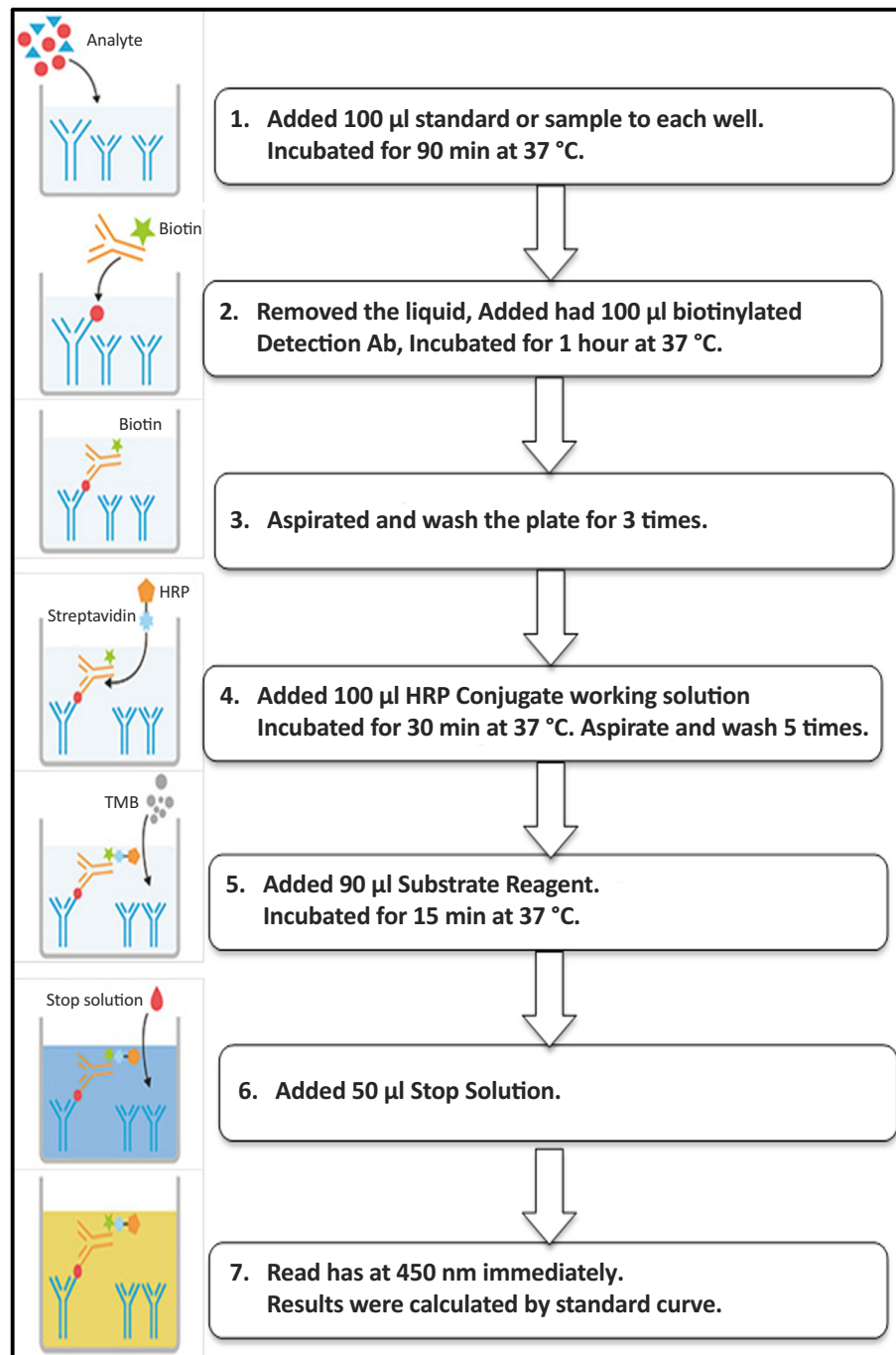


Figure 1. Method for assay procedure of IL-6 by using ELISA.

Statistical Analysis

Data were summarised, analyzed, and presented using GraphPad Prism 9.2.0 and Microsoft Office Excel 2013. Numeric data were expressed as mean \pm standard deviation. Whereas, categorical data were expressed as numbers. One-way ANOVA test and unpaired t-test were used to compare the mean values among the different groups in the cases of normally distributed variables. Chi-square was used to evaluate the qualitative data. Bivariate correlation was carried out using Pearson's correlation coefficient. P-value was considered significant at p -value ≤ 0.05 .

Results

All results present in this study were measured as mean \pm standard deviation (SD).

Table 1. Comparison characteristics of Demographic and IL-6 in coronary artery disease with positive control and negative control.

Characteristic	Coronary Artery Disease <i>n</i> =30	Positive Control <i>n</i> =30	Negative Control <i>n</i> =30	<i>p</i> -value
Age (years)				
Mean ± SD	58.125 ± 9.83	42.57 ± 12.18	40.77 ± 8.64	<0.0001 O HS
Range	39 – 81	30 – 67	30 – 55	
BMI (kg/m²)				
Mean ± SD	30.59 ± 4.91	29.76 ±4.57	28.42 ± 3.51	0.1539 O NS
Range	24.03 – 44.30	21.29 – 39.66	20.99 – 33.66	
WHR				
Mean ± SD	0.96 ± 0.061	0.97 ± 0.083	0.93 ± 0.074	0.0743 O NS
Range	0.85 –1.089	0.81 – 1.145	0.76 – 1.062	
Gender				
Male, <i>n</i> (%)	17 (56.67 %)	20 (66.67 %)	20 (66.66 %)	0.6582 CNS
Female, <i>n</i> (%)	13 (43.33 %)	10(33.33 %)	10 (66.66 %)	
IL-6 (pg/mL)				
Mean ± SD	128.11± 28.38	111.95±28.44	78.41 ± 19.70	<0.0001O HS
Range	92.29 -192.29	61.06 -155.14	15.96 – 97.80	

n: number of cases; SD: standard deviation; BMI: Body mass index; WHR: waist-hip ratio; IL-6: Interleukin-6 O: one-way ANOVA; C: chi-square test; NS: not significant at p -value > 0.05 ; HS: highly significant at p -value ≤ 0.01 .

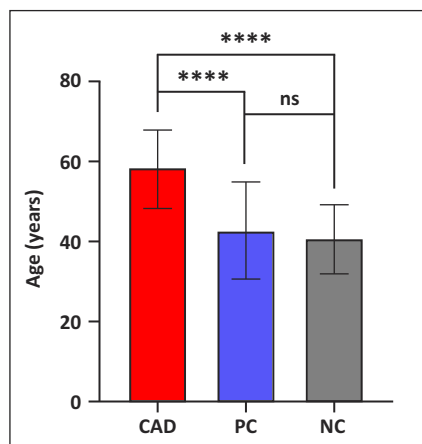


Figure 2. Estimation of the Age in patients with coronary artery disease (CAD) and positive control (PC) and negative control (NC). The figure shows a highly significant difference in patients with CAD compared with PC and NG, but no significant differences between NC and PC.

The results of this study were showed as the mean age of patients with CAD elderly (58.125 ± 9.83) years, but the age of PC younger was (42.57 ± 12.18) years, and that of NC was (40.77 ± 8.64) years. There was a highly significant difference in mean age between CAD, PC, and NC ($p < 0.0001$). At the same time, there was an insignificant difference between PC and NC, as shown in Figure (2).

The body mass index (BMI) kg/m^2 of patients with CAD was calculated to reach (30.59 ± 4.91) kg/m^2 , that of PC was (29.76 ± 4.57) kg/m^2 , and that of NC was (28.42 ± 3.51) kg/m^2 , and there was a difference among study group for the obesity measurement (MBI) but did not reach to significant difference as shown in Figure (3). A person's waist-to-hip ratio (WHR) measures their body proportionally to their hip circumference. Also, the WHR of patients with CAD was (0.96 ± 0.061), that of PC was (0.97 ± 0.083), and that of NC was (0.93 ± 0.074). The comparison among the study groups were showed no statistically significant differences as shown in Figure (4).

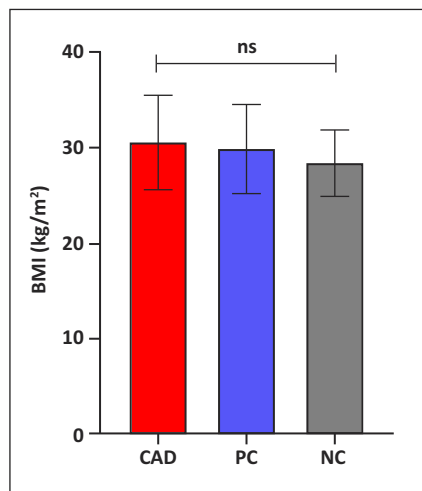


Figure 3. Estimation of mean body mass index (BMI (kg/m^2)) in patients with coronary artery disease(CAD) and positive control (PC) and negative control (NC).

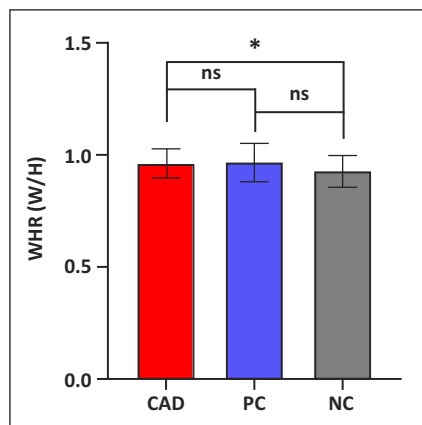


Figure 4. Estimation of waist-hip ratio (WHR) in patients with coronary artery disease(CAD) and positive control (PC) and negative control (NC).

The prevalence of CAD in men was considerably greater than in women. So, our results were indicated 17 (56.67 %) males and 13 (43.33 %) females. PC group was included a 20 (66.67 %) males and 10 (33.33 %) females and NC group was included a 20 (66.67 %) males and 10 (33.33 %) females. There was no significant difference in the frequency distribution of patients with CAD when compared with PC and NC, as shown in Figure (5).

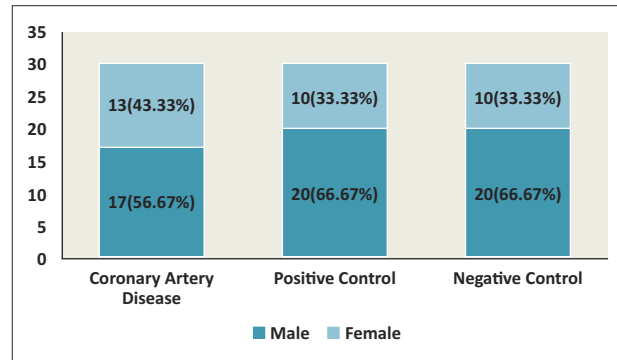


Figure 5. Bar chart shows the frequency distribution of patients with coronary artery disease and positive control and negative control according to gender.

The results of this study show increased levels of IL-6 (128.11 ± 28.38) pg/mL in patients with CAD as compared with PC and NC, (111.95 ± 28.44), (78.41 ± 19.70), pg/mL respectively. The results of our study showed a highly significant difference ($p < 0.0001$) in the concentrations of IL-6 as compared with PC. Also, a significant difference was present in mean values between CAD and PC ($p = 0.0289$). The results showed a highly significant difference in concentration of IL-6 in patients with CAD as compared to NC ($p < 0.0001$), There was a highly significant difference in the level of IL-6 in people between PC and NC ($p < 0.0001$). As shown in Figure (6).

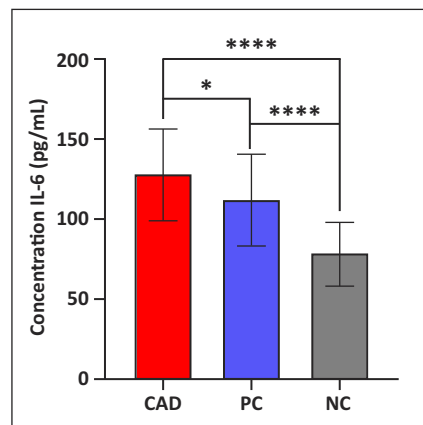


Figure 6. Estimation of cytokines concentrations Interleukin-6 (IL-6 pg/mL) in patients with coronary artery disease (CAD) and positive control (PC) and negative control (NC).

Discussion

In most industrialized countries, coronary artery disease (CAD) is caused predominantly by atherosclerosis of the coronary arteries and is one of the major causes of death. The prevalence of CAD varies according to age, gender, socioeconomic position, and other factors. Atherosclerosis is defined by a number of linked processes, including lipid dysregulation, thrombosis, inflammation, vascular smooth cell activation, platelet activation, endothelial dysfunction, oxidative stress, and genetic factors (26, 27). According to the current study's findings, the average age of the patients was 58.125 years, which was significantly older than the average age of PC 42.57 years and NC 40.77 years.

The findings are consistent with prior research (28), which discovered that the average age of patients with CAD was (55) years. Furthermore, the findings were consistent with the findings of research (29) and his colleagues, who discovered that the average age of patients with CAD was 64.72 years (55.6-73.8 years).

The findings of our investigation were confirmed by (30), who discovered that the elderly and the old population are especially vulnerable to CAD. The high prevalence of age-related CAD is caused in part by broader aging processes, such as accumulating morbidities, deteriorating homeostasis, and the long-term detrimental impact of CAD risk factors. Insidious alterations in CAD structure and function with increasing age are to blame for the illness (31). As we become older, our central arteries get stiffer, causing an increase in afterload stress, myocardial strain, and alterations in diastolic perfusion that can lead to functional declines as well as ischemia, heart failure, arrhythmias, and other CAD illnesses (32). Aortic impedance decoupling from ventricular pumping performance is increasing the prevalence of heart failure with an intact ejection fraction. Myocytes, endothelial cells, and pacemaker cells deteriorate with age, increasing the risk of coronary artery disease (CAD), heart failure, arrhythmias, peripheral artery disease, and cerebrovascular disease (CVD) (33). Each of these aging processes occurs at a different rate in different people, resulting in a wide range of CAD clinical outcomes. For some elderly people, CAD aging manifests itself simply as functional decrements. Because total CAD increases dramatically with age, cardiologists have the challenge of tailoring preventive and therapeutic priorities to each individual's circumstances (34). Our findings contradicted (35), which did not assist to explain the higher prevalence and severity of CAD described in the elderly.

Regarding BMI, the present study has revealed that the measurement of BMI in patients with CAD has significantly higher than that in the positive and negative control. These results have agreed with (9). They recorded that mean BMI in CAD patients was ≥ 25 and suggested that obesity is an independent risk factor for CAD in both genders. Also, the results agreed with (10), who found that BMI measurements have significantly associated with increased risk factors of cardiovascular and a higher risk profile. However, the study has disagreed with (11), who found a positive correlation between the severity of CAD with WHR but not with BMI. (12) reported that patients with higher levels of BMI were more likely to be associated with cardiovascular risk factors like hypertension, hypercholesterolemia, and diabetes mellitus.

The results of the current study were agreed with the survey (13), which recorded that the increased BMI has been associated with other significant health implications, including hypertension, diabetes mellitus, metabolic syndrome, and dyslipidemia, all of these independent risk factors for CAD. Our results were consistent with the study (14). They have showed that the people with increased BMI have greater prevalence, extent, and severity of CAD. The consequences of this study have inconsistent with the studies (15), (16), which have reported an inverse relationship between BMI and CAD.

The WHR has been used as an indicator or measure of health, fertility, and the risk of developing severe health conditions. (17). The present study did not find any significant difference between patients and each PC and NC. It disagreed with (11, 18, 19). They recorded a positive correlation between the severity of CAD with WHR. Also, the results disagree with (20), who found that the waist/height ratio was higher in patients with CAD; they observed that waist/height ratio was significantly correlated with the CAD.

CAD is a type of cardiovascular disease that is the leading cause of death globally and is responsible for most deaths among men and approximately one-third of all female deaths. However, this result may conflict with (21, 22), who have found that females are at higher risk of CAD development or progression than males. Also, these results have differed from the study (23), which exhibits that the absolute numbers of women living with and dying of CAD and stroke exceed those of men. Men generally develop CVD at a younger age and develop CAD than women (24), (25).

IL-6 is a pleiotropic cytokine that stimulates B-cell differentiation, thymocyte, and T-cell development, activates macrophages, stimulates hepatocytes to produce acute-phase proteins, and activates natural killer (NK) cells. IL-6 also has anti-inflammatory properties. By enhancing the activity of immunoregulatory cells during replication, IL-6 is anticipated to have a rejuvenating effect (54). IL-6 has both anti-inflammatory and pro-inflammatory properties. It is produced by immune cells and immune accessory cells such as monocytes and macrophages, as well as cardiovascular components like endothelium cells, vascular smooth muscle cells, and ischemic myocytes (55).

When patients with coronary artery disease were compared to positive and negative controls, the level of IL-6 was shown to be significantly higher in the current research. This conclusion is consistent with the findings of (56–58), who discovered that IL-6 is linked to atherosclerotic disease and primary cardiovascular outcomes, as well as the risk

of significant adverse cardiovascular events, cardiovascular mortality, and myocardial infarction (MI) (59). Serum IL-6 levels greater than 1 pg/ml in people with chest pain who were intermediate-risk (according to the atherosclerotic cardiovascular disease risk score) and were sent for coronary angiography were linked to severe CAD. Measuring IL-6 levels may be useful in reclassifying intermediate-risk persons into higher-risk categories (60).

According to research on IL-6 receptors, IL-6 blockage may give a unique therapy strategy for the prevention of coronary heart disease. To evaluate and find new therapeutic targets, large-scale clinical investigations and genetic testing in broad populations are still necessary (61, 62). Researchers discovered links between IL-6 levels and CAD severity, coronary events, mortality, and progression to heart failure in a meta-analysis (63). It was discovered that those who had pre-existing CAD had greater levels of IL-6 than those who did not have CAD and were unaffected by CAD treatment. However, none of these investigations discovered a link between IL-6 levels and the development of CAD. Inflammation has a significant influence on the progression of atherosclerosis. Several inflammatory biomarkers have been studied extensively and have been demonstrated to predict the development of CAD. Surprisingly, CRP is the most important inflammatory biomarker associated with an elevated risk of CAD development. CRP appears to be a less likely cause of CAD than IL-6. In their study, the area under the ROC curve of IL-6 for predicting CAD was larger than that of hs-CRP, indicating that it may be more accurate. This might be because IL-6 plays a more early and important role in the pro-inflammatory regulatory process (59).

In clinical situations characterized by tissue injury, such as infections, malignant neoplasms, ischemic disorders, and trauma, IL-6 production, a critical determinant of acute-phase protein synthesis, is increased. This pathophysiology may also explain why higher levels of inflammatory markers in the blood are associated with a higher risk of death (64). Much bench data from the same two decades has long suggested a variety of mechanisms linking IL-6 to plaque erosion and rupture, including activation of matrix metalloproteinases, which weaken the fibrous cap, activation of endothelial cells, which overexpress adhesion molecules, and induction of tissue factor, which leads to a prothrombotic environment (65).

IL-6 levels are not only related to the severity of the diseases, but they are also good predictors of future outcomes. Patients with unstable angina and a difficult in-hospital course, for example, had higher IL-6 levels than those who did not have problems (66). Pro-inflammatory cytokines may have a role in the breakdown and instability of atherosclerotic plaque in coronary arteries. The location of plaque rupture or erosion is marked by an inflammatory process, and pro-inflammatory cytokines increase the synthesis of matrix metalloproteinases, which are known to be involved in vascular remodeling and plaque disruption (67).

In terms of family history, genetic variables have a critical role in the susceptibility to coronary artery disease. Previously published research has demonstrated that various genetic variables have a significant influence on the development of these disorders. Because single nucleotide polymorphisms in the IL-6 gene promoter can change IL-6 expression and secretion, altering circulating levels may result in significant biological responses (68). Through circulation level variation, IL-6 might be regarded as a crucial regulator of cardiovascular disease beginning and progression (56, 69). carried performed research on 20 patients with acute coronary syndrome and 50 individuals with stable coronary artery disease They observed that greater IL-6 levels in the blood were linked to the development of atherosclerosis.

Conclusions

The serum levels of IL-6 were significantly elevated in patients with CAD as compared with the PC and NC.

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Conflict of Interest

The authors have no conflicts of interest regarding the publication of this article.

Adherence to Ethical Standards

The study was approved by the ethical committee at the University of Al-Qadisiyah (registration code CMUQ 3544 on 15.12.2020).

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