Original Article

Estimation of Predictive Cytokine Interleukin-18 Level in Serum of Ischemic Heart Disease Patients by Using Enzyme-Linked Immune Sorbent Assay

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Abstract

Background: Interleukin-18 (IL-18) is a pro-inflammatory marker with a challenging role in atherosclerosis. It is closely associated with atherosclerotic plaque instability and is, therefore, a good predictor of undesirable events in Acute Coronary Syndrome acute coronary syndrome (ACS). Described as an interferon-gamma-inducing factor by Th1 cells. **Objectives:** The present study was carried out aiming to estimate predictive cytokine IL-18 level in the serum of ischemic heart disease (IHD) patients. **Materials and Methods:** People diagnosed with IHD included 45 patients and 45 healthy individuals. Ages ranged from 40 to 73 years. The serum levels of IL-18 were then quantitatively assessed using the enzyme-linked immune sorbent assay (ELISA) technique. ELISA testing had been carried out in line with the manufacturer's instructions (MyBioSource). **Results:** Regarding the sex, the entire study's sample was male-dominant with male to female ratio of 1.7:1; cases and control groups were also male-dominant sex with a male-to-female ratio of 2:1 and 1.5:1, respectively, without significant differences ($P > 0.05$), which also reflecting the matching purpose of samples collection. In respect to the immunological parameters among the study's groups, it has been found that the mean level of IL-18 was significantly higher among the cases group (IHD) than that of the controls (1.92184 ± 0.420832 vs. 0.74107 ± 0.132552) (mol/L) with significant difference of 1.180778 ($t = 17.953$, df: 88, $P = 0.000$). **Conclusion:** In conclusion, IL-18 is a pleiotropic proinflammatory cytokine. Males were more frequently observed than females among IHD patients. The current study showed that the serum level of IL-18 among patients had significant relation with the number of coronary artery disease. The serum level of IL-18 can be identified as a strong independent predictor of death from cardiovascular causes in patients with CAD.

Keywords: Atherosclerosis, IL-18, ischemic heart disease

Introduction

Ischemic heart disease (IHD) is the most common type of heart disease, which causes substantial morbidity and incapacitation in the population. The problem of IHD is one of the leading healthcare issues of the twenty-first century.[1] IHD involves damage to the heart muscle caused by impaired blood flow in the coronary arteries. Myocardial ischemia is synonymous with atherosclerosis.[2]

Atherosclerosis is characterized by endothelial dysfunction, vascular inflammation, and the formation of atherosclerotic plaque. This buildup of atherosclerotic plaque causes an inadequate supply of oxygen to the myocardial tissue, leading to myocardial hypoxia. Consequently, the plaque rupture and atherothrombosis cause further narrowing of coronary arteries and almost occluding the blood flow, leading to fatal acute coronary syndromes.[3]

Pro-inflammatory cytokines (such as IL-1β, IL-6, IL-18, etc.) were produced and act upon immunocompetent cells, thereby initiating inflammatory responses. Many members of the research community make a point that increased

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levels of these cytokines reflect the activity and severity of the disease. The effects of cytokines were closely related to physiological (normal) and pathophysiological responses in the body. With that, there is a modulation of both local and systemic defenses.[4]

Interleukin 18 (IL-18) is a pleiotropic proinflammatory cytokine stimulating the production of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), IL-1, IL-2, cell adhesion molecules, and apoptosis-inducting factors, promoting proliferative activity of T-lymphocytes and lytic activity of natural killer cells (NK cells).[5]

In the Th1 paradigm, it works in concert with IL-12, whereas with IL-2 and without IL-12, it can stimulate the generation of Th2 cytokines from Th1 cells, NK cells, and NKT cells. IL-18 also plays a role in hemophagocytic lymphohistiocytosis, a life-threatening condition characterized by a cytokine storm that can be secondary to infections.[6]

It can be a pathogenetic factor in the development of diseases accompanied by acute and chronic inflammation, including atherosclerosis. In addition, elevated IL-18 levels are associated with other cardiovascular diseases (CVD), including acute coronary syndrome, type 2 diabetes, metabolic syndrome, and arterial hypertension. They are associated with CVD's adverse prognosis and high mortality.[7]

It is closely associated with atherosclerotic plaque instability and is, therefore, a good predictor of undesirable events in acute coronary syndrome (ACS).[8] Described as an IFN-γ inducing factor by Th1 cells.[9] IFN-γ plays a key role in atherosclerotic immune cell recruitment, foam cell formation, and plaque progression and stability.[10]

The proatherogenic effect of IL-18 can also occur in the absence of T cells—IFN-γ secreted by macrophages, NK cells, and Vascular Smooth Muscle Cell (VSMC) has been shown to be sufficient for disease progression. Differentiation of naive T cells to the Th1 phenotype is synergistically induced by IL-12 and IL-18.[7] Treatment with IL-18 inhibitors not only prevented plaque formation but also transformed it into a more stable plaque phenotype.[11]

Proinflammatory cytokine IL-18 is a proatherosclerotic cytokine. In patients with coronary artery disease (CAD), elevated IL-18 levels and IL-18 genetic variation have been associated with acute coronary events and cardiovascular death. Previously, it has been shown that IL-18 receptor subunits, as well as IL-18, are expressed in atherosclerotic lesions.[12] IL-18 has been associated with the development of subclinical atherosclerosis. The stability of atherosclerotic lesions appears to be influenced by IL-18 production in addition to the overall prevalence of atherosclerosis. The proatherogenic and atherosclerotic plaque destabilizing effects of IL-18 are most likely caused by the IL-18-dependent generation of IFN-c.[13]

Aim of the study:

to evaluate predictive cytokine IL-18 concentrations in the serum of IHD patients.

Materials and Methods

This investigation was conducted at a private laboratory. About 45 clinically diagnosed patients and 45 healthy controls from Ibn Al-Bitar Specialized Center for Cardiac Surgery in Iraq were included in the case study. Their ages ranged from 40 to 73. The samples were taken during the period from October 4 to December 31, 2022. The patient's consent was taken verbally for the process of collecting samples and conducting the research. Patients with any history of coronary artery bypass graft, percutaneous coronary intervention, severe valvular heart disease, and history of chronic heart failure or acute heart failure were excluded. Patients in the intensive care unit and in catheter lobbies. They all have CAD progression, all of them with angioplasty, diabetes mellitus, hypertension, and 90% of patients with smoking. All control individuals were basically healthy, with no CAD. All patients take drugs such as aspirin, insulin, crestor, heparin, and brilinta. Two groups made up the study groups. People diagnosed with IHD included 45 patients (30 males and 15 females) and 45 healthy individuals (27 males and 18 females). This study comprised all participants. Five milliliters of blood were drawn from the veins of each patient and the groups of healthy controls. A sample was placed in a gel tube and allowed to coagulate for around 30 min. Then, serum was isolated. The serum levels of IL18 were then quantitatively assessed using the enzyme-linked immune sorbent assay (ELISA) technique. ELISA testing had been carried out in line with the manufacturer's instructions (MyBioSource, USA). Each well's optical density was assessed using an ELISA reader at 450 nm, and the results for the concentrations of IL-18 were extrapolated from the standard curve.

Statistical analysis

In order to conduct the statistical analysis for this study, IBM SPSS version 25.0 were employed (Corporation, 2017). The data should be tested for normal distribution. Homogeneity and randomization were also calculated. Additionally, Pearson's chi-square test was used to establish the significant differences for nonparametric data, while mean and standard deviation, *t* test, table of ANOVA, and Pearson correlation were used to determine the distinctions that are substantial for parametric data.

The univariate logistic regression model and receiver operating characteristic (ROC) curve were used to identify the optimal cutoff value of immunological parameters of IL-18 as a predictive diagnostic marker for IHD. A *P* value of <0.05 was used as a statistical significance criterion throughout the study.

Ethical approval

Experimental designs were carried out based on the guidelines of the laboratory and according to the protocol approved by the Department of Microbiology, College of Medicine, Al-Iraqia University, Baghdad, Iraq, with the ethical clearance number 195 dated September 12, 2022.

Results

A total of 90 of 1:1 ratio of collected cases and controls samples were investigated, respectively, following inclusion and exclusion criteria. This current work included 45 patients and 45 healthy control groups. Regarding the sex, the entire study's sample was male-dominant with a male-to-female ratio of 1.7:1 (63.3%: 36.7%), as well as cases and control groups were also male-dominant sex with a male-to-female ratio of 2:1 (66.7%; 33.3%) and 1.5:1 (60%; 40%), respectively, without significant differences ($P > 0.05$) which also reflecting the matching purpose of samples collection [Table 1].

Age is the most potent independent risk factor for atherosclerosis. Aging causes changes in the walls of blood vessels, affecting the transport of oxygen and nutrients to the tissues. These changes make vessels stiffen, resulting in increased peripheral resistance. Over 85% of cardiovascular deaths occur in the elderly.[14]

With respect to the immunological parameters among the study's groups, it has been found that the mean level of IL-18 was significantly higher among the cases group (IHD) than that of the controls (1.92184 ± 0.420832 vs. 0.74107 ± 0.132552) with significant difference of 1.180778 (t = 17.953, df: 88, P = 0.000), respectively [Table 2] [Figure 1].

IL_18 as a predictive diagnostic test for ischemic heart diseases

Among a 90-study sample, the optimal cutoff value of IL-18 for detecting patients with high risk of developing IHDs was 216.67700 with a sensitivity of 97.8%, specificity of 84.1%, positive predictive value (PPV) of 97.81%, negative predictive value (NPV) of 97.72%, and excellent area under the ROC curve (AUC) of 0.997 ± 0.004 (P = 0.000) [Table 3] [Figure 2].

Discussion

The current study showed that the serum level of IL-18 among patients had significant relation with the number of CAD.

Data on baseline IL-18 concentrations were available for 90 patients. It has been found that the mean level of IL-18 was significantly higher among the cases group (IHD) than that of the controls (1.92184 ± 0.420832 vs. 0.74107 ± 0.132552) with a significant difference of 1.180778 (t = 17.953, df: 88, P = 0.000), respectively [Table 2].

The serum level of IL-18 can be a significant independent predictor of cardiovascular mortality in patients with

Table 1: Baseline characteristics of the study's sample (n = 90)

Characteristics	Study groups (IHD)			
	Cases (n = 45)	Control (n = 45)	Total (n = 90)	Significancy
Age (years)				
Mean (years) ± SD	57.29 ± 7.516	58.49 ± 7.683	57.89 ± 7.581	t = -0.749, df: 88, P = 0.456[a]
Range (min–max)	33 (40–73)	30 (40–70)	33 (40–73)	
Age (in groups)				
≤46	3 (6.7)	5 (11.1)	8 (8.9)	χ^2: 7.204, df: 4, P = 0.125[b]
47–53	14 (31.1)	4 (8.9)	18 (20)	
54–60	13 (28.9)	17 (37.8)	30 (33.3)	
61–67	11 (24.4)	15 (33.3)	26 (28.9)	
>67	4 (8.9)	+4 (8.9)	8 (8.9)	
Gender				
Female	15 (33.3)	18 (40)	33 (36.7)	χ^2: 0.431, df: 1, P = 0.512[b]
Male	30 (66.7)	27 (60)	57 (63.3)	

[a] Unpaired T-test

[b] Chi-square test

Table 2: Mean comparison of immunological parameter of interlukin-18 among study's groups (n = 90)

Immunological parameters (mean ± SD)	Study groups (IHD) (n = 90)		Mean differences	Significance [a]
	Cases (n = 45)	Control (n = 45)		
Interlukin-18	626.93653 ± 175.824542	181.56307 ± 34.245192	445.373467	t= 16.679, df:88, **P = 0.000**

[a]: Unpaired t test

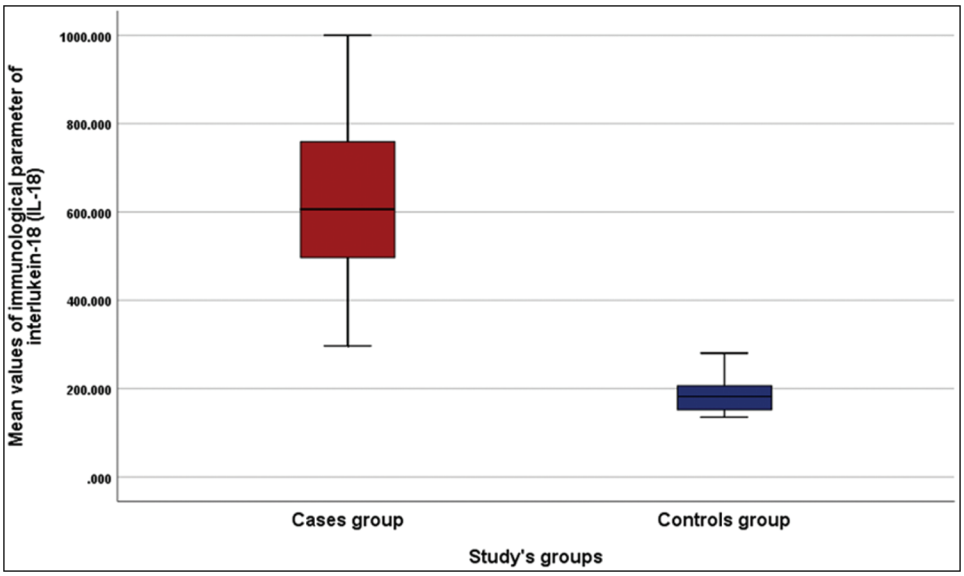

Figure 1: Mean comparison of immunological parameter of interlukin-18 among study's groups (n = 90)

Table 3: Predictive value of interleukin-18 (IL-18) for diagnosis of ischemic heart diseases among the study's sample (n =90)

Paramter	Validity of model						
	Sensitivity (Sn)	Specificity (Sp)	Positive predictive value (PPV)	Negative predictive value (NPV)	Accuracy	Area under the curve (AUC)	Significancy (P value)
Interlukine-18 (IL-18)	97.8	84.1	97.81%	97.72%	97.8	0.997	0.000

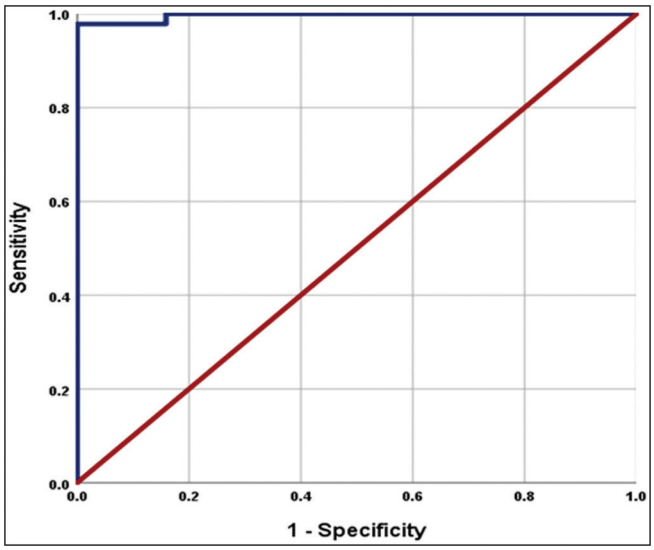

Figure 2: Receiver operating characteristic curve of ischemic heart diseases predicted by the immunological parameter of interleukin-18 (IL-18) among the study's sample (n = 90)

CAD, regardless of the clinical status at admission. This result strongly supports the experimental evidence of IL-18-mediated inflammation, allowing the acceleration and vulnerability of atherosclerosis.[8]

Higher IL-18 levels were consistently associated with male sex, higher BMI, diabetes, decreased renal function, and other inflammatory biomarkers. The IL-18 level at baseline was significantly associated with CV mortality independent of clinical characteristics and indicators of renal and cardiac dysfunction.[15]

Due to its pleiotropic characteristics, IL-18 is crucial for numerous signaling pathways, making it a key role in the development of atherosclerosis and a highly desirable research target. According to the cytokine milieu, the distinct cytokine IL-18, which has been studied in this work, can start a chain reaction of proinflammatory cytokines and activate Th1 or Th2 response. These two mechanisms are essential for accelerated atherosclerosis caused by pro-oxidants, advanced glycation products, immune response activation, and retention of circulating cytokines, which contribute to the pro-inflammatory state when renal function declines.[16] IL-18, which was first shown to be a factor that stimulates the production of IFN-γ, was discovered to be highly expressed in human coronary plaques and to be the cause of the destabilization of those plaques. Additionally, it was established in one study that young and middle-aged patients who had recently experienced an acute myocardial infarction had higher IL-18 concentrations in serum than age- and sex-matched control participants, demonstrating that concentration of this substance is higher in these patients and associated with the severity of coronary atherosclerosis.[17]

IL-18 and its receptors were detectable in cardiomyocytes in patients with CAD and surrounded by inflammatory infiltrates. It appears that the ischemic insult with the inflammatory response of this state is the main stimulator of IL-18 expression since increased mRNA of IL-18 concentrations were found in the serum of patients with CAD. The conversion of IL-18 to its active form by caspase-1 was boosted in the myocardium suggesting pathophysiological mechanisms of IL-18 have been involved in CAD.[18-21]

Conclusion

In conclusion, IL-18 is a pleiotropic proinflammatory cytokine. Males were more frequently observed than females among IHD patients. The current study showed that the serum level of IL-18 among patients had significant relation with the number of CAD. The serum level of IL-18 can be identified as a strong independent predictor of death from cardiovascular causes in patients with CAD.

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Conflicts of interest

There are no conflicts of interest.

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