

## Serum interleukin-18 and extent of coronary artery disease in unstable angina

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## Original Article

## Abstract

**BACKGROUND:** Interleukin-18 (IL-18) is an inflammatory marker with challenging role in atherosclerosis. The present study was carried out aiming to evaluate the association between IL-18 serum level and extent and severity of atherosclerosis among young patients with unstable angina (UA) who underwent coronary angiography.

**METHODS:** This cross sectional study was performed from July to October 2015 in Chamran heart center, Isfahan, Iran. 180 patients with UA in the age range of below 50 years entered the study. All demographic, past history, physical examination, electrocardiogram (EKG or ECG), and transthoracic echocardiogram (TTE) data were collected. Serum level of IL-18 was measured using enzyme-linked immunosorbent assay (ELISA) method. A coronary angiography was performed on all patients to evaluate the presence and the incidence rate of coronary artery disease (CAD).

**RESULTS:** Mean age of the patients was  $46.0 \pm 4.6$  years [ $47.4 \pm 4.3$  and  $45.9 \pm 4.9$  among patients with CAD and normal coronary, respectively ( $P = 0.040$ )]. Rate of severe CAD was greater among men compared to women with values 67.8% and 51.8%, respectively ( $P = 0.032$ ). The median [interquartile range (IQR)] value of serum IL-18 among patients with CAD [192.86 (128.03,325.75)] was higher than normal coronary subjects [172.81 (139.77,243.21)], however it was not significant ( $198.4 \pm 93.5$ ,  $P = 0.287$ ). A significant difference between serum IL-18 level and number of stenosis vessels was detected only among women ( $P = 0.032$ ).

**CONCLUSION:** Serum IL-18 level can predict the number of coronary arteries with significant stenosis among women with unstable angina.

**Keywords:** Interleukin-18, Unstable Angina, Atherosclerosis, Angiography

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## Introduction

Coronary artery disease (CAD) is the main cause of mortality in developed and under developing countries.<sup>1</sup> With increasing age, atherosclerosis is aggravated and finally causes clinical scenarios like stable and unstable angina (UA) and acute myocardial infarction (MI). It is estimated that atherosclerosis will be the first cause of death in the entire world by 2020.<sup>2</sup> Atherogenesis is a lifelong process.<sup>3,4</sup> It is documented that atherosclerosis and its most dramatic consequence, MI, is an inflammatory process.<sup>5,6</sup>

Beside traditional risk factors and behavioral factors like stress, inflammatory markers have

recently been proposed to have diagnostic and prognostic role in atherosclerosis and their association with CAD severity is controversial.<sup>7-9</sup> Interleukin-18 (IL-18), of the cytokine family, is a proinflammatory cytokine which is expressed mainly by macrophages and acts on its receptor on the membrane of endothelial cells, lymphocytes, smooth muscle cells (all components of the atherosclerotic plaque) and induces Interferon-gamma (IFN- $\gamma$ ) production, endothelial dysfunction and plaque instability.<sup>10,11</sup> Some studies have shown increased IL-18 mRNA expression in carotid unstable plaques and it has been shown that increased serum level of IL-18 is associated with

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increased carotid intima media thickness (CIMT).<sup>12,13</sup> In a prospective study, IL-18 was independent predictor of cardiovascular mortality with 3.3 fold increased risk of death among stable and unstable patients with high level of serum IL-18.<sup>14</sup>

Regarding the lack of data on the role of IL-18 in severity of atherosclerosis, the authors in the present study aimed to evaluate the association of serum IL-18 level with CAD presence and extent among young patients with UA.

## Materials and Methods

This was a cross sectional study performed from July to October 2015 in Chamran heart center, Isfahan, Iran. The inclusion criteria were patients younger than 50 years old referring to the hospital with diagnosis of UA. UA diagnosis was defined as the new-onset angina pectoris during the recent 4 weeks, angina pectoris lasting more than 10 minutes at rest or with minimal exertion, and crescendo pattern of known previous stable angina, all with or without dynamic ST segment depression or T wave inversion in electrocardiogram (EKG or ECG).

Patients with any history of MI, coronary artery bypass graft (CABG), percutaneous coronary intervention (PCI), severe valvular heart disease (VHD), severe uncontrolled systemic hypertension (systolic blood pressure  $\geq 220$  mmHg, diastolic blood pressure  $\geq 130$  mmHg), severe pulmonary arterial hypertension (PAH) (mean pulmonary artery pressure  $> 40$  mmHg), and history of chronic heart failure or acute heart failure were excluded. The study was approved by the Institutional Bioethics Committee (IEC), and informed written consent was obtained from all the patients. All patients meeting the inclusion and exclusion criteria entered the study protocol. All patients underwent complete history taking, physical examination, and transthoracic echocardiogram (TTE) by a cardiologist. Serial ECGs (each 30 minutes up to 3 times and during chest pain) were taken and interpreted by the same cardiologist. 5 ml of blood was taken via a peripheral venous line from all patients before catheterization procedure. After separation of serum, it was transmitted to the  $-70$  °C laboratory medium to measure the level of IL-18 with enzyme-linked immunosorbent assay (ELISA) method (using Humal IL18 ELISA BMS 267.2 and BMS 267.2 ten, Mender med system).

Hypertension was defined as having systolic blood pressure at least 140 mmHg, and/or diastolic blood pressure at least 90 mmHg, or being on antihypertensive drugs.<sup>15</sup> Fasting blood glucose  $\geq 126$  mg/dl or 2-hour postprandial plasma glucose

$\geq 200$  mg/dl or using anti-diabetic agents was defined as diabetes mellitus (DM).<sup>7</sup> Dyslipidemia was signified as total cholesterol  $> 200$  mg/dl, triglyceride  $> 150$  mg/dl, low-density lipoprotein (LDL)  $> 100$  mg/dl, or high-density lipoprotein (HDL)  $< 35$  mg/dl.<sup>7</sup> Patients who smoked daily were considered as current smokers.<sup>16</sup>

According to the American college of cardiology (ACC)/American heart association (AHA) guidelines for management of patients with non ST elevation acute coronary syndrome (NSTEMI-ACS), all the patients in the present study underwent coronary angiography via right femoral artery approach with Siemens system.<sup>17</sup> Angiography film was reported with two cardiologists who were blinded about the patient history and study protocol in different times.

In case of the lack of stenosis among patients, they were placed into the CAD-free group. Extent of CAD was defined as more than 75% stenosis of any of coronary arteries or branches and  $\geq 50\%$  stenosis of left main coronary artery (LMCA).<sup>18</sup>

The data were reported as rate (%) and mean  $\pm$  standard deviation (SD) or median [interquartile range (IQR)] for qualitative and quantitative data, respectively. Kolmogorov-Smirnov (K-S) test was performed to check the assumption of normality of distribution of the quantitative variables. Mann-Whitney test, independent sample t-test, and Kruskal-Wallis test were performed to assess the group differences in quantitative variables (where appropriate) and the chi-square test was used to evaluate and perform comparisons among the qualitative variables. Multinomial logistic regression model was performed to evaluate IL-18 quartile changes (trend of quartiles) among patients with different number of involved vessels in comparison to participants without any involved vessels. Statistical analysis was performed with SPSS software (version 15.0, SPSS Inc., Chicago, IL, USA). The results were assumed to be statistically significant if  $P \leq 0.050$ .

## Results

In this study, 180 patients with UA underwent coronary angiography. Patients were divided into two groups based on presence of CAD. 107 (59.9%) and 69 (40.1%) of the subjects had CAD and normal coronary angiogram, respectively. Mean age of the patients was  $46.0 \pm 4.6$  years ( $47.4 \pm 4.3$  and  $45.9 \pm 4.9$ ,  $P=0.040$  among patient with and without CAD, respectively). Severity of CAD was greater among men than women ( $P = 0.032$ ).

**Table 1.** Demographic characteristics of subjects

Variables	CAD (n = 107)	CAD-free (n = 73)	P
Age (years) [mean ± SD]	47.4 ± 4.3	45.9 ± 4.9	0.040*
Men [n (%)]	59 (57.3)	28 (40.6)	0.032**
Hypertension [n (%)]	38 (36.9)	16 (23.2)	0.058**
DM [n (%)]	29 (28.2)	7 (10.2)	0.004**
Dyslipidemia [n (%)]	50 (48.5)	20 (29.0)	0.010**
Smokers [n (%)]	35 (34.0)	14 (20.3)	0.051**
Family history of CAD [n (%)]	55 (53.4)	39 (56.5)	0.687**

SD: Standard deviation; DM: Diabetes mellitus; CAD: Coronary artery disease

\* Independent t-test; \*\* Chi-Square test

Table 1 shows the incidence of cardiovascular risk factors in both groups. There was no significant difference in atherogenic risk factors like hypertension, smoking habits, and family history of CAD between the two groups. However, the incidence of DM and dyslipidemia was greater in CAD group ( $P < 0.050$ ).

Median (IQR) of serum IL-18 (picog/ml) in CAD group was higher in comparison with the CAD-free group. This difference was not significant [192.9 (128.0,325.7) vs 172.8 (139.8,243.2),  $P = 0.287$ ].

There was no significant difference in median (IQR) of serum IL-18 (picog/ml) levels based on the atherosclerotic risk factors except in participants with no family history of CAD ( $P = 0.027$ ) (Table 2).

The serum level of IL-18 (picog/ml) differed significantly based on the number of involved vessels in women, but not in overall nor in men (Table 3).

Odds ratios 95% confidence interval [OR (95% CI)] of IL-8 quartile changes among patients with different number of involved vessels in comparison to those without any involved vessels was shown in table 4. There was no significant difference between OR (95% CI) of IL8 quartile changes among patients with different involvement in comparison

to normal participants, in addition, adjustment for age, sex, DM, hypertension, dyslipidemia was performed (Table 4).

## Discussion

This study showed that the serum level of IL-18 among patients with UA had significant relation with the number of coronary artery stenosis among women. In a basic animal study in 2002, Elhage et al. had noted IL-18 as a risk factor for atherosclerosis.<sup>19</sup> Chen et al. had shown that there was a direct relationship between serum IL-18 level and CAD severity among patients with UA as defined with a validated score.<sup>20</sup> Positive family history was correlated significantly to serum level of IL-18 among patients with CAD. This was in contradiction with the present study as there was no significant correlation between cardiovascular risk factors and IL-18. Blankenberg et al. in a prospective 5-year study on 10600 European men documented that serum IL-18 at baseline was a predictor for cardiovascular events and angina pain.<sup>21</sup> Although this study was performed only among men, it provided strong evidence for prognostic value of IL-18 as an inflammatory marker for cardiovascular events.

**Table 2.** Median interquartile range (IQR) for serum level of interleukin-18 (IL-18) (picog/ml) based on atherosclerotic risk factors among patients with and without unstable angina (UA)

Risk factors	CAD	CAD-free	P*
Hypertension	206.5 (136.6, 333.5)	230.4 (164.6, 305.2)	0.992
Lack of hypertension	178.1 (122.8, 308.4)	166.6 (130.5, 222.9)	0.373
DM	226.5 (129.5, 337.9)	170.7 (137.0, 286.6)	0.754
Lack of DM	186.7 (126.8, 292.8)	173.3 (140.4, 239.3)	0.441
Dyslipidemia	201.9 (130.5, 326.2)	170.2 (143.4, 250.5)	0.463
Lack of dyslipidemia	179.1 (125.5, 314.2)	173.8 (131.3, 242.1)	0.517
Smoking	179.1 (120.6, 343.9)	170.7 (143.0, 289.7)	0.737
Lack of smoking	195.5 (131.0, 313.5)	173.3 (122.5, 231.6)	0.195
Family history of CAD	162.9 (122.1, 247.1)	169.7 (143.8, 249.9)	0.470
Lack of family history of CAD	237.9 (138.1, 432.7)	181.2 (103.6, 243.2)	0.027

DM: Diabetes mellitus; CAD: Coronary artery disease

\* Mann-Whitney U test

**Table 3.** Median interquartile range (IQR) of serum level of interleukin-18 (IL-18) (picog/ml) based on the number of vessels involved and sex

Sex	Involvement	Median (IQR)	P*
Men	Normal vessels	173.3 (142.6, 270.1)	0.962
	One vessel	178.6 (117.7, 310.6)	
	Two vessels	159.8 (131.0, 327.6)	
	Three vessels	203.7 (123.2, 270.1)	
Women	Normal	168.6 (117.7, 233.7)	0.032
	One vessel	218.8 (138.5, 331.2)	
	Two vessels	261.3 (180.1, 338.5)	
	Three vessels	131.0 (114.1, 156.4)	
Total	Normal	172.8 (139.7, 243.2)	0.284
	One vessel	196.1 (126.5, 328.5)	
	Two vessels	206.5 (141.2, 333.5)	
	Three vessels	137.3 (122.5, 231.9)	

\* Kruskal-wallis test

IQR: Interquartile range

Corson reported the relationship of IL-18 with cardiovascular events as well.<sup>22</sup> Interleukin-6 (IL-6), another inflammatory marker from cytokines family, was evaluated in the study by Gotsman et al.<sup>23</sup> They stated that there was significant correlation between serum level of IL-6 and CAD severity. However, they did not evaluate the number of diseased epicardial vessels in contrast to the present study. IL-6 and IL-18 are from the same cytokines family, hence the results of the former study may be expanded to other interleukins (ILs). Mallat et al. evaluated the role of IL-18 in a wider group of patients with acute coronary syndrome (ACS) including UA and patients with acute MI.<sup>24</sup> They confirmed elevated level of IL-18 among these patients and showed its strong direct relationship with severity of left ventricular systolic dysfunction. Ridker and Silvertown proposed that inflammatory markers had significant effect on progression of CVD and taking strategies to reduce these markers

would decrease their burden in the future.<sup>25</sup>

### Conclusion

Serum IL-18 level can predict the number of coronary arteries with significant stenosis among women with UA. We recommend a larger trial in both sexes in a longitudinal cohort study that can predict estimation power of IL-18 for cardiovascular events.

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### Conflict of Interests

Authors have no conflict of interests.

**Table 4.** Odds ratios 95% confidence interval [OR (95% CI)] of interleukin-18 (IL-18) (picog/ml) quartile changes among patients with different number of involved vessels in comparison to those without any involved vessels

Involvement	Crude		Adjust 1		Adjust 2	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Normal vessels	1*	-	1*	-	1*	-
One vessel	1.14 (0.84-1.56)	0.386	1.13 (0.82-1.57)	0.440	1.06 (0.75-1.5)	0.718
2 vessels	1.29 (0.83-2.006)	0.258	1.30 (0.83-2.03)	0.245	1.20 (0.75-1.92)	0.432
3 vessels	0.87 (0.52-1.43)	0.593	0.86 (0.51-1.44)	0.578	0.81 (0.48-1.37)	0.444

\* Participants without any involved vessels are considered as the reference group.

OR (95% CI): Odds ratios 95% confidence interval; Crude: Without adjustment; Adjust 1: Age-sex adjustment; Adjust 2: Age-sex-diabetes-hypertension-dyslipidemia adjustment; Multinomial logistic regression analysis was performed.



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