Interleukin-8 and Interleukin-10 in Patients with Ischemic Heart Disease

Muayad Khalid Abdulkareem*, Aida R. Al.derzi ** ,Basil N. Saeed ***

ABSTRACT:

BACKGROUND:

Ischemic heart disease is the main cause of death throughout the world. Cytokines are key regulatory glycoproteins . They are intimately associated with atherogenesis and modulate plaque morphology and stabilization.

OBJECTIVE:

To study the role of pro-inflammatory Interleukin-8 , and anti-inflammatory interleukin-10 in patients with Acute coronary syndromes (ACS)(acute myocardial infarction and unstable angina).

METHODS:

This study included (55) patients with acute coronary syndrome, (30) patients with acute Myocardial infarction (24 males and 6 female) and 25 patients with unstable Angina (11 males and 14 females). This project has been carried out on patients at the coronary care unit (CCU) of Baghdad teaching hospital/ Medical City/ Baghdad, during the period from April to June 2010. A twenty five, apparently healthy individuals were taken as a healthy control group. Venous blood sample was taken from each subject. Levels of interleukin-8 and Interleukin-10 were estimated by using enzyme linked immunosorbant assay . {ELISA-kit for detection of IL-8 (BioSource Europe S.A. /catalogue number; KAP1301)} and {ELISA-kit for detection of IL-10 (BioSource Europe S.A./ catalogue number; KAP1321)}

RESULTS:

Plasma level of pro-inflammatory cytokine (interleukin-8) showed a significant elevation in patients with acute myocardial infarction (Mean ± SD =95.26 pg/ml ± 102.89 ;P value=0.004) and unstable angina (Mean ± SD =146.43 pg/ml ± 154.45 ;P value=0.019) in comparison to control group (Mean ± SD =47.75 pg/ml ± 36.67). Serum level of anti-inflammatory cytokine (interleukin-10) showed a significant decrease in unstable angina patients (Mean ± SD =16.95 pg/ml ± 11.92 ;P value=0.012) in comparison to control group (Mean ± SD =30.29 pg/ml ± 21.17),however, it showed no significant differences among acute myocardial infarction patients (Mean ± SD =27.73 pg/ml ± 23.16 ;P value=0.500) in comparison to control group. The ratio of pro-/anti-inflammatory cytokines (interleukin-8 / interleukin-10) was significantly elevated in acute coronary syndrome; acute myocardial infarction and unstable angina patients (P=0.001, P=0.019, P=0.001 respectively) .

CONCLUSION:

Interleukin-8 is an important pro-inflammatory cytokine in coronary artery disease which may contribute to the plaque destabilization in acute coronary syndrome especially unstable angina, and low level of anti-inflammatory interleukin-10 which is considers as a risk factor of development of acute events in unstable angina.

KEY WORDS: acute coronary syndromes, cytokines , interleukin-8 , interleukin-10.

INTRODUCTION:

Ischemic heart disease (IHD) is the main cause of death throughout the world, it responsible for more than seven million deaths per year [(1)], and in the near future it is predicted to be the leading cause of death worldwide [(2)] .The disease includes a wide spectrum of conditions, ranging from silent ischemia and exertion-

* Falluja General Hospital, Anbar , Iraq.
**Department of Microbiology ,College of Medicine, University of Baghdad, Iraq.
***Department of Medicine ,College of Medicine, University of Baghdad, Iraq.

induced angina, through unstable angina(UA), to acute myocardial infarction(AMI)[3].

Cytokines are key regulatory glycoproteins allied to inflammatory/immunological processes which modulate all aspects of vascular inflammation by altering the proliferation, differentiation and function of an extensive array of cell types[4]. Interleukin-10 is an acid-labile anti-inflammatory cytokine that limits and controls inflammatory response [5]. Interleukin-8 is a pro-inflammatory cytokine whose principal role in infection and inflammation appears to be the recruitment and activation of circulating and tissue neutrophils to the site of tissue damage [6]. IL-8 is produced by macrophages and other cell types such as vascular endothelium and can be secreted by any cells with toll-like receptors which are involved in the innate immune response[7,8,9]. Interleukin-10 is an anti-inflammatory cytokine that limits and controls inflammatory response [5]. It is secreted mainly by Th2 (CD4+), regulatory T cells, and activated macrophages that blocks activation of cytokines synthesis by Th1 cells, activated monocytes /macrophages and NK (natural killer) cells [10,11]. IL-10 is an example of a negative feedback regulator and a powerful immunosuppressants [12,13]. The biologic effects of IL-10 result from its ability to inhibit many of the functions of activated macrophages like (inhibiting the production of IL-12 by activated macrophages and dendritic cells). This cytokines is a critical stimulus for IFN-y secretion and is an inducer of innate and cell-mediated immune reactions against intracellular microbes. IL-10 functions to down-regulate all such reactions. Furthermore IL-10 inhibits the expression of co stimulators and class II major histocompatibility complex (MHC) molecules on macrophages and dendritic cells. Because of these actions, IL-10 serves to inhibit T cell activation and terminate cell-mediated immune reactions[11]. Thus the aim of this study is to determine level of IL-8 and IL-10 in acute coronary syndrome.

PATIENTS AND METHODS:

This project has been carried out on patients at the coronary care unit (CCU) of Baghdad teaching hospital/ Medical City/ Baghdad, during the period from April to June 2010. The diagnosis in every patient was done by a specialist in cardiology based on clinical presentation and history of ischemic heart disease, which was confirmed by ECG and cardiac enzymes .This study included (55) patients with ischemic heart disease, (30) patients with acute myocardial infarction (24 males and 6 female) and (25) patients with unstable angina (11 males and 14 females). These patients were admitted from Baghdad and different other Iraqi cities. A twenty five, age and sex matched, apparently healthy individuals were taken as a healthy control group. Venous blood sample was taken from each subject . Levels of interleukin-8 and interleukin-10 were estimated by using enzyme linked immunosorbant assay. Solid phase (sandwich) ELISA tests were used for estimation and quantitative determination of very low concentrations of IL-10 serum levels and IL-8 plasma levels. The two assays used monoclonal antibodies (MAbs) directed against distinct epitopes of IL-10 and IL-8. The plate was read at 450 nm against a reference filter set at 650 nm. The amount of substrate turnover was determined colourimetrically by measuring the absorbance, which is proportional to the IL-8 and IL-10 concentration. A calibration curve was plotted and IL-8 and IL-10 concentration in samples is determined by interpolation from the calibration curve (Figure 1) and (Figure 2).{(Catalogue numbers; KAP1321; IL-10-EASIA, BioSource Europe S.A.) and (KAP1301; IL-8-EASIA, BioSource Europe S.A.)}. The laboratory works have been done in Immunology Department of the Teaching Laboratory/ Baghdad Medical city.

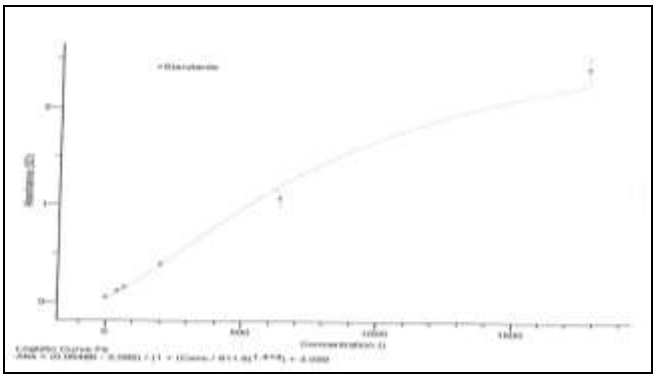

Figure 1:IL-8 logistic curve fit.

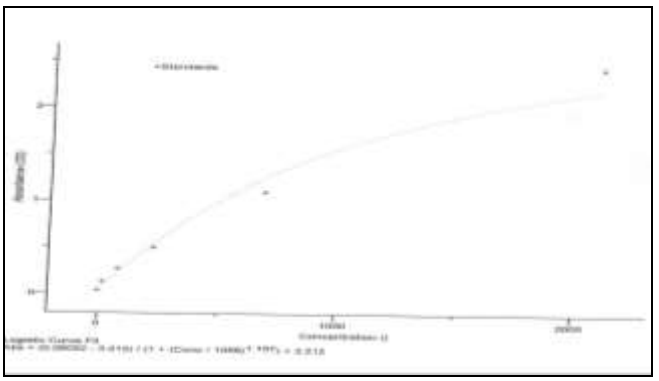

Figure 2 :IL-10 logistic curve fit.

STATISTICAL ANALYSIS:

Statistical Package for Social Science Version 17 (SPSS.v17) was used for data input and analysis. Discrete variables were expressed as number and percents and continuous variables as means ± standard deviation. Pearson chi square test for independence was used to test the association between discrete variables; Mann-Whitney was used as appropriate. To test the significance of difference between independent continuous variables: t test was used when the variables followed normal distribution, otherwise the significance of difference between two groups was assessed by non-parametric test (Mann-Whitney test).

In order to determine the optimal cut-off values and diagnostic performance of these IL-8 & IL-10, receiver operating characteristic curves (ROC) analysis were performed. ROC analysis was used to show an idea about the usefulness of the test and helps in comparing it to other tests. The closer the area to one (ideal test) the more valid it is. The larger the area under the curve (closure to one) the more valid the test, since there is a great gain in sensitivity for minimal loss in specificity. Optimal cut-off points for these risk factors were determined based on the convergence of sensitivity and specificity. Findings with P value of 0.05 or less were considered significant.

RESULTS:

Results showed that, acute coronary syndrome were common among male patients (Table 1). Mean age of patients with acute myocardial infarction and unstable angina was 58.7 and 55.1 years respectively and the majority of patients who developed UA and AMI were above the age of 40 years and there was a significant increase in the frequency of IHD with increasing age (Table 2). The risk factors that accelerate the incidence of acute coronary syndrome were studied. Concerning acute myocardial infarction and unstable angina, 50% and 76% of patients were hypertensive, 46.7% and 32% diabetic, 70% and 40 smokers ,13.3%

and 12% excess alcoholic and 56% and 60% had a positive family history of Ischemic heart disease respectively (Table 3) and (Figures 3,4,5,6). Plasma level of pro-inflammatory cytokine (interleukin-8) showed a significant elevation in patients with acute myocardial infarction (Mean ± SD =95.26 pg/ml ± 102.89 ;P value=0.004) and unstable angina (Mean ± SD =146.43 pg/ml ± 154.45 ;P value=0.019) in comparison to control group (Mean ± SD =47.75 pg/ml ± 36.67) (Table 4 and Table 5) and (Figures7,8,9). Serum level of anti-inflammatory cytokine (interleukin-10) showed a significant decrease in unstable angina patients (Mean ± SD =16.95 pg/ml ± 11.92 ;P value=0.012) in comparison to control group (Mean ± SD =30.29 pg/ml ± 21.17),however, it showed no significant differences among acute myocardial infarction patients (Mean ± SD =27.73 pg/ml ± 23.16 ;P value=0.500) in comparison to control group. The ratio of pro-/anti-inflammatory cytokines (interleukin-8 / interleukin-10) was significantly elevated in acute coronary syndrome; acute myocardial infarction and unstable angina patients (P=0.001, P=0.019, P=0.001 respectively) (Table 4,5) and (Figures 7,8 , 9).

Table 1: Frequency Distribution of Study Sample by Gender.

	AMI	UA		
	N = 30 (N%)	N = 25 (N%)	X^2	P value
Gender				
Male	24 (80.0)	11 (44.0)	7.868	0.020
Female	6 (20.0)	14 (56.0)		

N; number, %; percent, AMI; acute myocardial infarction, UA; unstable angina, X^2; chi square test statistic,.

Table 2: Frequency Distribution of study Sample by Age.

	AMI	UA		
	N = 30 (N%)	N = 25 (N%)	X^2	P value
Age Group (year);				
>40	28 (93.3)	23 (92.0)	10.440	0.005
≤40	2 (6.7)	2 (8.0)		

N; number, %; percent, AMI; acute myocardial infarction, UA; unstable angina, X^2; chi square test statistic,.

Table 3: Frequency Distribution of Patients with Acute Coronary Syndrome According to Certain Risk Factors.

	Type of Study Group				
	Acute Myocardial Infarction	Unstable Angina	Control	X^2	P
Cardiovascular Risk Factors	N=30 (N%)	N=25 (N%)	N=25 (N%)		
Hypertension	15 (50.0)	19 (76.0)	0 (0.0)	30.650 ^{B}	0.000
Diabetes mellitus	14 (46.7)	8 (32.0)	0 (0.0)	15.264 ^{B}	0.000
Smoking	21 (70.0)	10 (40.0)	3 (12.0)	18.864 ^{B}	0.000
Excess alcoholic	4 (13.3)	3 (12.0)	0 (0.0)	0.022 ^{B}	0.883
Family History of IHD	17 (56.0)	18 (60.0)	4 (16.0)	12.444^B	0.002

X^2; chi square test, N; Number, %; percent

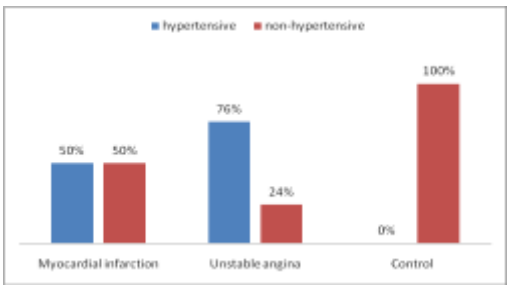

Figure 3: Frequency Distribution of Study Sample according to presence of Hypertension.

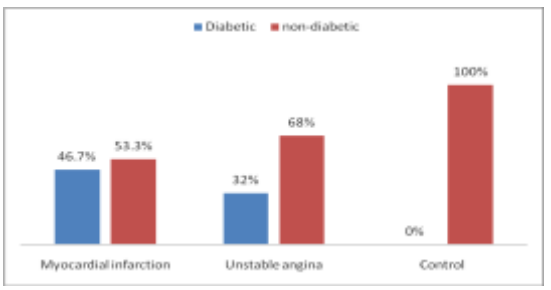

Figure 4: Frequency Distribution of Study Sample according to presence of Diabetes Mellitus.

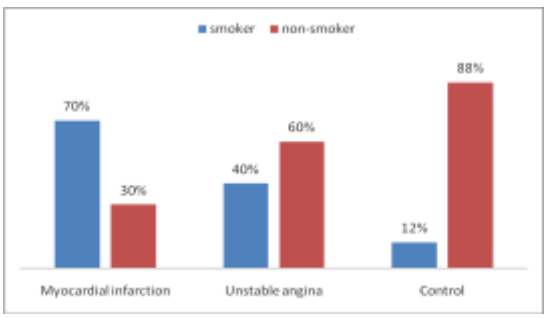

Figure 5: Frequency Distribution of Study Sample according to Smoking status.

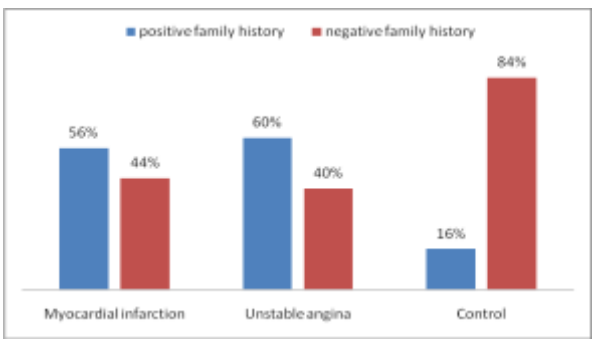

Figure 6: Frequency Distribution of Study Sample according to presence of Positive Family History of IHD.

Table 4: Levels of IL-8, IL-10, and IL-8 to IL-10 ratio for each study group expressed in mean ± standard deviation.

	N	Mean ± SD	Test Statistic*	P value
IL-8				
AMI	30	95.26 ± 102.89	203.000	0.004
Control	25	47.75 ± 36.67		
Unstable Angina	25	146.43 ± 154.45	192.000	0.019
Control	25	47.75 ± 36.67		
AMI	30	95.26 ± 102.89	350.500	0.671
Unstable Angina	25	146.43 ± 154.45		
IL-10				
AMI	30	27.73 ± 23.16	335.500	0.500
Control	25	30.29 ± 21.17		
Unstable Angina	25	16. 95 ± 11.92	185.000	0.012
Control	25	30.29 ± 21.17		
AMI	30	27.73 ± 23.16	284.500	0.124
Unstable Angina	25	16.95 ± 11.92		
IL-8 / IL-10 Ratio				
AMI	26*	3.61 ± 4.09	191.000	0.019
Control	22*	2.67 ± 3.33		
Unstable Angina	23*	7.02 ± 12.38	117.000	0.001
Control	22*	2.67 ± 3.33		
AMI	26*	3.62 ± 4.09	206.500	0.064
Unstable Angina	23*	7.02 ± 12.38		

SD; standard deviation, N; number, %; percent, IL-8; Interleukin 8, IL-10; Interleukin 10, IL-8 /IL-10; interleukin 8 to interleukin 10 ratio. AMI=Acute Myocardial Infarction.

* Mann-Whitney U

***** patients or controls who have been zero concentration of IL-8 or IL-10 were excluded during calculation of ratio.

Table 5 : Area under the receiver operating characteristic curve (ROC) between ACS groups and control group of the variables.

	Cytokines	AUC	P value
ACS			
	IL-8	0.712	0.002
	IL-10	0.379	0.083
AMI			
	IL-8	0.729	0.004
	IL-10	0.447	0.504
UA			
	IL-8	0.692	0.020
	IL-10	0.296	0.013

AUC; area under the curve, IL-8; Interleukin 8, IL-10; Interleukin 10, ACS; Acute coronary syndrome, AMI ;Acute myocardial infarction, UA; Unstable angina.

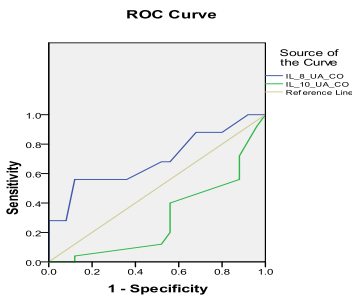

Figure 7: Receiver operating characteristic curve (ROC) showing the performance of each of interleukin 8 (IL-8) and interleukin 10 (IL-10) in Unstable Angina (UA) as if they are used as screening tests.(CO ;Control).

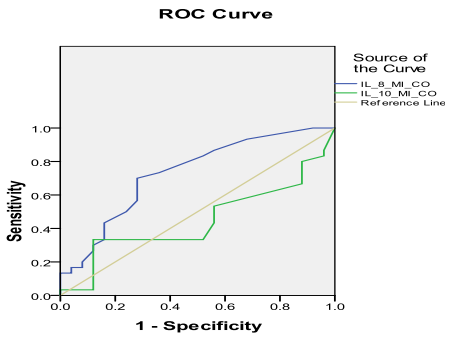

Figure 8: Receiver operating characteristic curve (ROC) showing the performance of each of interleukin 8 (IL-8) and interleukin 10 (IL-10) in Acute Myocardial Infarction (AMI) as if they are used as screening tests.(CO ;Control).

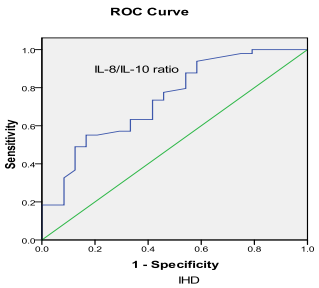

Figure 9: Receiver operating characteristic curve (ROC) showing the performance of (IL-8) to (IL-10) ratio in ACS (Unstable Angina and Acute Myocardial Infarction) as if they are used as screening tests

DISCUSSION:

In the current work , serum level of IL-8 was found to be significantly elevated in patients with AMI and those with UA (Table 4). Furthermore, Serum level was higher among patients with Acute coronary syndrome (ACS) (as consider together UA and AMI patients) in comparison to normal healthy subjects. These results were in agreement with a study done by Kanda et al. (1996) who demonstrated that evaluated serum levels of IL-8 was related to the clinical presentation of Coronary Artery Disease (CAD) and it was a useful marker for the detection of UA, as well as an earlier marker of AMI than changes in serum myoglobin, leucocytes, creatine kinase, or creatine kinase-MB[14]. Several reports demonstrated that serum levels of IL-8 were significantly higher in patients with UA or AMI than in healthy control subjects, suggesting that IL-8 is involved in the process of atherosclerosis by strongly expression of CXCR2 receptor on macrophage that infiltrate the atherosclerotic lesion which is a receptor for IL-8 [15] , and it may be a useful clinical predictor of unstable CAD[16,17].

There is an attention has been given to the role of IL-10 in the pathogenesis of atherosclerosis and CAD. In the present study, UA patients exhibited lower serum levels of IL-10 as compared with AMI patients group and healthy control subjects (Table 4), however, AMI patients showed elevation in pro-inflammatory (IL-8). In addition, pro-inflammatory (IL-8) to anti-inflammatory (IL-10)cytokines ratio showed significant greater elevation among patients with AMI and those with UA in comparison to control group, moreover,

analysis of IL-8 to IL-10 ratio showed significant difference between ACS patients and control group (Table 4). These results agree with that of Kadhim W A (2009), who suggested that reduced levels of IL-10 may favor plaque instability and the development of acute coronary events [18] . Anguera I et al. (2002) reported that lower levels of IL-10, with higher pro-inflammatory to anti-inflammatory cytokines ratio, were observed on admission of patients with UA who subsequently had cardiovascular events[19]. Furthermore, Rajappa M et al. (2009) found that AMI patients showed elevation in pro-inflammatory and anti-inflammatory cytokines, while UA is associated with low levels of serum IL-10, moreover, higher levels of anti-inflammatory cytokines IL-10 may be needed to provide protection in UA, and they concluded that these cytokines are markers of CAD and may be used for the identification of high-risk patients with UA/AMI [20].

CONCLUSION:

Interleukin-8 is an important pro-inflammatory marker act on coronary arteries which may contribute to the plaque destabilization in acute coronary syndrome especially unstable angina, and low level of IL-10 is a risk factor of development of acute events in unstable angina. Acute myocardial infarction patients showed elevation in IL-8 and nearly normal levels of IL-10; moreover, levels of IL-8 in patients with AMI is lower than those with UA; which may suggest that an increasing in concentration of IL-10 antagonized the action of IL-8 . These cytokines may be needed for identification of high risk patients with unstable angina and AMI.

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