Serum levels of interleukin (IL)-13, IL-17 and IL-18 in patients with ischemic heart disease

İskemik kalp hastalığı olan hastalarda serum interlökin (IL)-13, IL–17 ve IL–18 düzeyleri

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Abstract

Objective: It has been reported that the cytokines play an important role in the pathogenesis of cardiovascular diseases. The aim of this study was to evaluate the serum levels of interleukin (IL)-13, IL-17 and IL-18 in patients with ischemic heart disease (IHD) and also to clarify their association with traditional risk factors of disease.

Methods: A total of 60 patients with IHD as having acute myocardial infarction (AMI; n=30) or unstable angina (UA; n=30) and 30 sex- and age-matched healthy subjects as a control group were enrolled to this cross-sectional, case-controlled study. Serum samples were collected from all participants (for AMI patients at 3-5 days after events and for UA at admission time) and tested for the IL-13, IL-17 and IL-18 by use of ELISA method. Statistical analysis was performed using ANOVA, Student t, Kruskal-Wallis, Mann-Whitney U and Chi-square tests as appropriate.

Results: The frequencies of subjects with detectable levels of IL-13 were 6.7%, 20% and 33.3% in AMI, UA and control groups, respectively. The frequency of subjects with detectable levels of IL-13 in control group was significantly higher as compared to AMI group and total group of patients with IHD ($p<0.02$ and $p<0.05$, respectively). The mean serum levels of IL-17 in AMI group (6.68±1.2 pg/ml) and UA group (5.48±1.01 pg/ml) were significantly higher than that observed in control group (2.07±0.60 pg/ml; $p<0.005$ and $p<0.04$, respectively). Moreover, the mean serum levels of IL-18 in UA group (122.92±18.16 pg/ml) were significantly higher than in control group (67.82±5.98 pg/ml; $p<0.03$). The mean serum levels of IL-18 in IHD patients without a certain traditional risk factor including non-hypertensive patients (120.14±17.04 pg/ml), non-dyslipidemic patients (131.86±20.04 pg/ml), non-diabetic patients (111.96±14.71 pg/ml) and non-smoker patients (113.93±16.41 pg/ml) were significantly higher as compared to control group ($p<0.04$, $p<0.004$, $p<0.03$ and $p<0.03$, respectively). Although, the mean serum levels of IL-18 in patients with a certain traditional risk factor were higher in comparison to control group, but the differences were not significant. The means serum levels of IL-17 in patients with or without a certain traditional risk factor were also markedly higher as compared to healthy group.

Conclusions: These results showed that the higher serum levels of IL-17 and IL-18 were associated with IHD. The presence or absence of a certain traditional risk factors of IHD may influence the serum levels of cytokines. These findings may be considered to improve the predictive or prognostic values of inflammatory cytokines for IHD and also to design possible novel therapeutic approaches.

(Anadolu Kardiyol Derg 2009; 9: 75-83)

Key words: Acute myocardial infarction, unstable angina, interleukin-13, interleukin-17, interleukin-18

Özet

Amaç: Kalp ve damar hastalıklarının patogenezinde sitokinlerin önemli bir rol oynadığı rapor edilmektedir. Bu çalışmanın amacı iskemik kalp hastalığında (İKH) IL-13, IL-17 ve IL-18'in serum düzeylerini değerlendirmek ve bunların geleneksel risk faktörleri ile birlikteliğini açığa kavuşturmaktı.

Yöntemler: Akut miyokard infarktüsü (AMİ) (n=30) veya kararsız anginası (KA) (n=30) olan toplam 60 IKH'li hasta ile yaş ve cinsiyeti denk olan 30 sağlıklı kişi kontrol grubu olarak bu enine-kesitli, vaka-kontrollü çalışmaya dâhil edildi. Tüm katılanlardan (AMİ hastaları için olaydan 3-5 gün sonra ve KA için kabullerinde) serum örnekleri toplanarak ELISA yöntemi ile IL-13, IL-17 ve IL-18 ölçümü yapıldı. Uygunluklarına göre, istatistiksel analizinde ANOVA, Student-t, Kruskal- Wallis, Mann-Whitney U ve χ^2 testleri yapıldı.

Bulgular: Interlökin-13 saptanabilen kişi sıklığı AMİ, KA ve kontrol gruplarında, sırası ile %6.7, %20 ve %33.3 idi. Kontrol grubunda saptanabilen IL-13'lü kişilerin sıklığı AMİ ve İKH olan hastalar ile karşılaştırıldığında istatistiksel olarak daha fazla idi (sırası ile $p<0.02$ ve $p<0.05$).

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Akut miyokard infarktüslü gruptaki (6.68±1.2 pg/ml) ve KA grubundaki (5.48±1.01 pg/ml) ortalama IL–17 serum düzeyleri kontrol grubunda gözlemlenenden (2.07±0.60pg/ml) istatistiksel olarak daha yüksekti (sırası ile p<0.005 ve p<0.04). Ayrıca, KA grubundaki ortalama IL-18 serum düzeyleri (122.92±18.16 pg/ml) kontrol grubundan (67.82±5.98 pg/ml) istatistiksel olarak daha yüksekti (p<0.03). Normotansif hastalar (120.14±17.04 pg /ml), normolipidemik hastalar (131.86 ± 20.04 pg/ml), diyabeti olmayanlar (111.96±14.71 pg /ml) ve sigara içmeyen hastaların (113.93±16.41 pg/ml) dâhil olduğu belirli geleneksel risk faktörü taşımayan IKH olanlarda ortalama serum IL–18 düzeyleri kontrol grubu ile karşılaştırıldığında istatistiksel olarak daha yüksek bulundu (sıra ile p<0.04, p<0.004, p<0.03 ve p<0.03). Her ne kadar belirli geleneksel risk faktörü olan hastaların ortalama serum IL-18 düzeyleri kontrol grubu ile karşılaştırmada daha yüksek bulunsa da farklar istatistiksel olarak anlamlı değildi. Belirli geleneksel risk faktörü olan ya da olmayan hastaların ortalama serum IL-17 düzeyleri de sağlıklı grupla karşılaşmada oldukça yüksek bulundu.

Sonuç: Bu bulgular IL–17 ve IL–18 serum düzeylerinin IKH'da daha yüksek olduğunu göstermiştir. İskemik kalp hastalığında belirli geleneksel risk faktörlerinin varlığı ya da yokluğu serum sitokin düzeylerini etkileyebilir. Bu bulgular IHD'da inflamatuvar sitokinlerin prediktif ve prognostik değerlerinin arttığını düşündürür ve ayrıca yeni tedavi yaklaşımlarının olasılığı da akla gelmelidir *(Anadolu Kardiyol Derg 2009; 9: 75-83)*

Anahtar kelimeler: Akut miyokard infarktüsü, kararsız angina, interlökin-13, interlökin-17, interlökin-18

Introduction

It has been reported that the immunopathological and inflammatory processes play an important role in the initiation and development of ischemic heart disease (IHD). Accumulation of leukocytes including monocytes/macrophages, T cells, B cells and PMN cells has been demonstrated in atherosclerotic lesions (1, 2). T-helper (Th)-dependent responses in particular, exerts an important role in the pathogenesis of cardiovascular diseases (3). Upon antigenic stimulation, Th cells differentiate into Th1 and Th2 subsets, which are characterized by the release of distinct cytokines profile. Th1 cells secret cytokines including interferon (IFN)-γ and interleukin (IL)-2 whereas Th2 cells primarily secrete IL-4 and IL-10 and IL-13 (4). It has been reported that Th2 type cytokines including IL-4 and IL-10 inhibit Th1 responses, thereby, down-regulating Th1-dependent local inflammatory reactions (5, 6). The cytokine environment, which T cells encounter with it after antigen exposure determines subsequent T cell polarization. The IL-12 and IL-18 are produced by antigen presenting cells (such as macrophages and dendritic cells), inducing Th1 differentiation (4, 5).

The results of some clinical and experimental studies have demonstrated that the up-regulation of Th1-associated inflammatory response and down-regulation of anti-inflammatory responses of Th2-related cytokines are associated with cardiovascular diseases (3, 6-8).

The IL-17 is a proinflammatory cytokine, which is produced by a newly described effector T-cell subset, termed Th17 (9). The involvement of Th17 in autoimmune and inflammatory diseases has been reported in some studies (10), however, their participation in the pathogenesis of IHD have not been adequately investigated.

As mentioned, the Th cells have been categorized into several subsets including Th1, Th2 and Th17 cells. Although, there are some studies on the serum levels of cytokines such as IL-6, IL-8, IL-10, IL-12 and IL-17 in patients with IHD (11), the data on certain subtypes of Th cells are scarce and the relationship of cytokines with traditional risk factors of IHD is not also clarified.

Therefore, the aim of this study was to evaluate the serum concentrations of IL-13 (a Th2 type cytokine), IL-17 (a Th17 type cytokine) and IL-18 (an inducer of Th1 cells) in patients with IHD and also to clarify their association with traditional risk factors of disease.

Methods

A total of 60 patients (aged 40-60 years) with IHD who were admitted to Ali-ebne-Abitaleb hospital of Rafsanjani (a city that located in Kerman province in south- east of Iran) were enrolled to this cross-sectional, case-controlled study. Patients were classified into 2 groups according to well established criteria, as having acute myocardial infarction (AMI) (n=30) or unstable angina (UA) (n=30). The diagnosis of AMI was established according to the presence of two of the following criteria: i-prolonged chest pain compatible with AMI, ii-typical electrocardiogram changes, iii-raising of cardiac enzymes. Unstable angina was defined according to the Braunwald's classification and all patients had chest pain at rest with definite ischemic electrocardiographic changes such as ST-segment changes and/or T-wave inversion. Patients with UA were in class IIIB according to Braunwald's classification (12). A third control group was comprised of 30 sex- and age-matched subjects with similar geographic and socioeconomic background without IHD. The healthy control group was recruited among blood donors of Rafsanjani Blood Transfusion Center. Exclusion criteria were valvular heart disease, surgery, trauma within the prior month, cardiomyopathy, liver disease, renal failure, malignant diseases, other inflammatory disease (such as septicemia and pneumonia) and oral anticoagulant therapy. This study was evaluated and approved by the Ethical Committee of Rafsanjani University of Medical Sciences. Moreover, patients were recruited if they agreed for blood sampling and participation in the study.

Cytokines detections

In patients with AMI the serum concentrations of IL-13, IL-17 and IL-18 were measured during 3-5 days after admission. In patients with UA the measurements were done at admission time. Peripheral blood samples (2-4 milliliter) were collected from subjects of 3 groups and the serum was separated and stored at -20°C.

Serum IL-13, IL-17 and IL-18 levels were measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits (IL-13: Bender Medsystems, Vienna, Austria; IL-17: IBL, Hamburg, Germany; IL-18: Bender Medsystems, Vienna, Austria). The minimum detectable concentrations were 0.73 pg/ml for

IL-13, 2 pg/ml for IL-17 and 9.2 pg/ml for IL-18. Moreover, according to the manufacturer guidelines the coefficient of variations (CV) were 6%, 3.7% and 6.5% for assessment of IL-13, IL-17 and IL-18, respectively.

Statistical analysis

All the available data were analyzed by a computer program (SPSS, Chicago, IL, USA). Differences in variables were analyzed using Student t, ANOVA, Mann-Whitney U, Kruskal-Wallis and Chi-square tests as appropriate and p values of less than 0.05 were considered significant.

Results

Baseline characteristics of patients:

Baseline characteristics of AMI, UA and healthy control groups are shown in Table 1. There were no significant differences among 3 groups for the age and gender ratio. Moreover, no statistically significant differences were observed between both groups of patients with respect to the age or presence of traditional risk factors for atherosclerosis

Serum IL-13 levels in IHD and control groups

The frequency of subjects with detectable levels of IL-13 and mean serum IL-13 levels in healthy control and patients groups are demonstrated in Figures 1, 2and Table 2. Serum levels of IL-13 were detectable in 2/30 (6.7%) patients with AMI, in 6/30 (20%) patients with UA and in 10/30 (33.3%) of healthy subjects. Overall, serum IL-13 levels were detectable in 8/60 (13.3%) of total patients with IHD (AMI plus UA). Statistical analyses showed that the frequency of subjects with detectable levels of IL-13 in healthy control group was significantly higher as compared to AMI and total IHD groups ($p<0.02$ and $p<0.05$, respectively) (Fig. 1). The difference of the frequency of subjects with detectable levels of IL-13 between control and UA groups was not statistically significant. Although, the mean serum levels of IL-13 in control group was higher than those observed in patients groups, the differences were not significant (Fig. 2). No significant differences were also observed between men and women of control and patients groups with respects to both the frequency of subjects with detectable levels of IL-13 and the serum concentrations of IL-13 (Table 2).

The serum levels of cytokines according to traditional risk factors of IHD are summarized in Table 3 and Table 4. In diabetic or smoker patients the frequency of subjects with detectable levels of IL-13 was significantly lower than that observed in healthy control group ($p<0.02$). Indeed, serum levels of IL-13 were not detectable in any diabetic- or smoking patients. The frequency of subjects with detectable levels of IL-13 was also lower in hypertensive or dyslipidemic patients as compared to healthy control group but the differences were not significant. No significant differences were found between non-hypertensive, non-dyslipidemic, non-diabetic or non-smoker patients and control group with respect to the frequency of subjects with detectable levels of IL-13. Furthermore, the differences of the

Table 1. Baseline characteristics of study patients

Parameters*	AMI (n=30)	UA (n=30)	Control (n=30)
Age, years	48.8±7.9	49.7±7.3	49.8±7
Sex (Men/Women), n	15/15	15/15	15/15
Hypertension, n	14	12	0
Dyslipidemia, n	15	17	0
Diabetes mellitus, n	10	6	0
Current smoking, n	8	9	0
Medications:			
Aspirin, n	11	13	0
Statins, n	8	11	0

Data are presented as mean ± SD and numbers
ANOVA, Chi-square and Fisher exact tests
*No significant differences were observed between patients groups with respect to the age, sex or presence of risk factors
AMI - acute myocardial infarction, UA - unstable angina

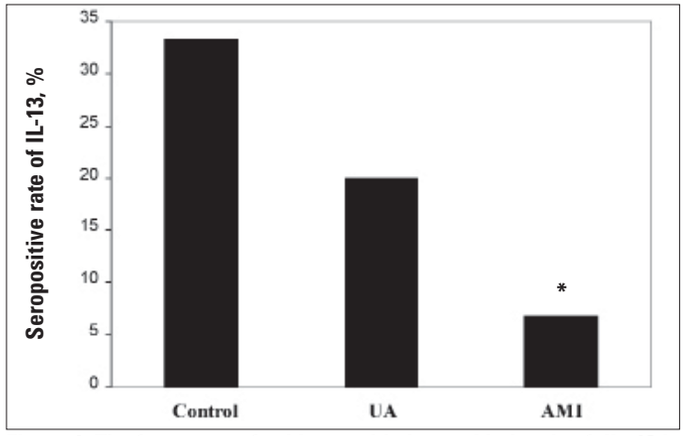

Figure 1. The frequency of subjects with detectable levels of IL- 13 in studied groups

* - $p<0.02$ differences are significant between healthy control and AMI groups
AMI - acute myocardial infarction, IL-13 - interleukin-13, UA - unstable angina

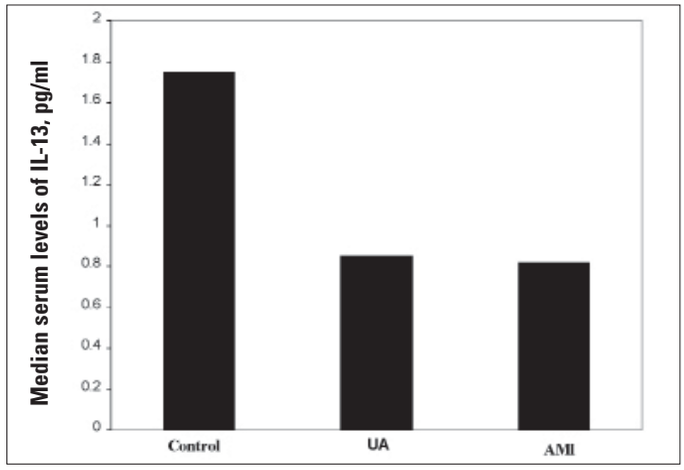

Figure 2. The median serum concentrations of IL-13 in studied groups

AMI - acute myocardial infarction, IL-13 - interleukin-13, UA - unstable angina

Table 2. Serum levels of IL-13, IL-17 and IL-18 in IHD and healthy groups according to gender

Groups*	Sex	No.	IL-18, pg/ml	IL-17 levels, pg/ml	IL-13 detectable rate, n(%)	IL-13, pg/ml
AMI	Male	15	103.50 ± 28.46	6.76 ± 2.17	0 (0%)	-------
	Female	15	91.56 ± 24.72	6.61 ± 1.28	2 (13.3%)	0.82 ± 0.24 0. 82 (0.65-1.00)
	Total	30	97.53 ± 18.55	6.68 ± 1.24♣	2 (6.7%)****	0.82 ± 0.24 0.82 (0.65-1.00)
UA	Male	15	117.78 ± 24.16	7.44 ± 1.72	2 (13.3%)	3.05 ± 2.61 3.05 (1.20-4.90)
	Female	15	128.06 ± 27.92	3.52 ± 0.83	4 (26.7%)	0.82 ± 0.69 0.5 (0.43-1.87)
	Total	30	122.92 ± 18.16***	5.48 ± 1.01**	6 (20%)	1.56 ± 1.72 0.85 (0.43-4.90)
IHD	Male	30	110.64 ± 18.39	7.10 ± 1.36	2 (6.7%)	3.05 ± 2.61 3.05 (1.20-4.90)
	Female	30	109.81 ± 18.63	5.07 ± 0.80	6 (20%)	0.82 ± 0.55 0.57(0.43-1.87)
	Total	60	110.22 ± 12.98*	6.08 ± 0.79♣♣	8 (13.3%)*	1.38 ± 0.53 0.82 (0.43-4.90)
Healthy	Male	15	73.15 ± 8.41	2.40 ± 1.31	4 (26.7%)	6.58 ± 10.70 1.75 (0.25-22.60)
	Female	15	62.49 ± 8.56	1.74 ± 0.46	6 (40%)	7.79 ± 14.9 7.2(0.25-38.25)
	Total	30	67.82 ± 5.98	2.07 ± 0.68	10 (33.3%)	7.31 ± 12.77 1.75 (0.25-38.25)

Data are presented as mean ± SD, median (minimum-maximum) values and numbers/percentages
ANOVA, Kruskal Wallis, Chi-square and Fisher exact tests
*-p<0.05, **-p<0.04, ***-p<0.03 , ****p<0.02, ♣ - p<0.005, ♣♣ - p<0.002 as compared with healthy controls group
AMI - acute myocardial infarction, IHD - ischemic heart disease, IL-13 - interleukin-13, IL-17 - interleukin-17, IL-18 - interleukin-18, UA – unstable angina

frequency of subjects with detectable levels of IL-13 between hypertensive-, dyslipidemic, diabetic or smoker patients were not statistically significant as compared to counterpart groups without certain risk factor (Table 4). The serum levels of IL-13 did not significantly influenced by the hypertension or dyslipidemia risk factors.

The serum levels of cytokines according to medication are summarized in Table 5. Administration of the statins did not influence the serum levels of IL-13. However, the frequency of subjects with detectable levels of IL-13 in aspirin-administrated patients was significantly (p<0.04) higher as compared with patients who did not received aspirin.

Serum IL-17 in IHD and control groups

In contrast to the IL-13, the cytokines IL-17 and IL-18 were detected in all subjects (Table 2). Statistical analyses showed that the mean serum concentrations of IL-17 in AMI and UA groups were significantly higher than that observed in healthy control group (p<0.005 and p<0.04, respectively) (Fig. 3). Moreover, the mean serum levels of IL-17 in total IHD patients was significantly higher than that observed in healthy subjects (p<0.002). No significant difference was observed between AMI and UA groups regarding the mean serum concentrations of IL-17.

In healthy control and IHD groups the mean serum levels of IL-17 in men were higher than that observed in women but the differences were not statistically significant (Table 2).

According to the traditional risk factors, the means serum levels of IL-17 in patients with a certain risk factor including hypertensive patients, dyslipidemic patients and smoker patients were significantly higher as compared to control group (p<0.05, p<0.03 and p<0.005, respectively). Moreover, the mean serum levels of IL-17 in diabetic patients were higher than that observed in healthy control group but the difference did not reach statistically significant. The means serum levels of IL-17 in patients without a certain traditional risk factor including non-hypertensive patients, non-dyslipidemic patients, non-diabetic patients and non-smoker patients were also significantly higher as compared to control group (p<0.001, p<0.001, p<0.001 and p<0.007, respectively). The differences of the means serum levels of IL-17 in non-hypertensive-, non-dyslipidemic, non-

Table 3. Serum levels of IL-13, IL-17 and IL-18 in patients with IHD according to traditional risk factors

Risk factors	RF status	No.	IL-18, pg/ml	IL-17 levels, pg/ml	IL-13 detectable rate, n(%)	IL-13, pg/ml
Without RF (Healthy group)	--------	30	67.82 ± 5.98	2.07 ± 0.68	10 (33.3)	7.31 ± 12.77 1.75 (0.25-38.25)
Hypertension	Positive	26	97.26 ± 19.56	5.05 ± 0.97	3 (11.5)	2.18 ± 2.35 1 (0.65-4.90)
	Negative	34	120.14 ± 17.04	6.87 ± 1.18	5 (14.7)	0.90 ± 0.62 0.5 (0.43-1.87)
Dyslipidemia	Positive	32	91.29 ± 16.43	5.09 ± 0.63	4 (12.5)	1.76 ± 2.10 0.82 (0.50-4.90)
	Negative	28	131.86 ± 20.04	7.21 ± 1.52	4 (14.3)	1.00 ± 0.67 0.85 (0.43-1.87)
Diabetes	Positive	16	105.46 ± 27.86	5.01 ± 1.41	0 (0)	--------
	Negative	44	111.96 ± 14.71	6.47 ± 0.96	8 (18.2)	1.38 ± 1.50 0.82 (0.43-4.90)
Smoking	Positive	20	102.81 ± 21.43	6.67 ± 1.68	0 (0)	-------
	Negative	40	113.93 ± 16.41	5.79 ± 0.86	8 (20)	1.38 ± 1.50 0.82 (0.43-4.90)

Data are presented as mean ± SD, median (minimum-maximum) values and numbers/percentages
IHD - ischemic heart disease, IL-13 - interleukin-13, IL-17 - interleukin-17, IL-18 - interleukin-18, RF - risk factor

Table 4. Statistical comparisons of the serum cytokine levels according to the presence or absence of a certain risk factor

Risk factors	Cytokine	Comparison of groups		
		Pos vs Cont	Neg vs Cont	Pos vs Neg
Hypertension	IL-18	0.39	0.04	0.55
	IL-17	0.05	0.001	0.2
	IL-13 (%)	0.08	0.08	0.90
	IL-13	0.44	0.26	0.86
Dyslipidemia	IL-18	0.27	0.004	0.06
	IL-17	0.03	0.001	0.14
	IL-13 (%)	0.08	0.16	0.85
	IL-13	0.36	0.30	0.91
Diabetes	IL-18	0.15	0.03	0.79
	IL-17	0.09	0.001	0.36
	IL-13 (%)	0.02	0.22	0.09
	IL-13	NC	0.30	NC
Smoking	IL-18	0.16	0.03	0.63
	IL-17	0.005	0.007	0.56
	IL-13 (%)	0.02	0.22	0.09
	IL-13	NC	0.30	NC

ANOVA, Student t, Kruskal-Wallis, Mann-Whitney U and Chi-square tests
Cont.-control (healthy group without any indentified risk factor), IL-13 - interleukin-13, IL-17-interleukin-17, IL-18 - interleukin-18, NC - not computed due to absence of the mean of one group, Neg. - negative (the patients group that have not certain risk factor), Pos.-positive (the patients group that have certain risk factor)

diabetic or non-smoker patients were not statistically significant as compared to counterpart groups with certain risk factor, although, this parameter was found to be higher in patients without certain traditional risk factor (Table 3, Table 4). The administration of statins and aspirin had no significant effects on the serum levels of IL-17 (Table 5).

Serum IL-18 in IHD and control groups

The means serum levels of IL-18 in studied groups are presented in Table 2 and Figure 4. Statistical analyses showed that the mean serum concentrations of IL-18 in UA and IHD groups were significantly higher than that observed in healthy control group ($p<0.03$, $p<0.05$, respectively). significant difference was observed between AMI and UA and control groups regarding the mean serum concentrations of IL-18. In healthy control and IHD groups the differences of the mean serum levels of IL-18 between men and women were not significant

The means serum levels of IL-18 in IHD patients without a certain traditional risk factor including non-hypertensive patients, non-dyslipidemic patients, non-diabetic patients and non-smoker patients were significantly higher as compared to control group ($p<0.04$, $p<0.004$, $p<0.03$ and $p<0.03$, respectively). The differences of the means serum levels of IL-18 in non-hypertensive, non-dyslipidemic, non-diabetic or non-smoker patients were not statistically significant as compared to counterpart groups with certain risk factor, although, this parameter was found to be higher in groups without certain traditional risk factor. Furthermore, the means serum levels of

IL-18 in hypertensive, dyslipidemic, diabetic or smoker groups were somewhat higher in comparison to healthy control group, but the differences were not statistically significant (Table 3, Table 4). The administration of statins and aspirin had no also significant effects on the serum levels of IL-18 (Table 5).

Discussion

The results of the present study showed that the mean serum levels of IL-17 and IL-18 were significantly higher in patients with IHD as compared with healthy control group. Moreover, the frequency of subjects with detectable levels of IL-13 in healthy control group was significantly higher in comparison to AMI and total IHD groups. Regarding IL-18, our results were consistent with results of other investigators (13, 14). Increased levels of IL-18 expression have been also observed in human atherosclerotic plaque and were associated with plaque instability (15). Moreover, it has been demonstrated that the IL-18 level is highly dynamic following AMI events, with a marked elevation in the early phase of AMI (16). However, in our results no significant difference was observed between mean serum concentration of IL-18 of AMI and healthy control groups. These observations may be due to a delaying in blood sampling following events. It should be noted that the increased serum IL-18 levels in patients with AMI may be attribute to the myocardial damages. However, it has been reported that the elevation of the plasma levels of IL-18 did no correlate with myocardial necrosis and creatine kinase enzyme levels (16).

The IL-18 may directly and/or indirectly influence the initiation and development of the cardiovascular diseases. It is defined as IFN-γ inducing factor and accordingly IFN-γ enhances the expression of both chemokines such as monocyte chemoattractant protein-1 (MCP-1) and also increases the expression of adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1)

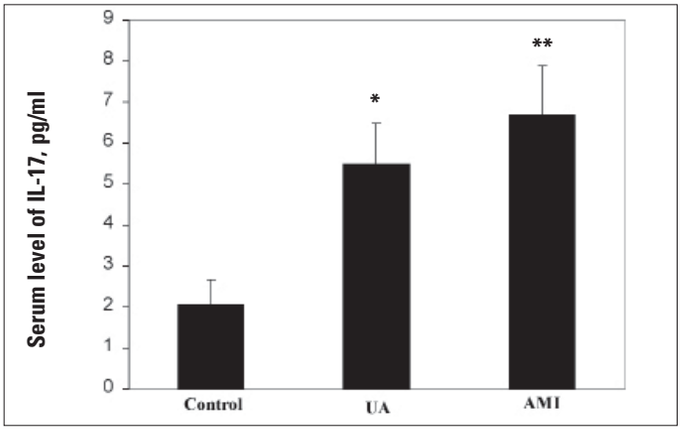

Figure 3. Mean serum concentrations of IL-17 in studied groups
*- p=0.005 and **-p=0.004 differences are significant as compared with control group
AMI - acute myocardial infarction, IL-17 - interleukin-17, UA - unstable angina

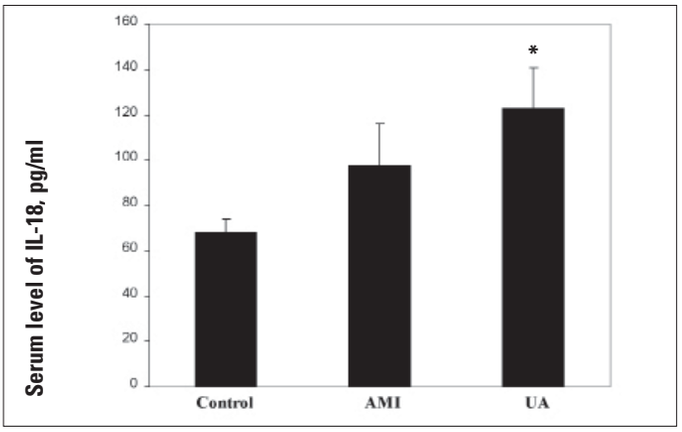

Figure 4. Mean serum concentrations of IL-18 in studied groups
*- p=0.003 differences are significant as compared with control group
AMI - acute myocardial infarction, IL-18 - interleukin-18, UA - unstable angina

Table 5. Serum levels of IL-13, IL-17 and IL-18 in patients with IHD according to medication

Medication*	Administration	No.	IL-18, pg/ml	IL-17 levels, pg/ml	IL-13 detectable rate, n(%)	IL-13, pg/ml
Statins	Positive	19	109.59 ± 21.95	5.23 ± 0.92	4 (21.1)	0.69 ± 0.34 0.57 (0.43-1.20)
	Negative	41	110.52 ± 16.22	6.47 ± 1.08	4 (9.8)	2.06 ± 1.97 1.43 (0.50-4.90)
Aspirin	Positive	24	113.44 ± 24.5	6.57 ± 1.41	6 (25)*	1.33 ± 1.76 0.57 (0.43-4.90)
	Negative	36	108.08 ± 14.48	5.75 ± 0.95	2 (5.6)	1.53 ± 0.473 1.53 (1.20-1.87)
No Medication (Control)	----------	30	67.82 ± 5.98	2.07 ± 0.68	10 (33.3)	7.31 ± 12.77 1.75 (0.25-38.25)

Data are presented as mean ± SD, median (minimum-maximum) values and numbers/percentages
ANOVA, Kruskal Wallis, Chi-square and Fisher exact tests
*- p=0.04 differences are significant as compared with group of patients without treatment
IHD – ischemic heart disease, IL-13 - interleukin-13, IL-17 - interleukin-17, IL-18 - interleukin-18

and vascular cell adhesion molecule-1 (VCAM-1) by endothelial cells (17, 18), which in turn may facilitate the influx of inflammatory cells. On the other hand, IL-18 itself has the potential to induce the expression of adhesion molecules and chemokines in an IFN-γ-independent manner (19). It induces the secretion of other proinflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-1 and IL-6, which were associated with cardiovascular disorders (20-22). Accordingly, our results confirmed that the IL-18 may have an important role in the pathogenesis of IHD.

The results of the present study showed that the mean serum levels of IL-18 were higher in patients groups without certain classical risk factor in comparison to counterpart groups that have got certain risk factor including hypertensive, dyslipidemic, diabetics or smoking patients. Moreover, the means serum levels of IL-18 in non-dyslipidemic, non-diabetic, non-smoker or non-hypertensive patients were significantly higher than that observed in healthy control group. Accordingly, it seems that the conventional risk factors and IL-18 may independently influence the cardiovascular events. In other word IL-18 should be consider as an independent risk factor for IHD.

The possible mechanisms responsible for the elevation of the serum levels of IL-18 remains to be clarify. It has been reported that the IL-18 gene expression induces by proinflammatory cytokines and also by some infectious-derived components such as lipopolysaccharides (23). An association between IHD and some infectious agents including Chlamydia pneumonia, Helicobacter pylori, herpes simplex virus, cytomegalovirus, hepatitis A, respiratory tract and dental infections has been reported in some epidemiological studies (2, 24). In our previous study higher seroprevalence of Chlamydia pneumonia was also observed in patients with IHD in comparison to healthy control group (25). Therefore, the infectious agents may directly and/or indirectly trigger the IL-18 production, which could also provide a potential link between infections and cardiovascular events.

The results of the present study also showed that the mean serum concentrations of IL-17 in patients with AMI and UA was significantly higher than that observed in control group. It has been demonstrated that a separate T-cell subset, termed Th17, produces IL-17 and the cytokine IL-23 has been introduced as a major inducer of IL-17 (10). Accordingly, the IL-17 and IL-23 axis should be investigated in further studies to clarify the role of this axis in the pathogenesis of cardiovascular diseases. Recently, it has been demonstrated that the number of circulating TH17 cells were significantly increased in patients with acute coronary syndrome (26). These observations are consistent with our results. IL-17 acts mainly as a proinflammatory mediator through a variety of mechanisms including stimulation of the production of other proinflammatory cytokines such as TNF-α, IL-1 and IL-6 as well as chemokines CXCL1 and CXCL2, induction of the expression of adhesion molecules such as ICAM-1, stimulation the production of C-reactive protein and nitric oxide (12, 13, 27, 28). IL-17 also mediates chemotaxis of neutrophils and monocytes to the sites of inflammation through the induction of chemotactic mediators such as IL-8, MCP-1and growth-related protein (Gro)-α (9, 27). Our results regarding higher serum levels of IL-17 in patients with IHD implicates that the IL-17 dependent mechanisms may be related with both UA and AMI events.

The results of the present study demonstrated that means serum levels IL-17 were higher in non-hypertensive, non-dyslipidemic, non-diabetic or non-smoker groups as compared to counterpart groups with certain conventional risk factor but the differences were not significant. Moreover, the means serum levels of IL-17 in patient groups with or without a certain conventional risk factor were higher than that observed in healthy control group. These findings represent that IL-17 may dependently (in the context of conventional risk factors) or independently (in the absence of conventional risk factors) associated with IHD events.

Our results also demonstrated that the frequency of subjects with detectable levels of IL-13 in healthy control group was significantly higher as compared to patients with AMI. The possible role of IL-13 in the IHD may be explained according to the classification of Th cells. Th cells have been categorized as Th1 and Th2 subsets with regard to their cytokine profiles. It should be also noted that Th1 and Th2 cytokines regulate each other function, so that IL-4 and IL-10 (Th2 cytokines) inhibit Th1 activation whereas IFN-γ (Th1 cytokine) suppresses Th2 development (5, 6). Th2-related cytokines are often recognized as anti-inflammatory mediators, because they can inhibit Th1-associated cell-mediated immune responses (5). It has been reported that Th1/Th2 imbalance may be involved in the pathogenesis of IHD so that the up-regulation of Th1-type cytokines (such as IFN-γ, IL-12 and IL-18) and down regulation of Th2-type cytokine IL-10 have been reported in patients with cardiovascular diseases (3, 6-8). Accordingly, lower frequency of subjects with detectable levels of IL-13 that observed in patients with IHD (especially AMI group) is consistence with up regulation and down regulation of Th1 and Th2 cells, respectively. It has been reported that IL-13 is a cytokine that elicits biological responses similar to IL-4 (29) which may suppresses Th1-dependent immune responses. Moreover, some activities such as the inhibition of IL-12 and TNF-α expression, down-regulation of NOS expression and down-regulation of fibrinogen production have been attributed to IL-13 activity (30-32). The participation of these parameters in the pathogenesis of cardiovascular diseases was reported (7, 8, 20, 32). Accordingly, IL-13 may be protective against IHD especially AMI.

The results of present study also demonstrated that in diabetic or smoker patients the frequency of subjects with detectable levels of IL-13 was significantly lower than that observed in healthy control group. Indeed, IL-13 was not detectable in any diabetic or smoker patients. Therefore, it seems that the diabetes and cigarette smoking risk factors may also influence cardiovascular events via down-regulation of IL-13 production. Consistent with our observations the lower IL-13 expression has been reported in bronchoalveolar lavage of smoker patients with chronic obstructive pulmonary disease (33).

Study limitations

It should be noted that our study has several limitations: First, measurement of cytokines was performed at once. These results could not give information respecting when the serum

cytokines elevated and how long the elevation of cytokines continued. However, previous studies have demonstrated that the IL-18 level is highly dynamic following AMI, with a marked increase in the early phase of AMI (16). Second, the effects of circulating cytokines on long-term clinical outcomes were not part of the protocol. However, estimating the time course of cytokines during the cardiovascular event might improve their prognostic value. Third, measurement of cytokines was performed on samples that were stored at -20°C. We cannot exclude the possibility of protein degradation. However, this should affect both cases and controls in a similar way. Forth, it should be also noted that the medication including statins and aspirin may influence the inflammatory cytokines patterns and accordingly the pre-treatment of some patients with these drugs may affect the results. Indeed, our results have demonstrated that the frequency of subjects with detectable levels of IL-13 in aspirin-administrated patients was significantly higher as compared with patients who did not receive aspirin. However, the observed results for IL-17 and IL-18 levels were not compatible with known anti-inflammatory effects of statins and aspirin. Furthermore, it has been demonstrated that the treatment of AMI patients with statins or ACE inhibitor has no effects on cytokine response (34). Therefore, the statins and aspirin medications may no influencing the inflammatory cytokines such as IL-17 and IL-18 in IHD.

It has been reported that elevated serum IL-18 level is a strong independent prospective risk factor for development of heart ischemic disease supporting that the cytokines may have an etiologic role for IHD. On the other hand, the results of some studies demonstrated that the serum level of IL-18 is significantly increased during the acute phase of coronary syndromes and gradually decreased (16), supporting that the early rise in serum cytokines levels may be a part of immune response against the protein released from the necrotic heart tissue. Thus, the cytokines may play a direct role in the pathogenesis of ischemic heart diseases or also may be formed during immunopathological responses after events.

Clinical implications

The examining the time course of inflammatory cytokines before or during the cardiovascular event might improve their predictive or prognostic values. The results of our study encourage further studies in these fields. Moreover, the manipulation of the cytokines production or their effects may be considered to design possible novel therapeutic approaches.

Conclusion

In conclusion, the results of this study demonstrated the lower frequency of subjects with detectable levels of IL-13 in AMI group, higher serum levels of IL-17 in both AMI and UA groups and higher serum levels of IL-18 in IHD patients, especially UA groups. Accordingly, hyperactivity of Th1 and Th17 cells and down-regulation of Th2 cells may be associated with IHD. Moreover, the presence or absence of a certain traditional risk factors of IHD may influence the serum levels of cytokines.

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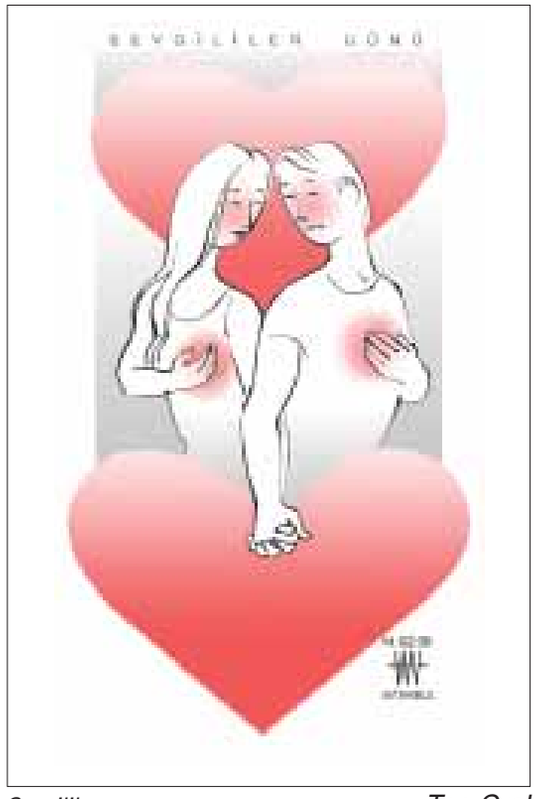

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