Cytokines Profile in Patients with Acute Myocardial Infarction

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Abstract

A total of 30 acute myocardial infarction patients and 30 age and sex-matched healthy controls were enrolled in this study. Patients group showed significant increase in serum levels of IL-1α, IL-2 and IL-6 as compared with controls (p<0.01 and P<0.05), and no statistically significant changes were found in serum levels of IL-4 and IL-10 between patients and controls. On the other hand there was positive linear correlation between IL-1α and IL-6 (r=0.787; P=0.004) and negative linear correlation between IL-10 and IL-6 (r=-0.601; P=0.049) in patients with acute myocardial infarction. In conclusion, the present study showed an increase production of pro-inflammatory cytokines and decrease production of anti-inflammatory cytokines in acute myocardial infarction patients.

Keywords: Acute myocardial infarction, Cytokines.

Introduction

Acute myocardial infarction (AMI) is a multi-factorial disease. AMI is often caused by unstable plaque in the pericardial coronary artery, in which inflammatory processes play a central role [1]. In this regard, several circulating pro-inflammatory molecules have been associated with thrombotic cardiovascular events including acute phase proteins, cellular adhesion molecules, and cytokines [2]. Local inflammatory cells can generate and release cytokines that have the potential to activate endothelium, transforming its natural anti-adhesive and anticoagulant properties. Furthermore, pro-inflammatory cytokines may reduce matrix synthesis and increase its degradation, favoring plaque rupture. Cytokines may enhance synthesis of endothelin in endothelial cells and macrophages, resulting in increased smooth muscle cell reactivity to local vasoconstrictors [3].

Animal experiments suggest that IL-6 and TNF-α play important role in the regulation of acute phase protein synthesis. Acute phase proteins, such as fibrinogen and factor VIII, are established risk factor for atherosclerosis. The serum cytokine IL-6 plays an important role in mediating inflammation and is a central stimuli for the acute phase response [4]. IL-1 has wide range of target cells including cardiomyocytes and vascular smooth muscle cells. It also induces prostanoid dependent hypotension in rabbits *in vivo* and stimulates human smooth muscle cells to secrete IL-6 [5]. Nogae and associates mentioned that treatment of rat hearts with IL-1α resulted in improved ventricular systolic pressure and overexpression of Mn^{2+} SOD resulting in reduced ischemia-reperfusion injury [6].

The effects of Th1 cytokines are counter balanced by Th2 cytokines, including IL-4 and IL-10, which inhibit Th1 responses, thereby, down-requlating Th1 dependent local inflammatory reactions [7]. IL-10 is a centrally operating anti-inflammatory cytokine that plays a crucial role in the regulation of the innate immune system. Studies using IL-10 deficient mice have shown that IL-10 has a protective role in atherosclerosis [8]. Further, it strongly deactivates the inflammatory host response and potently inhibits the production of pro-inflammatory cytokines. Pro- and anti-inflammatory cytokine ratios can signal the balance between pro- and anti-inflammatory forces [9 & 10].

The aim of study was to determine the levels of pro-inflammatory cytokines (IL-1α, IL-2 and IL-6), and anti-inflammatory cytokines (IL-4 and IL-10), and to detect the correlation among these cytokines in patients with AMI.

Patients and Methods
Patients groups

The current study comprised of thirty patients [9 females and 21 males; mean age 57.4±2.44 years, ranged between (22-

79)], they were attending the emergency departments in Ibn Al-Nafees Cardiac Specialty Teaching hospital and AL-Kindy Teaching hospital. The diagnosis of myocardial infarction was made on the base of WHO criteria [11].

Healthy controls groups

Included 30 healthy control were age and sex matched to patients, (8 females and 22 males; mean age 49.3±1.28 years, ranged between 25-60), who had no history or clinical evidence of AMI or any cardiac problems.

Determination of serum cytokines

The enzyme-linked immunosorbent assay (sandwich -ELISA) for determining serum cytokines were performed according to the protocol developed by BioSource, Europe S.A. company, *Belgium.*

Principle and procedure of ELISA and calculation of results:

The BioSource IL-s EASIA kit is a solid phase enzyme amplified sensitivity immunoassay performed on microtiter plate. Standards or samples containing ILs react with capture monoclonal antibody-1 coated on the microtiter well and with a monoclonal antibody-2 labeled with horse radish peroxidase. After an incubation period allowing the formation of a sandwich, the microtiter plate is washed to remove unbound enzyme labeled antibodies. Chromogenic solution (TMP+H2O2) is added and incubated, afterward the reaction is stopped with the addition of stop solution and the microtiter plate is then read at the 450 nm wavelength. The amount of substrate turnover is determined colourmetrically by measuring the absorbance which is proportional to the IL-s concentration. A standard curve is plotted and IL-s concentration in a sample is determined by interpolation from the standard curve.

Statistical analysis

Comparison of serum cytokines levels between patients and healthy groups were calculated by Mann-Whitney-test. Correlation between the cytokines was calculated by the spearman test [12].

Results

The present study revealed statistically significant elevation in median serum levels of IL-1α, IL-2 and IL-6 in patients (23.5, 2, 31.5Pg/ml) as compared to control group (0.0 Pg/ml), (($p<0.01$ and $P<0.05$). IL-2 levels was elevated in patients group but the differences was low not high statistically significant. Conversely, no significant differences were observed between patient and control group in median serum levels of anti-inflammatory cytokines (IL-4 and IL-10); ($p>0.05$), as clearly shown in Table (1) and Fig.(1). Moreover, there was positive linear correlation between IL-1α and IL-6 (r=0.787; P=0.004) and negative linear correlation between IL-10 and IL-6 (r=-0.601; P=0.049) in patients with AMI, Fig.(2 and 3).

Table (1)
Serum median levels of IL-1α, IL-4, IL-6, IL-10 (pg/ml) and IL-2 (IU/ml) in patients with AMI and healthy control groups.

Serum baseline cytokines	Patients NO.= 30	Control NO.= 30	P (Mann-Whitney Test)Value
IL-1α	23.5**	0.0	P=0.00
IL-2	2.0*	0.0	P=0.005
IL-4	0.0	0.0	P=0.865
IL-6	31.5**	0.0	P=0.00
IL-10	0.0	0.0	P=0.905

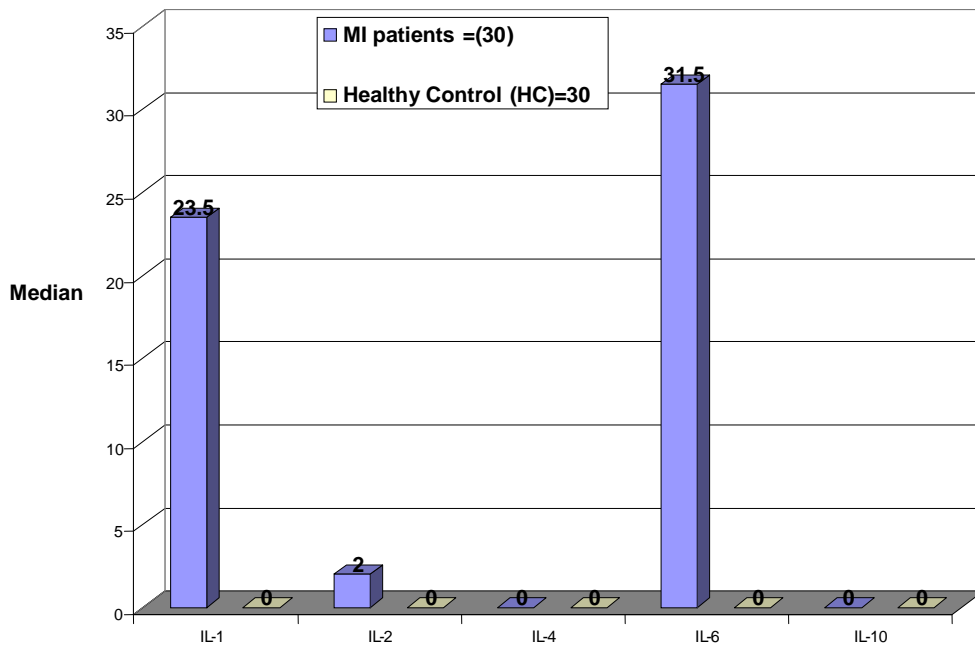**: High significant

*: Low
significant

***Fig.(1) The median serum concentration of IL-1α,IL-2, IL-4, IL-6, and
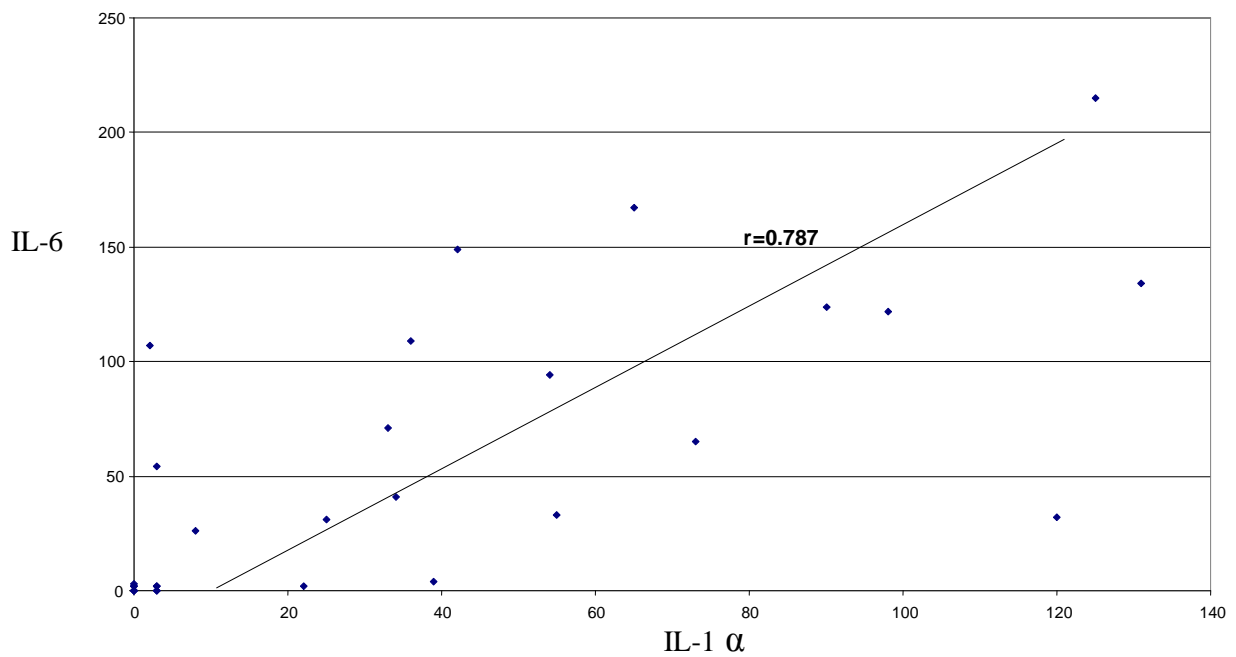IL-10 (pg/ml) between patient and control groups.***

Fig.(2) Linear correlation between Il-1α and IL-6 in patients with AMI.
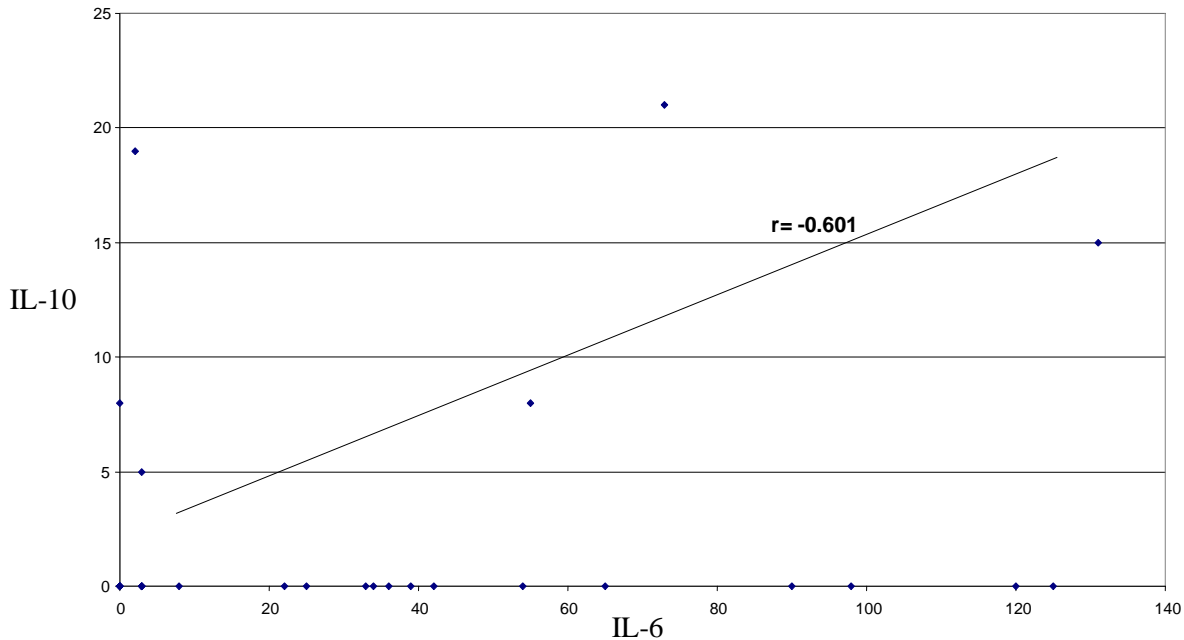
Fig.(3) Linear correlation between IL-10 and IL-6 in patients with AMI.

Discussion

Coronary atherosclerosis plaque disruption with consequent thrombosis is the major cause of acute coronary syndrome. Inflammatory mediators, which potentially have a major role in this process [10]. The results of the present study showed that the median serum levels of pro-inflammatory cytokines (IL-1α, IL-2 and IL-6) were significantly higher in patients with AMI as compared with healthy control group, and these findings are in agreement with other reports [13, 14, 15&16].

IL-1 induces prostanoid dependent hypotension in rabbits *in vivo* and stimulates human smooth muscle cells to secrete IL-6. In chronically ischemic myocardium where focal necrosis was documented, enhanced levels of IL-1 mRNAs were found indicating a role of this cytokine in myocardial inflammation [5]. In contrast with present results Heinisch etal, did not find elevation in IL-1α levels, but they noticed elevation in IL-β in AMI patients [17]. The current study revealed low increase in serum levels of IL-2 but this increasing was statistically significant. Similarly Katarzyna and colleagues also found that serum levels of IL-2 were significantly higher in patients with AMI and unstable angina when compared to stable angina and control group and they

mentioned that AMI is associated with long term increase of serum concentration of cytokines, and they pointed that the sudden coronary artery disease progression leading to acute coronary syndromes is triggered / accompanied by prolonged immune activation [14].

The high level of IL-6 in AMI patients observed in this study was comparable with other studies [15, 16 &18] who reported similar increase of this cytokine. Circulating IL-6 levels may be the result of a variety of stimuli, including various clinical risk factors that lead to the release of IL-6 from numerous cell types, including smooth muscle cells and macrophage/foam cells found in atheromatous plaques. Furthermore, human vascular smooth muscle cells express and secrete IL-6 after IL-1 stimulation [19]. This may be one explanation for the current result in Fig. (2), regarding the presence of positive linear correlation between serum levels of IL-1α and IL-6 in patients. Prior studies of AMI suggest that cytokines are preferentially produced by inflammatory cells in the pre-infarct zone, and persistent elevation of cytokines results from an increased infiltration of inflammatory cells. Thus, these data are consistent with the

hypothesis that inflammation plays a major role in atherosclerosis [20].

Less information is available regarding the role of anti-inflammatory cytokines in AMI. It recently has been demonstrated that the anti-inflammatory cytokines IL-4 and IL-10 may act as a protective factors in atherosclerosis [21]. Consistent with previous studies [9,16, 22 &23] the current results found no significant changes in serum levels of IL-4 and IL-10 in patients as compared with control. Correspondingly, Cheng *et al*, observed that there was no significant differences on the frequencies of IL-4 producing cells between patients and controls, so they concluded that Th1\Th2 functional imbalance exist in myocardial inflammation processes [7].

IL-10 is expressed in both early and advanced human atherosclerotic plaques and inhibits many cellular processes including metalloproteinase production and tissue factor expression, which may play a role in the clinical expression of atherosclerotic plaque rupture or erosion. IL-10 is a powerful suppressor of the immune response. It inhibits pro-inflammatory cytokines such as TNF-α and IL-6 [24 &25] and has multifaceted anti-inflammatory properties, including inhibition of prototypic pro-inflammatory transcription factors, *i.e.*, nuclear factor κB, which leads to the suppression of cytokine production [26]. Interestingly, this support the result of the present study in fig. 3, regarding the presence of negative correlation between IL-6 and IL-10. On the other hand, there were few investigations that found increase levels of IL-10 in AMI [8 & 9]. In other words, increased levels of IL-10 suggest good reperfusion, while low of IL-10 levels mean ischemic but not reperfused myocardium and is accompanied with increased levels of IL-6.

Limitations of previous studies on cytokine markers in AMI include the inclusion of only one or two cytokines that are pro-inflammatory or anti-inflammatory. Multiple cytokines are involved in the inflammatory process, and have overlapping, antagonistic, and synergetic effects on many cell types. In addition, they up-and down-regulate the production of other cytokines and inflammatory markers. In current study, we included three pro-inflammatory cytokines

and two anti-inflammatory cytokines. The limitation of the present study is the lack of follow-up data, mostly due to the lack of patient compliance. In conclusion, a relevant an imbalance in cytokine release is present in AMI, markedly favoring pro-inflammatory effects. Furthermore, the results also suggest that an imbalance between anti- and pro-inflammatory cytokines may act as a marker aid in predicting the prognosis of patients with AMI.

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الخلاصة

أحتشاء عضلة القلب الحاد هو واحد من أكثر امراض القلب شيوعا. تفعيل نظام المناعة ومصنوعاته (المدورات الخلوية) تلعب دورا حاسما في التسبب في مرض أحتشاء عضلة القلب الحاد. إن الهدف من الدراسة هو تحديد مستويات المدورات الخلوية الحاثة للالتهابات IL-2 ،IL-1α،6, والمدورات الخلوية المضادة للالتهابات-IL 10 , 4 IL- و كذلك دراسة العلاقة بين هذه المدورات الخلوية في المرضى الذين يعانون من مرض احتشاء عضلة القلب الحاد. المجموع الكلي كان ٣٠ مريض مصاب بمرض احتشاء عضلة القلب الحاد و ٣٠ شخص سليم، أعمارهم و أجناسهم متطابقة مع المرضى. في هذه الدراسة تم قياس المدورات الخلوية في المصل باستخدام تقنية الـ ELISA. مجموعة المرضى اظهرت زيادة معنوية في مستويات المصل للـ IL-6, IL-2 IL-1α -, مقارنة بمجموعة السيطرة (P<0.05), ($p < 0.01$ وعدم وجود تغيرات احصائية معنوية في المستويات المصلية للـ-IL 10 , 4 IL- بين المرضى والسيطرة. ومن ناحية اخرى هناك علاقة خطية موجبة بين IL-6, α , IL-1 (r=0.787; P=0.004) وعلاقة خطية سالبة بين IL-10 , IL-6. الدراسة الحالية لاحظت زيادة في انتاج المدورات الخلوية الحاثة ونقصان في انتاج المدورات الخلوية المضادة للالتهاب في مرضى احتشاء عضلة القلب الحاد. يستنتج من ذلك ان صلة الاختلال في تحرير المدورات الخلوية في المرضى موجود.

الكلمات الدليلية: احتشاء عضلة القلب الحاد، المدورات الخلوية.