

Measurement of Hepatocyte Growth Factor, Interleukin 6 and Tumor necrosis factor α in Acute Myocardial Infarction

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Abstract

Background: Early mortality rate due to acute myocardial infarction (AMI) is approximately 30%, and half of these deaths occur before reaching a hospital. The prevention and early detection play a key role in reducing mortality in AMI. Hepatocyte Growth Factor (HGF), Interleukin 6 (IL-6), and Tumor Necrosis Factor α (TNF- α) are most recent prognostic biomarkers for AMI. The present study was aimed to evaluate the level of these cytokines in AMI.

Methods: In this case control study, 39 patients with AMI were compared with 30 healthy subjects. Age, sex and other possible confounding factors were matched. AMI diagnosis was confirmed by typical symptoms, electrocardiogram changes and serum concentration of troponin I and creatine kinase-MB. The levels of TNF- α , IL-6 and HGF at baseline and 3 and 7 days later were measured using ELISA method.

Results: Levels of IL-6, TNF- α and HGF increased over time after AMI with ST-segment elevation in study group. The baseline IL-6 levels in AMI group were significantly higher than control group ($P < 0.05$).

Conclusions: The results of this study suggest that baseline levels of IL-6 as well as serial changes of serum IL-6, TNF- α and HGF concentrations can be used as potential diagnostic biomarkers in AMI.

Keywords: acute myocardial infarction, hepatocyte growth factor, interleukin 6, TNF- α

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Introduction

One of the major causes of mortality and morbidity worldwide, especially in many developed countries is coronary artery disease (CAD).¹ In some countries CAD is the cause of nearly half (48%) of the deaths, and as a result loss of the productivity of life. If no serious action is taken, the number of years of productivity life lost to CAD will increase to 64% for Brazil and even more dramatically to 95% for India, by 2030 as compared to 2000.² According to the World Health Organization, it is predicted that by 2030, 23.6 million deaths per year will be due to cardiovascular diseases.¹ A large proportion of CADs occur because of the atherosclerosis. Although the etiology of atherosclerosis is complex and poorly known, there is growing evidence that inflammation plays a central role in developing atherosclerotic lesion, which is in turn widely considered to be related to an inflammatory process.³

It has been proven that both behavioral risk factors (such as tobacco use, unhealthy diet, physical inactivity, and harmful use of alcohol) and metabolic risk factors (such as diabetes, hypertension, high blood lipids, overweight and obesity) possess a crucial role in the etiology of atherosclerosis.⁵ The fragility of the atherosclerotic plaque is associated with the inflammation and plays a significant role in destroying of the fibrous cap, which is the main pathophysiological event for the progression of acute coronary syndromes (ACS) such as unstable angina (UA) and acute myocardial infarction (AMI).⁶ Recent studies showed that some inflammatory cytokines (IL-6, TNF- α and IL-10) were predictors for cardiovascular outcome.^{7, 8} During the first 48 hours of an ACS, IL-6 has been proved a great predictor of a major adverse cardiovascular event over the first month after the initial coronary event and the first year.⁹ It has been observed

that circulating IL-6 concentrations is positively associated with the risk of CAD. Also, high IL-6 levels during acute coronary syndromes (ACS) may be associated with worse long-term prognosis.¹⁰ Some studies have indicated that IL-6 and CRP are powerful predictors of risky coronary events in patients with UA as well as independent risk factors for future MI. In addition, levels of TNF- α were associated with high risk of recurrent coronary events and it is also able to be potential markers for prediction of cardiovascular events.¹¹⁻¹³

Hepatocyte growth factor (HGF) is a cytokine with multipotent actions.¹⁴ It has multiple activities in various tissues during the development of diverse diseases and regulates cell growth, cell motility, and morphogenesis of various types of cells. HGF is also a sensitive biomarker for early diagnosis of acute myocardial infarction that between the known biomarkers, it rises the earliest and very promptly returns to normal values.¹⁵ Some study showed that HGF levels on admission in patients with AMI were higher than control values and peaked on 7th day.^{17, 18} In addition, it has been demonstrated that serum HGF concentrations in early stage might reflect the extent of myocardial damage in AMI patients. HGF was increased within three hours after onset of chest pain in most of AMI patients.¹⁶ Therefore, HGF is useful as a prognostic indicator in patients with AMI and reflects the clinical course of disease.

The aim of this study, is finding cytokine levels and serial changes of HGF, IL-6, and TNF- α in the patients with ST elevation AMI.

Methods and materials

We conducted a case control hospital-based study on 39 patients with AMI who were referred to the Ghaem academic hospital in Mashhad (northeast of Iran) and 30 healthy control subjects. AMI was diagnosed based on typical chest pain lasting 30min, with ST-T segment elevation >0.2 mV in 2 or more contiguous leads on a standard 12-lead electrocardiogram and an increase in Troponin and Creatine Kinase MB activity that was at least twice the upper limit of normal. Healthy control subjects (n = 30) were recruited from among subjects who were referred to for an annual checkup or pre-employment examination as they were assumed not to have CAD and gave no history of cardiovascular symptoms. Also, patients who had history of antiplatelet and statin medication were excluded from control group.

Case and controls subjects were age, and sex matched. Clinical signs and laboratory results (Troponin I and Creatine Kinase MB) of patients with AMI were documented.

The levels of these cytokines were evaluated at three points in the patients with AMI. In addition, the possible relationship between these three factors with echocardiographic findings and Killip class were evaluated. Killip classification is defined based on the Killip's article in 1967.¹⁹

The Laboratory Measurement of Cytokines and biochemical parameters

Venous blood samples were taken from patients immediately before the administration of heparin at the time of admission, and 3 and 7 days later. Serums were separated from blood samples by centrifugation, and were stored at -70°C. ELISA cytokines have been used for HGF, IL-6, and TNF- α measurement.

Fasted blood samples (20 ml) were collected into plain Vacutainer™ tubes, for cytokines and growth factor measurements, lipid profile and those for measurement of fasting blood glucose was taken into Vacutainer™ tubes containing fluoride-oxalate.²⁰

Total cholesterol (TC), low density lipoprotein cholesterol (LDLC), high density lipoprotein cholesterol (HDL), Triglyceride (TG) and fasting blood sugar (FBS) were measured using routine techniques using a Cobas auto-analyser system (ABX Diagnostics, Montpellier, France).²⁰

The results of the FBS <110 mg/dL were interpreted normal values, FBS between 110 and 126 mg/dL were considered as impaired fasting glucose (IFG), and FBS >126 mg/dL were considered as Diabetes Mellitus, according to the American Diabetic Association criteria.²⁰

Statistical analysis:

The Statistical Package for Social Sciences (SPSS version 16) was used for data analysis. The Kolmogorov-Smirnov test was used to assess normality. Descriptive statistics (frequency, mean, and standard deviation) were determined for all variables. Values are reported as mean \pm SD for normally distributed variables (or Median and IQR for not normal distribute variable). Baseline demographics and clinical characteristics were compared among groups using t-student test, chi-square test, and/or Fisher exact test as appropriate. A P-value <0.05 was regarded as statistically significant.

Ethical Issue:

The study was conducted in accordance with the principles of Declaration of Helsinki 1996 version and Good Clinical Practice standards. The study protocol, informed-consent form, and the other study related documents were reviewed and approved by Human Research Ethics Committee of Mashhad University of Medical Sciences.

Results

The main age of case group subjects was 55.74 \pm 13.08 and in the control group was 64.9 \pm 10.5 with no significant difference between groups in sex, smoking habit, opium addiction, hypertension, hyperlipidemia, diabetes mellitus, and stroke history (P>0.05) (table1).

In case group 41% (16 subjects) had history of unstable angina whereas in control group nobody had this history and there was a significant difference between groups (P<0.001) (table1).

In the biochemical components of blood analysis there were no significant difference between groups in FBS, LDLC, HDLC, TC, TG, BUN, Creatinine, Hemoglobin, and Hematocrit (P>0.05) (table1).

According to the electrocardiograph analysis most of patients in case group (more than 77%) had inferior wall ST elevation myocardial infarction (MI) followed by anterior wall ST elevation MI (table2).

In cytokine assay between groups at baseline, IL-6 in case group was significantly higher than control group (P=0.001) but differences in HGF and TNF- α are not significant (P>0.05) (table 1). In addition, serial cytokine assay in case group showed that serum levels of IL-6, TNF- α and HGF have increased significantly over time (P<0.05)(table 3).

Table 1- basic characteristics of case and control subjects

group Variable		Case N=39	Control N=30	P value
Age		55.74±13.08	64.9±10.5	0.477
Sex	Male	26(66.7)	18(60)	0.567
	Female	13(33.3)	12(40)	
Smoking	Present	12(30)	10(33.3)	0.874
	Absent	27(70)	20(66.7)	
Addiction history	Present	7(17.9)	8(26.6)	0.611
	Absent	32(82.1)	22(73.4)	
Hypertension	Present	19(48.7)	8(26.6)	0.840
	Absent	20(51.3)	22(73.4)	
Diabetes Mellitus	Present	4(10.25)	3(10)	0.743
	Absent	35(89.75)	27(90)	
Hyperlipidemia	Present	9(23)	10(33.3)	0.344
	Absent	30(77)	20(66.7)	
Stroke History	Present	2(5.1)	1(3.33)	0.953
	Absent	37(94.9)	29(96.6)	
Unstable angina history	Present	16(41)	0(0.0)	<0.001
	Absent	23(59)	30(100)	
Killip classification	I	32(82)	-	-
	II	7(18)	-	
Ejection fraction	<30%	7(18)	-	-
	30-40%	7(18)	-	
	40-50%	22(57)	-	
	>50%	3(7)	-	
End diastolic	Grade I	28(72)	-	-
Left ventricular function	Grade II	11(28)	-	
FBS (mg/dl)		120.12±25.66	110±24.1	0.345
TC (mg/dl)		162.88±38.70	165±40.67	0.061
LDL-C (mg/dl)		96.77±35.130	100±37	0.084
HDL-C (mg/dl)		41.55±8.25	43±9.54	0.093
TG (mg/dl)		152.59±84.674	160±87.47	0.099
BUN		27.50±8.88	25±7.98	0.455
Creatinine		0.994±0.288	0.85±0.3	0.833
Hemoglobin		12.63±1.79	13.5±1.94	0.433
Hematocrit		39.194±4.67	42±4.5	0.532
Base Line	HGF	1913±2101	1294±562	0.1
	TNF- α	39.59±119	17±7	0.27
	IL-6	132.26±86.41	52±13	0.001

Data in this table shown as: Mean \pm SD and percentage in parentheses.

^a FBS: Fasting blood sugar

^b TC: total cholesterol

^c LDL-C: Low density lipoprotein- cholesterol

^d HDL-C: High density lipoprotein- cholesterol

^e TG: triglyceride

Table 2- electrocardiogram abnormal findings

Lead	Abnormal Findings	No.(%)
Lateral leads	Present	14(36)
	Absent	25(64)
Anterior leads	Present	18(46)
	Absent	21(54)
Inferior leads	Present	30(77)
	Absent	9(23)
Right lead	Present	8(20)
	Absent	31(80)
Posterior lead	Present	7(18)
	Absent	32(82)

Table 3- Cytokine assay in Case group during hospital stay^a

	Base line	3rd days after AMI	7th days after AMI	P ₀ ^b	P ₁ ^c	P ₂ ^d
HGF _(pg/mL)	1360(1225-1601)	1810(1310-2516)	1913(1565-4367)	0.006	0.006	0.019
TNF- α _(pg/mL)	3.2(2.9-3.6)	3.3(3-3.7)	3.3(3.1-3.8)	0.012	0.008	0.006
IL-6 _(pg/mL)	102(86-142)	167(145-408)	203(162-590)	<0.001	<0.001	<0.001

^a Data shown as Median (Interquartile range)

^b P₀ is P value of comparing between base line via 3rd days after AMI.

^c P₁ is P value of comparing between base line via 7th days after AMI.

^d P₂ is P value of comparing between 3rd days via 7th days after AMI.

Discussion

The main findings of this study are that (1) IL-6 increases in early phase after AMI and that (2) serial elevation in serum levels of IL-6, TNF- α and HGF occurs after AMI.

It had been proved previously that thrombus formation induces the HGF circulating and it is increasing after AMI.²¹ Also HGF serum level is higher in angina pectoris and old myocardial infarction.²² Soeki et al. showed that HGF levels in patients with AMI were higher than control values at the time of admission. Also, levels of HGF on day 7 after AMI were higher than baseline levels¹⁸. In the present study, although difference between baseline HGF in case group and control group was not significant, HGF levels of case group in day 3 was higher than baseline and in day 7 was higher than baseline and 3rd day. Serial increased levels of HGF over time, have not been reported previously.

Cytokine IL-6 is the major procoagulant that is secreted by numerous cells such as monocytes, lymphocytes, fibroblasts, endothelial cells and smooth muscle.²³ Puhakka et al. found that IL-6 has increased immediately and transiently after AMI.²⁴ In a case-control study with 1179 surviving AMI cases and 1528 healthy controls, it was proved that in men, MI risk was higher with increasing IL-6 levels.²⁵

Increased IL-6 in the first 48 hours after ACS is associated with prognosis of future cardiovascular events.²³ In addition, IL-6 in the patients with refractory unstable angina are higher than those with stable angina.²⁶ A study that was conducted on 38 patients with stroke showed that IL-6 levels are increased in stroke with mechanisms of atherosclerosis plaque rupture.²⁷ We found same result in our study; IL-6 was higher in case group than controls at the admission time. It also increased serially on 3rd day and 7th day after admission.

TNF- α can be produced by many cell types such as macrophage cells, lymphoid cells, mast cells, endothelial cells,

cardiac myocytes and adipose tissue. It is known as a regulator of inflammatory cells, and as a pyrogenic agent. TNF- α is involved in almost all aspects of the inflammatory process of atherosclerosis.²⁸ High levels of TNF- α in the patients after AMI is an independent risk factor in cardiovascular events occurring again.²⁸ It was shown before that TNF- α increase significantly on admission and 24 hours post admission in AMI patients comparing to control group.²⁹ Although high levels of TNF- α on admission time were not confirmed in our study, serial increase of this cytokine over time was observed.

Conclusion

We have reported increased levels of IL-6, TNF- α and HGF during AMI with ST-segment elevation patients. At the admission time, the IL-6 levels were significantly higher than the control group. So, besides the clinical signs and symptoms of AMI, measuring IL-6 may be helpful for diagnosis of this disease. It seems that further studies are needed to determine sensitivity and specificity in larger number of patients.

Conflict of Interest Statement

The authors indicate no potential conflicts of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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