

Tropical Journal of Pharmaceutical Research April 2016; 15 (4): 827-831

ISSN: 1596-5996 (print); 1596-9827 (electronic)

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Available online at <http://www.tjpr.org><http://dx.doi.org/10.4314/tjpr.v15i4.23>

Original Research Article

Effects of coagulation factors and inflammatory cytokines on development of acute myocardial infarction in patients younger than 60 years

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Received: 15 October 2015

Revised accepted: 9 March 2016

Abstract

Purpose: To investigate the effects of coagulation factors and inflammatory cytokines on acute myocardial infarction (AMI) development in patients younger than 60 years.

Methods: In this study, 60 patients admitted to The First Affiliated Hospital of Dalian Medical University (Dalian, China) with AMI and 30 other subjects matched with the patients for age and ethnicity but without AMI were enrolled. Blood samples were collected from the AMI patients and the control subjects after a 12-h fast. Subsequently, the levels of coagulation factors (F II (FII), VII (FVII), VIII (FVIII), fibrinogen (Fg) and von Willebrand factor (vWF) in plasma were analyzed by enzyme-linked immunosorbent assay (ELISA). The protein expression levels of these coagulation factors were determined by Western blot analysis. Inflammatory factors including C-reactive protein (CRP), tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) were also measured by ELISA.

Results: FII, FVII, FVIII, Fg and vWF levels in plasma were significantly higher in AMI patients compared with control subjects ($p < 0.01$). Furthermore, the protein expression levels of FII, FVII, FVIII, Fg and vWF were also significantly up-regulated in AMI patients compared with those in control subjects. Additionally, no significant difference was observed in CRP between AMI patients and control subjects ($p > 0.05$). However, TNF- α and IL-6 levels in the plasma of AMI patients were significantly higher than those in control subjects ($p < 0.05$).

Conclusion: The results reveal that the pathogenesis of AMI in patients younger than 60 years might be closely related to the high levels of coagulation factors and inflammatory cytokines in the blood.

Keywords: Coagulation factor, Inflammatory cytokines, Acute myocardial infarction, C-reactive protein, Tumor necrosis factor- α , Interleukin-6

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Currently, coronary heart disease (CHD) has become one of the leading global threats to human health [1]. Acute myocardial infarction (AMI) is the major clinical complication of CHD and the main cause of mortality in patients [2]. Although great improvements have been achieved in the diagnosis and treatment of AMI,

the short- and long-term mortality rates in AMI patients still remain high [3,4]. In addition, the onset age of AMI is becoming increasingly low [5,6]. Therefore, it is of great significance to investigate the pathogenesis of AMI in patients younger than 60 years.

Epidemiological studies have revealed that some coagulation factors including coagulation factor

(F) II (FII), VII (FVII), VIII (FVIII), fibrinogen (Fg) and von Willebrand factor (vWF), are closely related to thrombus formation and CHD [7,8]. In addition, the systemic inflammation response has been documented in patients with AMI, and includes the elevation of circulating inflammatory cytokines such as C-reactive protein (CRP), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) [9,10]. However, to the best of our knowledge, there are few reports regarding the effects of coagulation factors and inflammatory cytokines on the occurrence and development of AMI in patients younger than 60 years. The present study was aimed to investigate the effects of coagulation factors and inflammatory cytokines on the development of acute myocardial infarction in patients younger than 60 years, findings that would be beneficial for the accurate diagnosis and effective treatment of AMI in the clinic.

EXPERIMENTAL

Chemicals and reagents

Human anti-prothrombin (FII), anti-FVII, anti-FVIII, anti-Fg and anti-vWF monoclonal antibodies were obtained from Abcam (Cambridge, UK); CRP, TNF- α and IL-6 ELISA kits were purchased from Pierce/Endogen Co. (Rockford, IL, USA). FII, FVII, FVIII, Fg and vWF enzyme-linked immunosorbent assay (ELISA) kits were purchased from Pierce/Endogen Co. (Rockford, IL, USA). All other reagents used in this study were of analytic grade.

Patients and controls

Sixty patients (39 males and 21 females) admitted to The First Affiliated Hospital of Dalian Medical University (Dalian, China) with a diagnosis of AMI, defined according to the guidelines of the WHO MONICA protocol [11], were studied. A group of 30 control subjects (19 males and 11 females) matched with the patients for age and ethnicity, were also enrolled in the present investigation. No control subject had episodes of cardiovascular disease and none used antihypertensive or lipid-lowering drugs. The characteristics of AMI patients and controls are shown in Table 1, and no obvious difference of the characteristics was observed between the two groups.

Venous blood samples were collected from the patients during hospitalization and the control subjects after a 12-h fast. Subsequently, 3.8 % sodium citrate solution (1:9, v/v) was added to the blood, and the samples were centrifuged

(3000 rpm, 15 min). The isolated plasma was snap-frozen and stored at -80 °C before measurement. All our experimental protocols were approved by the ethical committee of The First Affiliated Hospital of Dalian Medical University (Ref no. 20120918-h1). All the patients and controls were residents of the region of Dalian and gave their consent to participate in the study.

Table 1: Baseline demographics and disease characteristics of AMI patients and control subjects

Variable	Control	AMI
Sex (M/F)	19/11	39/21
Body Weight (kg)	53.73 \pm 4.97	51.85 \pm 6.16
Age	54.74 \pm 5.05	52.03 \pm 7.21
Smoking (%)	60	51.67
Hypertension (%)	46.7	50
Diabetes (%)	6.7	10
TC (mmol/L)	3.48 \pm 0.82	3.69 \pm 0.98
TG (mmol/L)	1.82 \pm 0.63	2.08 \pm 0.73
Blood sugar (mmol/L)	5.21 \pm 1.14	6.03 \pm 1.96

Determination of FII, FVII, FVIII, Fg and vWF levels in plasma

Coagulation factors including FII, FVII, VIII (FVIII), Fg and vWF in the plasma were analyzed by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Determination of FII, FVII, FVIII, Fg and vWF protein levels

Total protein samples were separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) and then transferred to a polyvinylidene difluoride (PVDF) membrane. The proteins were probed with the appropriate primary antibodies and secondary antibodies conjugated with horseradish peroxidase subsequently. Next, they were visualized using chemiluminescence peroxidase reagents. To normalize the protein loading, β -actin was used as the internal control.

Determination of CRP, TNF- α and IL-6 levels in plasma

Furthermore, inflammatory cytokines including CRP, TNF- α and IL-6 were also determined by ELISA according to the manufacturer's instructions.

Statistical analysis

Data are expressed as mean \pm standard deviation (SD). Student's t-test was used to perform statistical analysis of the data.

Differences were considered statistically significant at $p < 0.05$.

compared with those in the control subjects (Figure 2).

RESULTS

CRP, TNF- α and IL-6 levels in plasma

Levels of coagulation factors in plasma

As seen in Figure 1, the ELISA results indicate that the plasma levels of coagulation factors, including FII, FVII, FVIII, Fg and vWF, were significantly higher in AMI patients than in control subjects ($p < 0.01$). Furthermore, the western blot assay results also revealed protein expression levels of FII, FVII, FVIII, Fg and vWF were up-regulated obviously in AMI patients

The plasma levels of inflammatory factors including CRP, TNF- α and IL-6 are shown in Figure 3. Our results showed that there were no significant differences in the release of CRP between AMI patients and the control subjects ($p > 0.05$). Interestingly, the TNF- α and IL-6 levels in the plasma of AMI patients were significantly higher than those of control subjects ($p < 0.05$). Our results showed that the release of inflammatory cytokines, including TNF- α and IL-6, is associated with pathogenesis of AMI patients younger than 60 years.

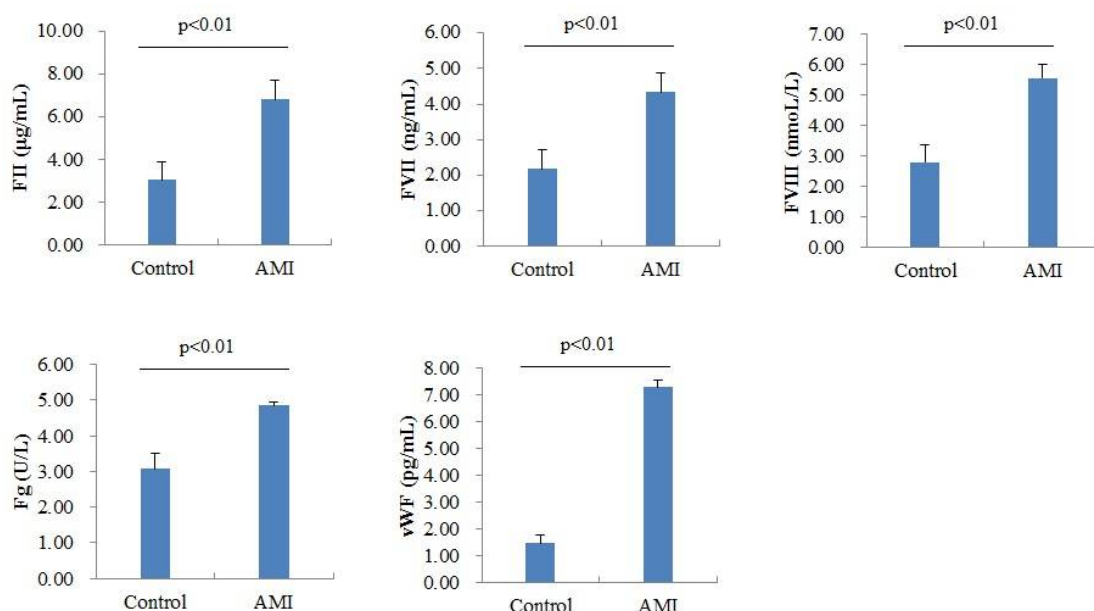


Figure 1: FII, FVII, FVIII, Fg and vWF levels in plasma. Data are expressed as mean \pm SD ($p < 0.01$), compared with control group

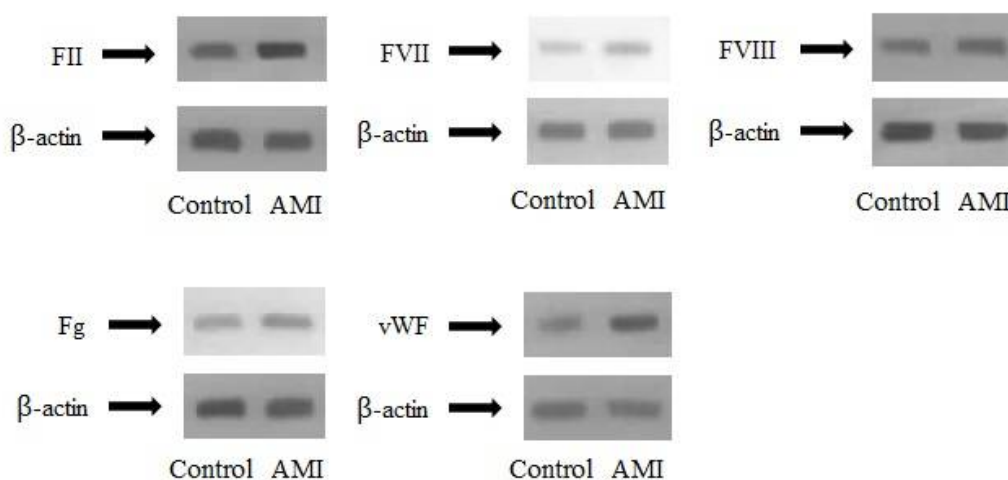


Figure 2: Protein expressions FII, FVII, FVIII, Fg and vWF in plasma of AMI patients

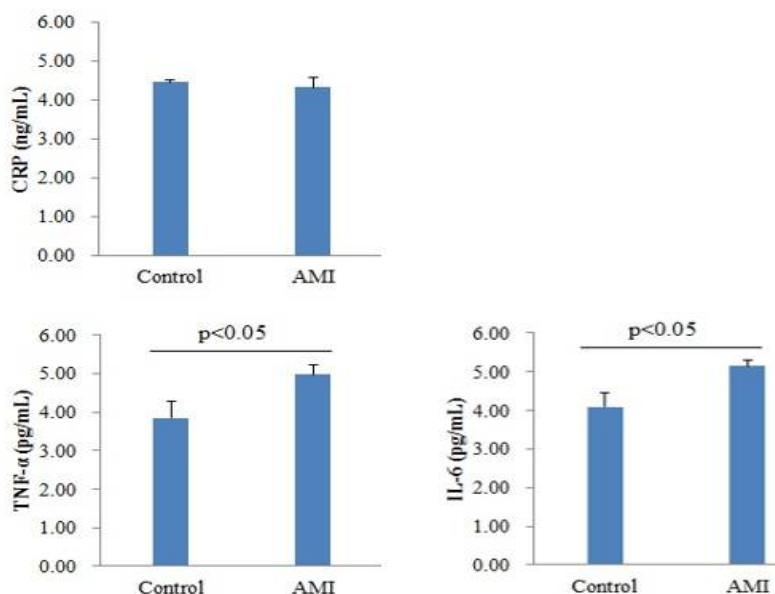


Figure 3: Plasma levels of CRP, TNF- α and IL-6. Data are expressed as mean \pm SD ($p < 0.05$), compared with control group

DISCUSSION

Recently, it had been reported that the AMI) is a frequent cause of death or severe permanent disability, and the age of AMI patients is becoming increasingly young [12,13]. Thus, the present study was designed to investigate the possible pathogenic mechanism of AMI in patients younger than 60 years related to coagulation factors and inflammatory cytokines.

AMI is associated with the coagulation and thrombotic occlusion of coronary vessels in the presence of unstable atheroma [14]. FII, FVII, FVIII, Fg and vWF are important coagulation factors in the blood coagulation process. FII (Prothrombin) plays a central role in the coagulation mechanism, and it can be activated to FIIa (thrombin). Fg could then change into fibrin due to the activation of FIIa [15]. FVII is the initiation factor of the exogenous coagulation pathway, and its abnormalities in the plasma may increase the risk of myocardial infarction and sudden death [16]. FVIII is involved in the second stage of the coagulation process, and it is also involved in the activation of coagulation factor X (FX). In addition, vWF and FVII could form a complex to stabilize and protect the activity of FVIII [17]. Several literature sources have proved that these coagulation factors play important roles in the development of vascular disease and AMI [18-21]. Therefore, we evaluated the plasma levels of coagulation factors in AMI patients and control subjects. Our results indicate that the high levels of coagulation factors in plasma (FII, FVII, FVIII, Fg and vWF) were closely related to

the development of AMI in patients younger than 60 years.

Increasing investigations have demonstrated that inflammation plays an important role in the pathogenesis of CHD. Inflammatory cytokines including CRP, TNF- α and IL-6 mediate adverse cardiovascular events in patients with CHD and they are used as indices for diagnosing CHD [22,23]. In our present study, the levels of inflammatory cytokines, including CRP, TNF- α and IL-6, in plasma were determined. Our results showed that the levels of TNF- α and IL-6 in AMI patients were higher than those control subjects, indicating that high levels of TNF- α and IL-6 might be related to the development of AMI in patients younger than 60 years.

CONCLUSION

Findings from this study reveal that the pathogenesis of AMI in patients younger than 60 years might be closely related to the high levels of coagulation factors and inflammatory cytokines in the blood. In addition, the results of the present study would be beneficial for accurate diagnosis and effective treatment of AMI in clinic.

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