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*Research Paper*

# The serum levels of MMP-9, sICAM-1, CRP and WBC increased in patients with acute coronary syndrome

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## Abstract

**Objective:** To evaluate the association of the peripheral levels of the defined inflammatory markers with different types of acute coronary syndrome (ACS) and stable angina, and the role inflammation played in the pathogenesis of ACS. **Methods:** For understanding the variation of serum concentrations of matrix metalloproteinase-9 (MMP-9), soluble intercellular adhesion molecule-1 (sICAM-1), C-reactive protein (CRP), and white blood cell count (WBC) and their association with ACS, 90 patients with coronary heart disease (CHD) and 30 healthy volunteers were recruited. The enrolled people were assigned into four equal groups, including acute myocardial infarction (AMI) group, unstable angina pectoris (UAP) group, stable angina pectoris (SAP) group and healthy control group. The serum levels of MMP-9 and sICAM-1 were measured with ELISA kits, CRP were measured with immunoturbidimetric assay, and WBC number were assessed all before any treatment was administrated. **Results:** (1) The serum levels of MMP-9, sICAM-1, CRP and WBC in the patients with ACS were significantly higher than those in the control group ($P < 0.01$). (2) Compared with control group, patients with SAP only had higher serum level of sICAM-1 ($P < 0.01$). While the levels of MMP-9, CRP, and WBC had no significant difference between them ($P > 0.05$ all). (3) Significant positive correlation between the serum levels of MMP-9 and sICAM-1 and CRP and WBC all were observed in the patients with ACS ($P < 0.05$). **Conclusion:** The elevation of serum concentrations of inflammatory markers including MMP-9, sICAM-1, CRP and WBC were associated with initiation and progression of ACS, and they may help predicting cardiovascular events.

*Keywords:* acute coronary syndrome; matrix metalloproteinase-9; soluble intercellular adhesion molecule-1; C-reactive protein; white blood cell count

## INTRODUCTION

Acute coronary syndrome (ACS) is characterized by the activation of systemic and local inflammatory mediators [1,2], so many researchers believe that inflammation plays a critical role in the pathogenesis of ACS and their clinical consequences [3]. Local inflammation in the arterial wall may promote vascular smooth muscle apoptosis, attenuate interstitial collagen synthesis, increase fibrin cap degradation, and all these may enhance plaque vulnerability. Eventually, the plaque rupture, then platelet adhesion and thrombus formation occurs, resulting in clinically defined ACS [4]. However, the correlation between many soluble inflammatory proteins and cells and their association with severity of this syndrome haven't been extensively studied. Our present study was to evaluate the association of the peripheral levels of some inflammatory markers, as matrix metalloproteinase-9 (MMP-9), soluble intercellular adhesion molecule-1 (sICAM-1), C-reactive protein (CRP), and white blood cell count (WBC), with different type of coronary heart disease (CHD), and the role inflammation played in the pathogenesis of ACS.

## MATERIALS AND METHODS

### Materials

We investigated a series of 90 patients with

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CHD(49 males and 41 females, aged 40 to 83 years) referred to the Department of Cardiology, the First Affiliated Hospital to Medical College of Shantou University from December 2004 to June 2005. Then they were assigned into three groups, including AMI group (30 patients), UAP group (30 patients), and SAP group(30 patients). The diagnosis was based on the presence of symptoms and signs of ischemic, biochemical evidence of myonecrosis, and electrocardiographic evidence of ischemia, as well as supportive coronary angiographic findings. Moreover, one equivalent group of 30 healthy volunteers were recruited (15 males and 15 females, aged 43 to 74 years) as a control. Exclusion criteria were presence of peripheral vascular diseases, significant co-morbid cerebral, hepatic, or renal diseases, hypertension, diabetes mellitus, or cancer, inflammatory disorders including infections and collagen vascular diseases, and use of steroid or anti-inflammatory drugs. All blood samples for assays had been drawn before any treatment was given. All patients and healthy volunteers gave informed consent.

### Methods

Blood samples for measurements were drawn as soon as the patients and controls had hospital admission and before any treatment was given. Serum was obtained by centrifugation of vacutainer clotted tubes at 3000 rpm for 10 minutes. All serum samples were stored at −70℃ until analysis. Serum levels of MMP-9 and sICAM-1 were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (Sigma), CRP samples were analyzed by latex particle-enhanced immunoturbidimetric assay with SYNCHRON LX20 Clinical System (Beckman), and the WBC were also determined before treatment.

### Statistical Analysis

Data were expressed as mean±SD. Differences between groups were analyzed by the t-test, and correlation coefficients were determined to evaluate the correlation between circulating levels of MMP-9, sICAM-1, CRP and WBC. A value of $P<0.05$ was considered to be statistically significant. Statistical analyses were performed with SPSS 11.0 statistical software.

## RESULTS

### Baseline characteristics

Each group displayed similar demographic and baseline characteristics except that serum levels of TG and TC in the patients with AMI and UAP were higher than those in the control group($P < 0.05$, ***Tab 1***).

### The alteration of serum levels of MMP-9, sICAM-1, CRP and WBC in the patients with ACS and SAP compared with those in control group

The serum concentrations of MMP-9, sICAM-1, CRP and WBC in the patients with AMI and UAP were significantly elevated than those in the control group ($P < 0.01$). However, compared with control group, patients with SAP only had higher serum level of sICAM-1 ($P < 0.01$), but similar levels of MMP-9, CRP, and WBC($P > 0.05$ all, ***Tab 2***).

### The correlation between the serum levels of MMP-9 and sICAM-1 and CRP and WBC in the patients with ACS

When AMI and UAP group were combined together as one ACS group, the significant positive correlation between the serum levels of MMP-9 and

***Tab 1*** **Baseline demographics and clinical characteristics of the study population** ($\bar{x} \pm s$)

| | CONTROL ($n$=30) | SAP ($n$=30) | UAP ($n$=30) | AMI ($n$=30) | $F$ | $P$ |
|---|---|---|---|---|---|---|
| Age(year) | 59.2 ± 7.1 | 62.3 ± 8.8 | 63.1 ± 10.7 | 62.4 ± 6.9 | 1.46 | 0.23 |
| Sex(M/F) | 15/15 | 16/14 | 17/13 | 16/14 | 1.57 | 0.21 |
| BMI(kg/m2) | 24.4 ± 2.5 | 26.2 ± 2.7 | 24.9 ± 2.5 | 25.5 ± 2.7 | 2.01 | 0.14 |
| SBP(mmHg) | 125 ± 13 | 125 ± 15 | 133 ± 16 | 124 ± 12 | 1.29 | 0.33 |
| DBP(mmHg) | 76 ± 10 | 79 ± 11 | 80 ± 12 | 79 ± 10 | 1.25 | 0.39 |
| TG(mmol/L) | 1.66 ± 0.57 | 1.57 ± 0.51 | 2.53 ± 0.64* | 2.69 ± 0.82* | 10.31 | 0.003 |
| TC(mmol/L) | 4.61 ± 0.81 | 4.74 ± 0.83 | 5.82 ± 0.92* | 5.51 ± 0.84* | 8.24 | 0.005 |
| HDL-C(mmol/L) | 1.24 ± 0.46 | 1.18 ± 0.25 | 1.22 ± 0.27 | 1.23 ± 0.47 | 1.41 | 0.27 |
| LDL-C(mmol/L) | 2.81 ± 0.71 | 2.82 ± 0.64 | 2.83 ± 0.64 | 2.74 ± 0.83 | 1.39 | 0.28 |
| FBS(mmol/L) | 5.26 ± 0.89 | 5.24 ± 1.37 | 5.04 ± 0.91 | 5.19 ± 0.78 | 1.25 | 0.39 |
| BUN(mmol/L) | 5.53 ± 1.95 | 6.17 ± 1.77 | 6.23 ± 2.49 | 5.78 ± 1.31 | 1.16 | 0.47 |
| Cr(μmol/L) | 115.57 ± 34.29 | 118.31 ± 42.12 | 121.98 ± 37.33 | 117.75 ± 33.92 | 2.33 | 0.11 |
| UA(μmol/L) | 377.34 ± 112.75 | 359.01 ± 124.19 | 342.15 ± 122.37 | 367.84 ± 104.46 | 1.15 | 0.47 |
| GPT(U/L) | 29.4 ± 10.9 | 33.3 ± 11.6 | 28.3 ± 9.5 | 33.6 ± 8.9 | 1.21 | 0.43 |

*$P < 0.05$ *vs.* control by Q-test

***Tab 2*** **Comparison of serum levels of MMP-9, sICAM-1, CRP and WBC in patients with CHD with those in control group** ($\bar{x} \pm s$)

| | MMP-9(ng/ml) | sICAM-1(ng/ml) | CRP(mg/L) | WBC($\times 10^9$/L) |
|---|---|---|---|---|
| CONTROL($n$=30) | 46.09 ± 31.86 | 94.27 ± 43.74 | 4.71 ± 2.16 | 5.34 ± 1.27 |
| SAP($n$=30) | 39.25 ± 27.69 | 131.60 ± 59.88** | 5.09 ± 1.72 | 5.59 ± 1.98 |
| UAP($n$=30) | 139.80 ± 32.08** | 249.30 ± 23.82** | 12.22 ± 4.21** | 9.17 ± 1.64** |
| AMI($n$=30) | 247.83 ± 36.87** | 341.51 ± 31.84** | 15.71 ± 4.72** | 13.71 ± 4.27** |
| $F$ | 23.58 | 76.91 | 33.69 | 21.62 |
| $P$ | < 0.01 | < 0.01 | < 0.01 | < 0.01 |

**$P < 0.01$ *vs.* control by Q-test

sICAM-1 ($r = 0.234, P = 0.031$), MMP-9 and CRP ($r = 0.436, P = 0.007$), MMP-9 and WBC ($r = 0.471$, $P = 0.006$), sICAM-1 and CRP($r = 0.253, P = 0.029$), sICAM-1 and WBC ($r = 0.272, P = 0.023$), and CRP and WBC ($r = 0.503, P = 0.004$) all were observed in the patients with ACS($P < 0.05$, ***Table 3***).

***Tab 3*** **Pearson correlation between serum levels of MMP-9 and sICAM-1 and CRP and WBC in the patients with ACS**

| | MMP-9 | sICAM-1 | CRP |
|---|---|---|---|
| sICAM-1 | 0.234* | | |
| CRP | 0.436** | 0.253* | |
| WBC | 0.471** | 0.272* | 0.503** |

*$P < 0.05$; **$P < 0.01$

## DISCUSSION

Activation of inflammatory pathways plays an important contributory role in coronary plaque instability and subsequent rupture, which can lead to the development of acute coronary syndrome(ACS)[5]. Elevated levels of serum inflammatory markers such as C-reactive protein (CRP) represent independent risk factors for further cardiovascular events[6].

Recent studies have suggested that inflammation plays a central pathogenic role in the initiation and progression of coronary atheroma and its clinical consequences[7]. Inflammatory markers are the mediators of cellular inflammation and promote local inflammation in the arterial wall, which may lead to vascular smooth muscle apoptosis, degradation of the fibrin cap and plaque rupture. Platelet adhesion and thrombus formation then occur, resulting clinically in unstable angina or myocardial infarction[8]. Our investigation evaluated the variation of serum levels of some inflammatory markers, as MMP-9, sICAM-1, and so on, and demonstrated that the elevation of serum levels of those markers were associated with initiation and progression of ACS, and they may help predicting cardiovascular events.

MMP-9 is aimportant member of a zinc-dependent metalloendoproteinases family that are involved in the degradation of extracellular matrix components. This enzyme, like MMP-2 its closest homologue in the family, is classified as a gelatinase. It is mainly expressed and secreted as protease precursor(by activated inflammatory cells, like macrophage, T cells, and neutrophil cells) which can cleave a variety of proteins including many components of the extracellular matrix such as collagens Ⅰ, Ⅱ, Ⅳ, and Ⅴ, entactin, and elastin [9-10]. So the increased expression and enhanced activity of MMP-9 can result in excess degradation of collagen. In patients with atherosclerosis, this may cause loss in mechanical integrity of plaque tissue that favors rupture. And physical disruption of vulnerable plaque often underlies acute coronary syndromes [11]. Nomoto K *et al* [12] reported that the MMP-9 level was significantly higher in the ACS group than in the chronic coronary artery disease group and the healthy control group. According this, they concluded that plaque destabilized by MMP-9 produced in response to inflammation participates in the mechanism of acute coronary syndrome. This study also showed a marked elevation of the MMP-9 level in patients with ACS. This suggested that the increase of MMP-9 level may be associated with pathopoiesis of ACS.

ICAM-1 was found on endothelial cells and leukocytes, but most ICAM-1 in the serum come from activated endothelium. Its pronounced adhesive ability could enhance the binding of leukocytes on the endothelium. It is important in the immigration of leukocytes from the circulation into the intima tissue [13,14]. This could contribute to the destruction of atherosclerotic plaque. Their denudation would be an extensive stimulus for thrombus formation in the artery, furthermore result in clinical ACS [15]. The soluble forms of ICAM-1 (sICAM- 1) are produced through enzymolysis, so its serum level reflects the expression of ICAM-1, considered to be markers of inflammatory endothelial activation [16]. Besides having been found in many inflammatory processes, elevated concentration of sICAM-1 was reported as well during ACS[17]. This investigation also found the higher serum level of sICAM-1 in patients with ACS

than those in controls. These data sets hint that the serum level of sICAM-1 may be a marker of instable extent of atheromatous plaque. Following the elevation of ICAM-1 level, inflammatory cells accumulate at the local vessel wall, from which most MMP-9 release, thus, the plaque would become more unstable.

Further more, in consideration of the important role that inflammatory processes play in determining plaque stability; recent works have focused on whether plasma inflammatory markers may help improve risk stratification. Of these markers, CRP has been the most widely studied, and there is now robust evidence that CRP is a strong independent predictor of future cardiovascular events, i.e., myocardial infarction (MI), stroke and death in patients with angina and in apparently healthy subjects [18]. Moreover, it has been suggested that CRP may not only portend the vulnerability of an atherosclerotic plaque but also directly and actively participate in both atherogenesis[19] and atheromatous plaque disruption [20]. In addition, many investigators have reported that WBCs, especially monocytes/macrophages, destabilize coronary artery plaques and an elevated WBC count is a risk factor of ACS [21,22]. In accordance with these data, the significant higher serum CRP and WBC levels in patients with ACS than those in SAP and control groups in our study indicate that elevated serum CRP and WBC levels may be simple but effective markers of deciding clinical prediction and prognosis of ACS.

According to our study, the concentration of MMP-9,sICAM-1,CRP and WBC increased as the disease deteriorates. Although our results may not be obviously similar as to other studies, owing to different assay methods, objects or other test variables. We do predict however, that the levels of these inflammatory markers may be used to assess ACS severity. Besides this, we found further significant positive correlations between the serum levels of MMP-9, sICAM-1, CRP and WBC in the patients with ACS. This demonstrated that the interaction between inflammatory markers lie in ACS, which worsens local inflammation in the arterial wall, and promotes plaque rupture and acute thrombosis. So we hypothesize that the combined detection of these inflammatory markers may more exactly predict the occurrence, severity and prognosis of acute cardiovascular events. And in order to stipulate these factors precisely, more extensive investigations may be needed.

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