Abstract: The study aimed to investigate the involvement of cerebral microcirculation turbulence after subarachnoid hemorrhage (SAH). Wistar rats were divided into non-SAH and SAH groups. Autologous arterial hemolysate was injected into rat's cisterna magna to induce SAH. Changes of pial microcirculation within 2 hours were observed. It was found that there were no obvious changes of the diameters, flow velocity and fluid state of microvessels in non-SAH group. With the exception of rare linear-granular flow in A4 arteriole, linear flow was observed in most of the arterioles. There was no blood agglutination in any of the arterioles. After SAH, abnormal cerebral pial microcirculation was found. Spasm of microvessels, decreased blood flow, and agglutination of red blood cells occurred. Five minutes following the induction of SAH, the diameters of the arterioles and venules significantly decreased. The decreased diameters persisted for 2 hours after cisternal injection. Decreased flow velocity of venules was found from 5 to 90 minutes after induction of SAH. Spasm of the basilar artery and increased brain malondialdehyde were also found after SAH. We concluded that cerebral microcirculation turbulence plays an important role in the development of secondary cerebral ischemia following SAH.

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Dynamic alterations of cerebral pial microcirculation during experimental subarachnoid hemorrhage

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Abstract

The study aimed to investigate the involvement of cerebral microcirculation turbulence after subarachnoid hemorrhage (SAH). Wistar rats were divided into non-SAH and SAH groups.
Autologus arterial hemolysate was injected into rat’s cisterna magna to induce SAH. Changes of pial microcirculation within 2 hours were observed. It was found that there were no obvious changes of the diameters, flow velocity and fluid state of microvessels in non-SAH group. With the exception of rare linear-granular flow in A4 arteriole, linear flow was observed in most of the arterioles. There was no blood agglutination in any of the arterioles. After SAH, abnormal cerebral pial microcirculation was found. Spasm of microvessels, decreased blood flow, and agglutination of red blood cells occurred. Five minutes following the induction of SAH, the diameters of the arterioles and venules significantly decreased. The decreased diameters persisted for 2 hours after cisternal injection. Decreased flow velocity of venules was found from 5 to 90 minutes after induction of SAH. Spasm of the basilar artery and increased brain malondialdehyde were also found after SAH. We concluded that cerebral microcirculation turbulence plays an important role in the development of secondary cerebral ischemia following SAH.

**Key words**: subarachnoid hemorrhage; secondary cerebral ischemia; microcirculation; rat.
Introduction

Delayed ischemic neurological deficits contribute to the severe outcome of subarachnoid hemorrhage (SAH). Up to now, the causes of this condition are not clearly revealed. Although the phenomenon of cerebral vasospasm, which is angiographically characterized as the persistent luminal narrowing of the major extraparenchymal cerebral arteries, is well known and has been investigated extensively lately [Manno, 2004; Dreier et al., 2002; Grasso, 2004], it can not fully explain the ischemic brain injury after SAH. Cerebral microcirculation maintains normal function of the cerebral tissue. This process may be disturbed in the event of cerebral injury under various pathological conditions. A good understanding of cerebral microcirculation and the pathogenesis of cerebral injury after SAH facilitates the development of new preventive and therapeutic approaches to SAH. Although an acute increase in microvascular permeability [Germano et al., 2000] and platelet aggregation in the cerebral microvasculature [Sehba et al., 2005] have been noted, the alterations of cerebral microcirculation following SAH are poorly defined and remain debatable [Nihei et al., 1991; Uhl et al., 2003; Park et al., 2001]. The present study was undertaken to investigate the changes of cerebral pial microcirculation following SAH using a novel rat model.

Materials and methods

Experimental animals and grouping

This study was approved by the Institutional Review Board of Taishan Medical College. Twenty-four Wistar rats of both sexes, weighing 330g to 380g, were purchased from Experimental Animal Center of Shandong University. The animals were housed in groups of five per cage at a constant temperature (24 ± 1°C) and humidity (60 ± 5%), and were given
free access to food and water before and after the experiment. The animals were divided randomly into non-SAH and SAH groups. Twelve rats were included in each group.

*Induction of SAH*

The room temperature was kept at 26±2°C. The rats were anesthetized by an intraperitoneal injection of chloral hydrate (10% solution, 350 mg/kg) and normal body temperature was kept with a heating lamp. The left femoral artery was cannulated and 0.4 ml blood was drawn into a heparinized microinjector (310, Stoelting Co. Ltd, USA). The blood was frozen at –80°C for 15 minutes. Then, the frozen blood was thawed at 37°C and the autologus arterial hemolysate was obtained.

The rat’s head was fixed in a stereotaxic frame (51600, Stoelting Co. Ltd, USA) to maintain a head down position of 30°. An incision was made into the midline of the skull. The skin and musculature were carefully separated using an operating microscope to expose the atlantooccipital membrane. The microinjector was mounted on the manipulating arm of the stereotaxic instrument with a needle connected to a three-way stopcock to allow the measurement of intracranial pressure (ICP). The needle was lowered into the cisterna magna under direct vision. To induce SAH, 0.3 ml arterial hemolysate was injected into the cistern very slowly over 20 min at a constant rate. The non-SAH rats were manipulated in the same way, but 0.3ml of normal saline, not arterial hemolysate, was injected into the cistern.

*Blood gas monitoring*

At different times, arterial blood samples were collected from the left femoral artery via the three-way stopcock. The blood samples were used for detection of blood gases, in which arterial pH, partial pressure of oxygen (PaO₂), partial pressure of carbon dioxide (PaCO₂) were involved.
Detection of arterial pressure

The right femoral artery was exposed and cannulated with a cannula connected to a three-way stopcock. The cannula was advanced distally into the abdominal aorta. The pressure module 1 (DA100C) of a Biopac system (MP150, USA) was connected to the cannula. The mean arterial blood pressure (MABP) was monitored and calculated automatically by the computer software program AcqKnowledge ver 3.7.2.

Observation of cerebral pial microcirculation

The rat’s head was fixed in a stereotactic frame. An incision was made along the midline of the skull and the parietal bones were exposed. A small hole, with a diameter of 0.6 cm, was drilled on the skull of the rat at the point 3.0 mm behind the bregma and 3.0 mm to the sagittal suture. The thin inner bone layer, the dura and arachnoid were carefully dissected. The observation window was perfused with artificial cerebrospinal fluid at a constant temperature of 37°C. The cerebral pial microcirculation was determined by a videomicroscopic system (WX-8A, Hengda optical & electronic Co., Xuzhou, China). The captured videos and images were saved as AVI and TIFF files and analyzed using the microcirculation software. The diameters of appropriate arterioles and venules were determined by an auto tracking measurement combined with an image camera method. The flow velocity of venules was detected with a flying-spots method. The environment temperature was maintained at 26±2°C.

Measurement of diameter of basilar artery

Diameters of the basilar artery were measured via a transclivus approach. Briefly, the trachea of the rat was exposed, transected and cannulated. The animal was mechanically
ventilated with room air (with a frequency of 60 cycles/min). Under visual guidance through the operating microscope, the clivus was exposed. A 15-20 mm² window was created by a dental drill to visualize the basilar artery. The window was perfused with warm artificial cerebrospinal fluid. The basilar artery diameters at the vertebrobasilar junction, middle of the pons and upper pons just proximal to the bifurcation, were determined. The average of the three measurements was used as a basilar artery diameter in the rat.

Detection of malondialdehyde content of brain tissues

The rat was euthanized following the operation and the brain was harvested. The left cortex was dissected and homogenized with a normal saline. The malondialdehyde content was determined by a sulfurbarbital acid method. The malondialdehyde kits were obtained from Jiancheng Institute of Biologic Engineering (Nanjing, China).

Data analysis

The experimental data were expressed as mean ± standard deviation (SD). The software SPSS 11.0 for windows was used for the statistical analysis. Comparisons of data were made using one-way analysis of variance (ANOVA) and a student t test. The p<0.05 was considered to statistically significant.

Results

General findings

In non-SAH and SAH groups, all of the parameters of arterial blood gas analysis, including pH, PaO₂ and PaCO₂ maintained within the normal range during the observation period. No significant differences were found between the two groups (Table 1).
After the cisternal injection of autologus arterial hemolysate or saline, the MABP increased 30 minutes after induction of SAH \((P<0.05)\). There was no significant difference between non-SAH group and SAH group. MABP returned to normal level one hour following cisternal injection (Table 1).

*Alterations of cerebral pial microcirculation*

In non-SAH rats, the diameters of pial arterioles and venules, the flow velocity, and the fluid state did not alter significantly. The surfaces of the microvessels showed clear appearances (Fig 1A). With the exception of rare linear-granular flow in some of the A4 arterioles, linear flow was observed in most of the arterioles. Blood flowed rapidly through microvessels. There was no obvious cell congregation during the experiment. In SAH rats, the pial arterioles and venules constricted markedly (Fig 1A & Fig 1B). The blood flow was slower than before. Severe agglutination of red blood cells occurred in the microvessels. In some of the microvessels, the blood flow was oscillated and ceased.

Diameters of pial arterioles and venules, flow velocities of blood in the venules are shown in Fig. 2, 3, and 4. In SAH group, the diameters of arterioles decreased to 60.8% of the baseline by the end of cisternal injection of arterial hemolysate \((p<0.05)\) and maintained a low level throughout the observation period. Within 2 hours after the SAH establishment, the diameters of the arterioles varied from 57.9% to 79.3% of the baseline value \((p<0.05)\). No recovery in the diameter was observed.

In SAH group, the diameters of venules reduced to 70.0% of the baseline value \((p<0.05)\). The constriction of pial venules lasted for 2 hrs after cisternal injection \((p<0.05)\). In the SAH group, the flow velocities of blood in pial venules at 5, 30, 60 and 90 minute intervals following the cisternal injection were reduced significantly as compared with those in non-SAH group \((p<0.05)\).
Alterations of diameter of basilar artery

The diameter of basilar artery was 330.2±28.0 μm in non-SAH group, and 142.1±31.7 μm in SAH group. The diameter of basilar artery in SAH group was 43.1% of that in non-SAH group (p<0.05).

Alterations of brain malondialdehyde

The malondialdehyde content was 16.4±3.0 nmol/mg protein in non-SAH group, and 32.2±4.5 nmol/mg protein in SAH group (p<0.05).

Discussion

After onset of SAH, both early and delayed cerebral ischemia may occur. The delayed cerebral ischemia is of more importance, because it correlates with the neurological deficit and the severe outcome of SAH [Grasso, 2004]. Oxyhemoglobin (OxyHb) and other cell components, which are released from the lysed red blood cells, are responsible for the occurrence of delayed cerebral ischemia [Grasso, 2004; Asano, 1999]. As the investigations in patients with SAH are restricted, a variety of animal models have therefore been established. The most commonly used method for producing SAH in rats is based on the introduction of fresh autologus arterial blood into cisterns, in which the delayed cerebral ischemia is not well simulated [Megyesi et al., 2000; Sun et al., 2003]. In this experiment, cisternal injection of the frozen and thawed blood was used to induce SAH. Although no specific analysis was conducted in this study on the changes of blood component after freezing of the blood, a small loss of red blood cells and depletion of granulocytes is expected. This is because even at -25°C, a 8% loss of red blood cells and removal of granulocytes could be found [Nicholls 1989, Farrugia 1992]. After the cisternal injection of
the autologus arterial hemolysate, significant spasm of basilar artery was found. Therefore, the rat model of SAH in this experiment seems more suitable for studying the cerebral ischemia within two hours of SAH.

Existing data show that the spasm of large cerebral arteries is not always consistent with the outcome of SAH patients. In many patients who developed neurological deficit, cerebral vessel spasm did not seem to be severe, as judged by transcranial Doppler and angiography [Veelken et al., 1995]. There might be some other factors involved in the development of cerebral ischemia and neurological deficits in these patients.

Although the cerebral microcirculatory changes during SAH have been investigated, previous studies have yielded conflicting results. Statistically significant increase of cerebral blood volume in patients with SAH and cerebral vessel spasm has been detected by different methods [Grubb Jr et al., 1977; Hino et al., 1989]. The increased cerebral blood volume has been thought to be due to maximally dilated peripheral arterioles, and the weak response to vasodilating stimuli, such as hypercapnia or acetazolamide, has been thought to be attributable to the maximal dilation of cerebral microvessels. However, recent studies have shown contradictory findings. Yundt et al. [1998] showed that cerebral blood volume was significantly reduced in patients with SAH and cerebral vessel spasm compared with normal volunteers and that severe vasospasm caused the parenchymal vessels to have reduced the capacity for autoregulatory vasodilation, resulting in decreased cerebral blood volume.

The pial microcirculation is a direct part of cerebral microcirculatory bed, and can be easily observed [Ishikawa et al., 1998; Nihei et al., 1991; Uhl et al., 2003; Park et al., 2001; Satomura et al., 2004; Ueda et al., 2003]. It was found in the present study that the pial arterioles and venules constricted remarkably after induction of SAH, which might attribute to the decreased cerebral blood perfusion. As the parameters of arterial blood gases maintained within normal range, the alterations of diameters of arterioles and venules might
be resulted from the condition of SAH. It has been demonstrated that endothelin-1 was increased [Zimmermann and Seifert, 1998; Sun et al., 2000; Juvela, 2002] and nitric oxide was decreased [Wolf et al., 1998; Sun, et al., 2000; Pradilla, et al., 2004] after SAH in different animal models and humans. In the present study, malondialdehyde, a product of oxygenic free radical metabolism [Bariskaner et al., 2003], increased significantly after induction of SAH. It was also found in our study that the blood flow velocity was reduced and severe agglutinations of red blood cells occurred in the microvessels after SAH. Blood flow stasis, oscillation and contraflow could also be found in some of the microvessels. The findings of our study suggest the involvement of the cerebral microcirculatory turbulence, including spasm of the microvessels and the deterioration of blood flow state, in the development of cerebral ischemia following SAH. However, its accurate mechanism remains to be clarified.

**Acknowledgments**

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References


Table 1. Arterial blood gas analysis after cisternal injection.

<table>
<thead>
<tr>
<th>Group</th>
<th>pH</th>
<th>PaO2 (mmHg)</th>
<th>PaCO2 (mmHg)</th>
<th>MABP (mmHg)</th>
</tr>
</thead>
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<tr>
<td>non-SAH</td>
<td>Baseline</td>
<td>7.39±0.02</td>
<td>126.9±8.2</td>
<td>34.2±3.5</td>
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<tr>
<td></td>
<td>0.5h</td>
<td>7.40±0.02</td>
<td>124.2±9.3</td>
<td>32.4±5.7</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>7.39±0.01</td>
<td>123.6±4.8</td>
<td>33.8±4.8</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>7.38±0.03</td>
<td>124.3±5.1</td>
<td>33.1±3.9</td>
</tr>
<tr>
<td>SAH</td>
<td>Baseline</td>
<td>7.38±0.02</td>
<td>125.9±3.8</td>
<td>32.5±4.9</td>
</tr>
<tr>
<td></td>
<td>0.5h</td>
<td>7.39±0.02</td>
<td>123.6±5.0</td>
<td>33.3±3.1</td>
</tr>
<tr>
<td></td>
<td>1 h</td>
<td>7.38±0.03</td>
<td>122.3±5.1</td>
<td>35.3±4.2</td>
</tr>
<tr>
<td></td>
<td>6 h</td>
<td>7.40±0.04</td>
<td>123.8±9.1</td>
<td>31.8±4.1</td>
</tr>
</tbody>
</table>

*P<0.05 vs Baseline
Figure legends

Figure 1. Pial microvessels before (1A) and 30 min after (1B) of cisternal injection of frozen blood in the SAH group, inducing vasospasm.

Figure 2 Alterations of diameters of pial arterioles (μm, mean ±SD) after cisternal injection in the two groups. *P<0.05 vs Baseline or non-SAH

Figure 3 Alterations of diameters of pial venules (μm, mean ±SD) after cisternal injection in the two groups. *P<0.05 vs Baseline or non-SAH

Figure 4 Alterations of the flow velocity of pial venules (μm/sec, mean ±SD) after cisternal injection in the two groups. *P<0.05 vs non-SAH
Figure 1
Fig 2

Time after cisternal injection

Diameters (μm)

non-SAH
SAH
Time after cisternal injection
Baseline
5min
10min
30min
60min
90min
120min
Diameters (μm)
Fig 3
non-SAH
SAH

* * *
Time after cisternal injection

Flow velocity (μm/sec)

non-SAH
SAH

Fig 4
Levels of pregnancy-associated plasma protein A in patients with coronary artery disease

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Running title: pregnancy-associated plasma protein A and coronary artery disease

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Abstract

**Purpose:** This study was designed to investigate the levels of pregnancy-associated plasma protein A (PAPP-A) or insulin-like growth factor -1 (IGF-1) in patients with acute coronary syndrome.

**Methods:** Serum PAPP-A and IGF-1 was measured with biotin–tyramide-amplified enzyme immunoassay and Enzyme Linked Immunooserbent Assay, respectively, in patients with ST elevation acute myocardial infarction (STEMI, n=12), unstable angina (UAP, n=15), and stable angina (n=15). PAPP-A and IGF-1 was also measured in 16 healthy subjects (control group).

**Results:** The serum levels of PAPP-A in the STEMI (16.9±10.3 mIU/L) and UAP group (15.2±10.5 mIU/L) were higher than in the stable angina (8.5±3.1 mIU/L) or control group (8.4±2.0 mIU/L, P<0.01). The serum levels of IGF-1 in the STEMI (132.3±40.9 µg/L) and UAP group (127.3±36.0 µg/L) were also higher than in the stable angina (44.9±18.5 µg/L) or control group (67.7±24.5 µg/L, P<0.01). There were no significant differences in serum levels of PAPP-A or IGF-1 among the single, double and three vessel lesion groups (P>0.05). The serum levels of PAPP-A (19.9±10.1 mIU/L) and IGF-1 (153.2±52.4 µg/L) after PCI were higher than those before PCI (15.1±10.0 mIU/L and 91.4±51.0 µg/L, respectively, P<0.01). A significant positive correlation was found between PAPP-A and IGF-1 levels in the STEMI and UAP group before PCI (r=0.48 P<0.01).

**Conclusion:** PAPP-A and IGF-1 are elevated in patients with acute coronary syndrome. They may be used as biomarkers for vulnerable plaques in patients with coronary artery disease. Whether post-PCI elevation of IGF-1 can be used to predict restenosis of coronary arteries remains to be seen.
**Key words**: coronary artery disease; pregnancy-associated plasma protein A; insulin-like growth factor-1; percutaneous coronary intervention

**Introduction**

Rupture of vulnerable plaque and subsequent thrombus formation in the coronary artery are considered to be responsible for the pathogenesis of acute coronary syndrome.\(^1\) Percutaneous coronary intervention (PCI) has become one of the main treatment measures for acute coronary syndrome.\(^2,3\) Successful PCI reduces the incidence of death, myocardial infarction and hospitalisation in patients with acute coronary syndrome.\(^2,3\) However, restenosis of targeted coronary arteries remains a major downside of PCI.\(^2,3\)

Recent studies have demonstrated that pregnancy-associated plasma protein-A (PAPP-A) is associated with the rupture of vulnerable plaque. It may play an important role in the occurrence and development of acute coronary syndrome.\(^4\) Several studies showed that insulin-like growth factor-1 (IGF-1) is relative to atherosclerosis and coronary artery disease.\(^5\) Some experimental studies have confirmed that PAPP-A and IGF-1 participate in the progress of restenosis after PCI.\(^6\) This study measured the serum levels of PAPP-A and IGF-1, at the same time, coronary angiography were performed in patients with coronary artery disease to investigate the relationship between PAPP-A, IGF-1 and the severity of coronary lesions, and the effect of PCI on PAPP-A, IGF-1

**Subjects and Methods**

**Study Subjects**
The study was approved by the institution review board of the First Affiliated Hospital of Zhengzhou University. Informed consent was obtained from all participants before the study. Between January 2006 and October 2006, 42 patients (27 men, mean age 62.5±12.5 years, range 40-78) were recruited from the Department of Cardiology and the Department of Emergency Medicine the First Affiliated Hospital of Zhengzhou University. All patients had coronary artery disease confirmed by coronary angiography. Out of the 42 patients, 12 were ST-elevation acute myocardial infarction (STEMI), 15 were unstable angina pectoris (UAP) and 15 were stable angina pectoris (SAP). PCI (angioplasty followed by stent implantation) was performed in 24 of these patients with STEMI and UAP.

Sixteen healthy subjects (10 men, mean age 56.1±10.3 years, range 42-76) were also recruited for the measurement of serum PAPP-A and IGF-1.

STEMI was defined as prolonged chest pain accompanied by ischemic ST-T elevation. It was confirmed by elevated plasma creatine kinase- MB (CK-MB) of more than twice the upper limit of the normal range, and by a troponin-I level of more than 0.5ng/ml. UAP was defined as chest pain at rest with either ST-segment depression of at least 0.1 mV or T-wave inversion in two or more continuous electrocardiographic leads. There was no elevation of CK-MB or troponin-I. 7

SAP was diagnosed as chest pain of at least six month’s duration accompanied by evidence of severe coronary artery disease on coronary angiography and by the absence of clinically evident ischemic episodes during the week preceding arteriography. 8

Angiographically severe coronary artery disease was defined by the presence of one or more stenosis of at least 50% in any major coronary artery. Those who had a previous history of cardiomyopathy, valvular heart disease, tumor, inflammatory diseases, liver and renal disfunction, cerebral vessels, and peripheral angiopathy were excluded from the study, as were those who were pregnant.
Both STEMI and UAP patients were managed with intravenous heparin before PCI. Thrombolytic therapy with streptokinase was used in the three STEMI patients who did not receive PCI. Other drugs used in all three groups of patients included beta-blockers, aspirin, nitrates, statins and angiotensin-converting enzyme inhibitors.

**Measurements of PAPP-A and IGF-1**

In patients with acute coronary events, venous blood was drawn within one hour of the onset of chest pain, and approximately 30 min before PCI. In patients with stable angina and underwent coronary angiogram or PCI, venous blood was drawn four hours before and four hours after coronary angiography or PCI. Within 30 min of blood collection, blood samples were centrifuged at 1600 revolutions/min per minute for 5 min. The abstracted serum was stored at -70°C. PAPP-A concentrations were determined by means of a biotin–tyramide-amplified enzyme immunoassay, as previously described, with a limit of detection of 0.03 mIU/L. Inter-observer variation of these measurements was less than 7%. Enzyme Linked Immoiserbent Assay (ELISA) was used to measure the serum levels of IGF-1 in patients with coronary artery disease and control group. IGF-1 concentration was measured by an IGF-I ELISA kit (Shanghai Senxiong Technology Industry Co. Ltd, Shanghai, China). Inter-observer variation of these measurements was less than 5%.

**Statistical Analysis**

Statistical analysis was performed with SPSS 10.0 software. Data were expressed as mean ± SD. Data between groups were compared by student t test. Categorical data was compared with Chi-square test. Comparison among the four groups was performed by analysis of variance of univariate (ANOVA). The relationship between serum levels of IGF-1 and
PAPP-A was analyzed by univariate linear relation. Correlation coefficient was tested by T-test. $P<0.05$ was considered as statistical significant.

**Results**

**General findings**

Table 1 shows the clinical characteristics of the study subjects. Among the four groups, there was no significant difference in sex, age, smoking history, family history of coronary artery disease, diabetes, hypertension and blood cholesterol levels ($P>0.05$).

**Comparisons of the mean level of serum PAPP-A, IGF-1 between groups**

As shown in Table 2, the levels of PAPP-A and IGF-1 in the STEMI and UAP groups were higher than the SAP and control group ($P<0.01$). There was no significant difference in PAPP-A between the SAP and control groups ($P>0.05$).

**Relationship between PAPP-A, IGF-1 and severity of vascular lesions**

The serum levels of PAPP-A and IGF-1 in single, double and three vessel disease group were higher than that in control group (Table 3, $P<0.05$). However, there was no significant difference in the serum levels of PAPP-A and IGF-1 among the three patient groups (Table 3, $P>0.05$).

**Comparisons of the serum levels of PAPP-A, IGF-1 before and after PCI**

As shown in Table 4, the serum levels of PAPP-A and IGF-1 after PCI were higher than that of before PCI ($P<0.01$).

**Correlation between the levels of PAPP-A and IGF-1 in AMI and UAP group.**
A significant positive correlation was found between the serum levels of PAPP-A and IGF-1 in STEMI and UAP group before PCI (r=0.48  P<0.01).

**Discussion**

The major findings of the this study are: 1) The serum levels of PAPP-A or IGF-1 in the STEMI and UAP group were higher than in the stable angina or in the health subjects; 2) The serum levels of PAPP-A or IGF-1 were similar between patients with stable angina and health subjects; 3) There were no significant differences in serum levels of PAPP-A or IGF-1 among patients with single, double or three vessel lesions; 4) The post-PCI levels of PAPP-A or IGF-1 were higher than those before PCI; 5) Before PCI, the PAPP-A levels were positively correlated with IGF-1 in the STEMI and the UAP group.

The results from this study provide further support to a recent study by Bayes-Genis and colleagues \(^9\), who also found that PAPP-A was abundantly expressed in plaque cells and extracellular matrix of ruptured or eroded unstable plaques of coronary arteries. They did not detect PAPP-A in the stable plaques, \(^9\) whereas in our study, only a very low level of PAPP-A was found in patients with stable angina and in health participants. These results indicate that increased levels of PAPP-A may reflect the instability of atherosclerotic plaques, and that PAPP-A may be used as a new biomarker for acute coronary syndrome.

Recent evidence suggests IGF-1 plays an important role in development of atherosclerosis. Both IGF-1 and its receptor are highly expressed in the atherosclerotic lesions.\(^10\) In unstable atherosclerotic plaques IGF-1 messenger RNA expression is greater than in the stable plaques.\(^10\)

The present study did not attempt to measure IGF-1 directly from the coronary lesions but the serum levels of IGF-1 in patients with unstable angina and ST elevation myocardial infarction were higher than in patients with stable angina or health subjects. In addition, a
positive correlation was found between serum levels of IGF-1 and PAPP-A prior to PCI. These results suggest that similar to PAPP-A, IGF-1 is involved in the pathogenesis of acute coronary syndrome and may also be used as a biomarker for vulnerable plaques.

It is unclear how IGF-1 is also elevated in patients with acute coronary syndrome, but previous studies on human fibroblasts suggested that PAPP-A acts as the enzyme cleaving IGF-binding protein 4, an inhibitor of the action of IGF. Therefore PAPP-A may increase the availability of IGF-1. In addition, the vascular smooth muscle cells at the site of injury or vulnerable plaque may increase the expression of IGF-1.

In the present study, the serum levels of PAPP-A and IGF-1 following PCI are higher than that before PCI. Although there is no clear explanation for the post-PCI elevation in PAPP-A or IGF-1, it is likely that the mechanical injuries to the atheromatous plaques in the coronary arteries following angioplasty and stenting have caused the release of these materials to the blood stream. The clinical significance of the post-PCI increase in PAPP-A and IGF-1 is unknown. Given the reported association between restenosis and IGF-1 in clinical and experimental settings, it is reasonable to hypothesize that patients who had high post-PCI levels of IGF-1 may have increased risk of coronary restenosis. However, this hypothesis can only be proven through long-term follow up in a large patient population.

In conclusion, this relatively small study has demonstrated that in patients with STEMI or unstable angina, there is an increased serum level of PAPP-A and IGF-1. PCI elevates serum PAPP-A and IGF-1 even further. PAPP-A and IGF-1 may be used as biomarkers for vulnerable plaques in patients with coronary artery disease. Whether IGF-1 elevation post-PCI is associated with an increased risk of restenosis of coronary arteries remains to be determined.
Acknowledgement: This work was supported by a grant from the Natural Science Foundation of Henan Province of China (NO: 0511041900).
References


<table>
<thead>
<tr>
<th>Table 1: Clinical Characteristics of STEMI, UAP, SAP and the control group</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STEMI</strong></td>
</tr>
<tr>
<td>group</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Age year</td>
</tr>
<tr>
<td>Male %</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>--------------------------------</td>
</tr>
<tr>
<td>Hypertension %</td>
</tr>
<tr>
<td>Family history of CAD %</td>
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<tr>
<td>Diabetes %</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
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</table>
Table 2: Comparisons of the level of serum PAPP-A and IGF-1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>PAPP-A (mIU/L)</th>
<th>IGF-1 (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>STEMI group</td>
<td>12</td>
<td>16.9±10.3</td>
<td>132.3±40.9</td>
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<tr>
<td>UAP group</td>
<td>15</td>
<td>15.2±10.5</td>
<td>127.3±36.0</td>
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<tr>
<td>SAP group</td>
<td>15</td>
<td>8.5±3.1*△</td>
<td>44.9±18.5*△</td>
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<tr>
<td>Control group</td>
<td>16</td>
<td>8.4±2.0*△</td>
<td>67.7±24.5*△</td>
</tr>
</tbody>
</table>

STEMI: ST elevation myocardial infarction; UAP: unstable angina, SAP: stable angina. Compare with STEMI group, * P<0.01  Compare with UAP group, △ P<0.01.
Table 3 Comparisons of serum PAPP-A and IGF-1 in single, double and triple-vessel disease

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>PAPP-A (mIU/L)</th>
<th>IGF-1 (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single vessel lesion group</td>
<td>12</td>
<td>15.09±10.15 *</td>
<td>91.54±49.04*</td>
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<tr>
<td>Double vessel lesion group</td>
<td>17</td>
<td>16.07±10.81 *</td>
<td>90.46±51.08*</td>
</tr>
<tr>
<td>Triple-vessel lesion group</td>
<td>13</td>
<td>17.30±10.01 *</td>
<td>92.40±50.02*</td>
</tr>
<tr>
<td>Control group</td>
<td>16</td>
<td>8.35±2.01</td>
<td>67.69±24.54</td>
</tr>
</tbody>
</table>

P value: 0.077 0.064

Compare with control group, *P<0.01
Table 4. Comparisons of the serum level of PAPP-A and IGF-1 before and after PCI

<table>
<thead>
<tr>
<th>Time</th>
<th>n</th>
<th>PAPP-A (mIU/L)</th>
<th>IGF-1 (μg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before PCI</td>
<td>24</td>
<td>15.1±10.0</td>
<td>91.4±51.0</td>
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<tr>
<td>After PCI</td>
<td>24</td>
<td>19.9±10.1</td>
<td>153.2±52.4</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>0.005</td>
<td>0.001</td>
</tr>
</tbody>
</table>