Serum Matrix Metalloproteinase 9 as a Marker for the Assessment of Severe Acute Pancreatitis

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CHEN, P., YUAN, Y., WANG, S., ZHAN, L. and XU, J. Serum Matrix Metalloproteinase 9 as a Marker for the Assessment of Severe Acute Pancreatitis. Tohoku J. Exp. Med., 2006, 208 (3), 261-266 —— Previous studies have shown that matrix metalloproteinase 9 (MMP-9) degrades basement membrane components in inflammation, but the change of serum MMP-9 level in the progression of acute pancreatitis remains unclear. The aim of our study was to assess the value of MMP-9 as a prognostic marker in acute pancreatitis. The prospective study included 10 patients with severe acute pancreatitis (SAP) and 10 patients with mild acute pancreatitis. The study also enrolled 10 healthy individuals as control. The serum MMP-9 level, serum C-reactive protein (CRP) level, serum tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)) level and acute physiology and chronic health evaluation (APACHE) II score were measured at 1 hr and 48 hrs after admission. APACHEII scores and serum MMP-9, TNF-\(\alpha\) and CRP levels were significantly increased in patients with SAP compared to those with mild acute pancreatitis and control subjects at 1 hr after admission (\(p < 0.01\)). When the states of illness were improved, the levels of the above-mentioned markers were decreased in patients with SAP at 48 hrs after admission (1 hr vs 48 hrs, \(p < 0.01\) or \(p < 0.05\)). Furthermore, significant positive correlation was found between serum MMP-9 level and serum TNF-\(\alpha\) level, serum CRP level or APACHEII score in patients at 1 hr after admission (MMP-9/TNF-\(\alpha\), \(r = 0.956\); MMP-9/CRP, \(r = 0.935\); MMP-9/APACHE II score, \(r = 0.957\); \(p < 0.01\)). These results suggest that MMP-9 is involved in the deterioration of SAP and serum MMP-9 level is a valuable assessment marker for the severity of SAP. ——— matrix metalloproteinase-9; tumor necrosis factor \(\alpha\); C-reactive protein; acute physiology and chronic health evaluation II score; acute pancreatitis

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Acute pancreatitis represents a potentially lethal disorder with no specific treatment. The mortality rate in patients with severe acute pancreatitis (SAP) may be as high as 20% to 40%, and this figure remains steady despite intense research on the source of overwhelming inflammatory responses (Raraty et al. 2004). Cytokines are primarily produced within the pancreas. They are coordinately designed to control cellular functions and interactions in an autocrine or paracrine fashion. When excess production occurs, they may be released into the systemic circulation.
Recent studies have shown that tumor necrosis factor $\alpha$ (TNF-$\alpha$), interleukin (IL)-1, macrophage migration inhibitory factor (MIF) and other cytokines might determine the severity and prognosis of acute pancreatitis (McKay et al. 1996; Kingsnorth et al. 1997; Sakai et al. 2003; Yavuz et al. 2004). Improved survival and attenuation of the local and distant inflammatory changes in experimental models of acute pancreatitis were seen when cytokine antagonists were administered. But even in the groups receiving anticytokine treatment, the mortality rates are still as high as around 50%, suggesting that other mediators might be involved in the pathogenesis of SAP (Norman et al. 1995; Chen et al. 2000; Matsuda et al. 2005).

Matrix metalloproteinase-9 (MMP-9) is a structurally related endopeptidase and is Zn$^{2+}$-containing enzyme that degrades wide range of extracellular matrix (ECM) components, including collagen types IV and V, different types of gelatin, fibronectin and elastin (Visse et al. 2003). MMP-9 is synthesized by many cell types, including connective tissues, endothelial, epithelial, and hematopoietic cells, and macrophages (Johnatty et al. 1997; Gibbs et al. 1999). Its proteolytic activity is thought to be necessary for a variety of functions, such as macrophage extravasation or migration (Xie et al. 1998), angiogenesis (Jadhav et al. 2004), wound healing (Rosenberg et al. 2001; Jorgensen et al. 2003), and tissue remodeling (Atkinson et al. 2003). A recent study has shown the increased expression of MMP-9 in response to proinflammatory cytokines, such as TNF-$\alpha$ and IL-1 (Johnatty et al. 1997). This increase could accelerate breakdown of ECM and might contribute to the pathogenesis of these diseases.

Therefore, these findings suggested that MMP-9 might be involved in generation of serum markers of ECM and might play an important role in the pathogenesis of acute pancreatitis. But the changes of serum MMP-9 level in patients with acute pancreatitis was unclear. In the present study, we measured serum MMP-9 level and evaluated the correlation between serum MMP-9 level and acute physiology and chronic health evaluation score II (APACHE II score), serum C-reactive protein (CRP) level or serum TNF-$\alpha$ level in patients with acute pancreatitis, to assess the predictive value of serum MMP-9 level for the severity of acute pancreatitis.

**Materials and Methods**

*Subjects and study protocol*

The prospective study included 20 consecutive patients with acute pancreatitis admitted to the emergency unit of our hospital from August 2001 to August 2004. The study enrolled 10 patients with SAP, 10 patients with mild acute pancreatitis and 10 healthy control individuals. The diagnosis of acute pancreatitis was in accordance with the criteria of “Clinical Diagnosis of Acute Pancreatitis and Criteria for Grading” formulated by the Chinese Medical Association (The pancreatic surgery academic group of Chinese Medical association surgical branch 1997). The diagnosis of mild acute pancreatitis was based on the increases (at least three times the upper the normal level) in serum amylase and/or lipase level, evidence of pancreatic swelling by imaging techniques and exclusion of other possible causes of abdominal pain, and no or slight organ dysfunction. The definition of SAP was based on the patients with acute pancreatitis, who were associated with one or more major local or systemic complications, such as systemic inflammatory response syndrome, acute respiratory distress syndrome, renal failure, desiccated intravascular coagulation, shock, pancreatic abscess, and pancreatic necrosis. The studies excluded the following patients: those died after 48 hrs of hospitalization and those with pancreatic tumor, surgery, trauma or pregnancy-induced acute pancreatitis. All patients were given current effective therapeutic measures. There were 19 males and 11 females, ranging in age from 33 to 70 years, with an average age of 48. The Committee on Research Ethics approved the study. Blood samples were obtained at 1 hr and 48 hrs after admission.
Measurements

The blood samples were centrifuged 3,000 rpm at 4°C for 15 min. The supernatants were decanted and frozen at -70°C until the time of assay. The study evaluated APACHE II score on each patient by the previously described method of Knaus et al. (1981). Serum CRP level was determined by immunoturbidimetric method. The serum MMP-9 and TNF-α levels were evaluated by enzyme-linked immunosorbent assay using Quantikine human MMP-9 and TNF-α assay kits (Endogen, MA, USA).

Statistical analysis

All values were expressed as mean ± S.E.M. Statistics were done by SPSS program at 11.5 version. The unpaired Student’s t-test or one-way analysis of variance (ANOVA) was used for comparison. Correlation analysis was generated from linear regression model. P value of < 0.05 was considered as statistically significant.

RESULTS

The present study showed that serum MMP-9 level was significantly increased from median of 246.5 ± 26.8 ng/ml in control subjects to 470.8 ± 51.4 ng/ml and 329.5 ± 54.0 ng/ml in patients with SAP and mild acute pancreatitis at 1 hr after admission, respectively (p < 0.01, for both). Serum MMP-9 levels showed significant difference between the patients with SAP and those with mild acute pancreatitis at 1 hr after admission (p < 0.01). On the other hand, serum MMP-9 level was decreased at 48 hrs after admission in patients with SAP (350.4 ± 41.5 ng/ml) and those with mild acute pancreatitis (275.6 ± 34.2 ng/ml) after effective therapeutic measures (1 hr vs 48 hrs, p < 0.01, for both). At 48 hrs after admission, serum MMP-9 level showed no difference in mild acute pancreatitis compared to those with control (48 hrs, 246.5 ± 26.8 ng/ml), but was still higher in SAP than that in mild acute pancreatitis and control (p < 0.01) (Fig. 1).

Fig. 2 showed that APACHE II scores were significantly increased in patients with SAP (13.6 ± 4.3) compared to those with mild acute pancreatitis (5.6 ± 2.1) and control subjects (4.3 ± 0.2) at 1 hr after admission (p < 0.01, for both). APACHE II scores were decrease in SAP group (8.7 ± 5.2) at 48 hrs after admission (1 hr vs 48 hrs, p < 0.05), but were still higher in SAP than those in mild acute pancreatitis (4.5 ± 1.3) and control (4.3 ± 0.2) at 48 hrs after admission (p < 0.01, for both).

At 1 hr after admission, serum CRP level was higher in SAP (3.6 ± 0.4 μg/ml) than that in mild acute pancreatitis (0.9 ± 0.1 μg/ml) or control (0.7 ± 0.1 μg/ml) (p < 0.01, for both). After effective treatment, serum CRP level was significantly decreased in SAP group (0.9 ± 0.3 μg/ml)
at 48 hr after admission (1 hr vs 48 hrs, \( p < 0.01 \)). Therefore, serum CRP level was not different in SAP group compared to that in mild acute pancreatitis (0.7 ± 0.3 μg/ml) and control (0.7 ± 0.1 μg/ml) at 48 hrs after admission (\( p > 0.05 \)) (Fig. 3).

Serum TNF-\( \alpha \) level was significantly higher in SAP (1.29 ± 0.14 μg/l) than that in mild acute pancreatitis (0.87 ± 0.13 μg/l) and control (0.32 ± 0.08 μg/l) at 1 hr after admission (\( p < 0.01 \), for both). After treatment, serum TNF-\( \alpha \) level was decreased in SAP (0.83 ± 0.13 μg/l) (1 hr vs 48 hrs, \( p < 0.01 \)) and mild acute pancreatitis (0.69 ± 0.19 μg/l) (1 hr vs 48 hrs, \( p < 0.05 \)) at 48 hrs after admission. Serum TNF-\( \alpha \) level showed no difference between SAP and mild acute pancreatitis at 48 hrs after admission (\( p > 0.05 \)), but was still higher in SAP and mild acute pancreatitis than that in control subjects (0.32 ± 0.08 μg/l) at 48 hrs after admission (\( p < 0.01 \), for both) (Fig. 4).

The correlation analysis showed that serum MMP-9 level had a positive correlation with all laboratory profiles that were used in the development of acute pancreatitis at 1 hr after admission (MMP-9/TNF-\( \alpha \), \( r = 0.956 \), \( p < 0.01 \); MMP-9/CRP, \( r = 0.935 \), \( p < 0.01 \); MMP-9/APACHE II score, \( r = 0.957 \), \( p < 0.01 \), respectively).

**DISCUSSION**

APACHE II score, which is a nonspecific scoring system and a health status indicator, is applied in the evaluation of acute pancreatitis. Previous studies have shown that acute pancreatitis with APACHE II scores greater than 7 were likely to have a severe course (Gurleyik et al. 2005). The score system is complex, somewhat difficult to perform and is less accurate for identification of local complications. But in the present study, APACHE II score was used as the most important step for precise diagnosis of severity of acute pancreatitis.

Previous studies reported that serum CRP and TNF-\( \alpha \) levels were both increased in SAP, and have close correlation with the severity of SAP (Paajanen et al. 1995; De Beaux et al. 1996). It has been proposed that TNF-\( \alpha \) might play a central role in acute pancreatitis and might mediate the systemic complications of the disease (Paajanen et al. 1995). A previous study confirmed that high concentrations of TNF-\( \alpha \) caused intravascular thrombosis and disseminated hemorrhagic necrosis of tissues (Whicher and Evans 1990). On the other hand, TNF helped to mediate many systemic septic responses, such as fever, hypotension, shock, catabolic hormone release and multiple organ injury (Fiers 1991), which were all hallmarks of SAP. Therefore, in our study, serum TNF-\( \alpha \) level could assess the severity of acute pancreatitis.
CRP is the best established and most available predictor of severity for acute pancreatitis. CRP is a proven predictor of severity for acute pancreatitis. The sensitivity and the positive predictive values of serum CRP level in patients with SAP have been reported to be 83% to 100%, and 37% to 77%, respectively (Mayer et al. 1984). In the present study, significantly higher serum levels of CRP in patients with SAP have indicated that this easy detectable marker reflects the severity of the disease.

It has been reported that MMP-9 activated neutrophil and promoted leukocyte-endothelial cell adhesion and consequently, neutrophil trafficking into inflamed tissues (Fernandez-Patron et al. 2001). The increased proteolytic activity of MMP-9 might further mediate pathological inflammation (Opdenakker et al. 2001). On the other hand, Porter et al. (2004) demonstrated that TNF-α induced endothelial cell to release MMP-9, which disrupted the basement membranes of vascular endothelial barrier and increased vascular permeability. Therefore, the inhibition of MMP-9 expression offers a potential pharmacologic and therapeutic target for halting the final biologic outcome of inflammation.

In the present study, serum MMP-9 level was significantly higher in patients with SAP than that in patients with mild acute pancreatitis and control subjects. The possible explanation for higher serum level of MMP-9 was that the increased proinflammatory cytokines in SAP (TNF-α, IL-1β, IL-8) can stimulate MMP-9 release (Johannaty et al. 1997; Wright and Friedland 2004). On the other hand, anti-inflammatory cytokines (IL-10, IL-4), which reduced MMP-9 expression (Wang et al. 1996), were decreased in SAP (Mentula et al. 2004; Ohmoto and Yamamoto 2005). So the inhibitory effect of anti-inflammatory cytokines on MMP-9 expression were weakened in SAP. Meanwhile, in our study, a significant correlation was found between serum MMP-9 level and APACHE II score, serum CRP level or serum TNF-α levels in patients with acute pancreatitis at 1 hr after admission. The results indicate that serum MMP-9 level might reflect the severity of acute pancreatitis.

CONCLUSION

In summary, serum MMP-9 level was remarkably increased in patients with SAP, but was decreased after improvement of the disease states. We suggest that MMP-9 could be used as a valuable assessment marker for the course and severity of SAP.

References


