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探討慢性腎病變病人血清及尿液中基質金屬蛋白水解酵素、胞漿素原活化劑或其抑制劑與腎功能之相關性研究

研究成果報告(精簡版)

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Relationships between circulating matrix metalloproteinase-2 and -9 and renal function in patients with chronic kidney disease

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Abstract

Background: It has been proven that extracellular matrix turnover is involved in the pathogenesis of various renal fibrosis diseases. Matrix metalloproteinase-2 and -9 (MMP-2 and -9) are the extracellular matrix degrading enzymes that are believed to play important roles in renal diseases. However, the relationship of circulating levels of MMP-2, -9 and serum creatinine in the patients of chronic kidney disease (CKD) has not yet been investigated.

Methods: Gelatin zymography and ELISA were employed to measure MMP-2 and MMP-9 activities in the plasma samples of 60 CKD patients and 40 control subjects.

Results: Serum creatinine concentrations and MMP-2 activities were significantly higher ($p<0.001$) while MMP-9 activity and creatinine clearance (CCr) were significantly lower ($p<0.05$ and $p<0.001$, respectively) in CKD patients, as compared with those of control subjects. In addition, serum creatinine concentrations correlated with MMP-2 activity ($R=0.288$, $p<0.05$) and inversely correlated with that of MMP-9 ($R=0.344$, $p<0.01$).

Conclusions: This study demonstrated a correlation between MMP-2, -9 and serum creatinine in CKD patients to suggest that MMP-2 and MMP-9 might contribute in the pathogenesis of CKD.

Keywords: Matrix metalloproteinase; Serum creatinine; Chronic kidney disease

1. Introduction

Renal insufficiency, characterized by a loss of renal function, can be either acute or chronic. When it is acute, renal function is lost within a relatively short period of time. Most often this situation is reversible and does not lead to scarring. In contrast, chronic renal insufficiency is accompanied by a permanent loss of functional nephrons, causing a slow loss of renal function and eventually leading to the development of chronic kidney disease (CKD) [1,2]. CKD, defined as the presence of kidney damage or a decreased level of kidney function for a period of ≥ 3 months, occurs when the tiny filters in the nephrons are damaged; this interferes with the kidneys' ability to remove wastes from the body. Despite of various causes, diabetes and high blood pressure are common causes for chronic kidney disease [3–5].

It is well known that extracellular matrix (ECM) turnover plays a critical role in the processing of CKD and remodeling of ECM is an important physiologic feature of normal growth and development. Many diseases, including

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CKD, have been associated with an imbalance between ECM synthesis and degradation, which may result in an accumulation of ECM molecules [6,7]. The major regulators of ECM degradation in the glomerulus are matrix metalloproteinases (MMPs) [8]. Thus, changes in MMPs expression or activity may directly translate into an altered ECM turnover, which may lead to glomerular scarring and a decline in renal function [7,9,10].

MMPs are a large family of zinc-requiring enzymes, which include interstitial collagenases, stromelysins, gelatinases, elastases, and membrane-type matrix metalloproteinases (MT-MMP) [11]. They synergistically degrade ECM components, and as such, are involved in a variety of pathophysiological processes (including embryonic development, angiogenesis, invasive cell behavior, inflammation, wound healing and fibrosis) in which tissue remodeling plays a major role. Recent studies have established that MMPs play a role in the development of glomerular sclerosis [12] and a number of glomerulonephritis [13–15]. In addition, several experimental models of interstitial fibrosis have demonstrated changes in the production of MMPs [16,17].

The gelatinases (MMP-2 and MMP-9) are a subfamily of MMPs that share the ability to degrade basement membrane types IV and V collagens, aggrecan, elastin and gelatins [11]. It was initially suggested that they were involved in the pathogenesis of CKD. Recently, various studies have shown that MMP-2 and -9 were involved in both normal renal development and glomerular diseases [7]. In the developing kidney, MMP-2 mRNA expression is limited to the mesenchyme. However, MMP-2 protein has been found in immature nephron structure undergoing epithelial differentiation [18,19]. In renal diseases, it has been shown that increased plasma levels of MMP-9 precede the appearance of microalbuminuria in noninsulin-dependent diabetes mellitus [20]. In addition, a marked decrease in MMP-2 mRNA expression was detected in glomeruli of diabetic patients [21].

Based on the key role of ECM turnovers in the processing of CKD and the vital involvement of MMP-2 and -9 in normal renal development and glomerular diseases [7,8,17], the aim of this study was to investigate the clinical implications of circulating MMP-2 and MMP-9 plasma levels in patients with CKD.

2. Materials and methods

2.1. Subjects and specimen collection

A total of 100 subjects, including 40 control subjects and 60 CKD patients enrolled from Chung Shan Medical University Hospital, Taichung, Taiwan, were recruited into this study. Venous blood samples were obtained from all patients and placed in tubes containing EDTA, immediately centrifuged, and stored at −80 °C. Biochemical markers of renal function, including serum creatinine and CCr, were obtained for every sample in the routine biochemistry laboratory and estimated by the Cockcroft–Gault equation [22], respectively. Clinical characteristics of patients were summarized in Table 1.

Table 1
Characteristics and studied parameters of patients in this study

	Control subjects (n=40)	Chronic kidney disease (n=60)	p value
Age (year)	40.4±2.7	60.5±1.9	N.S.
Gender, M/F	40 (20/20)	60 (29/31)	N.S.
Serum creatinine (mg/dl)	0.77±0.03	6.58±0.38	<0.0001
Creatinine clearance (ml/min)	105.1±5.66	11.28±0.75	<0.0001
MMP-2 (ng/ml)	51.56±3.54	98.43±3.13	<0.0001
MMP-9 (ng/ml)	109.41±8.03	79.04±6.41	<0.005

2.2. Determination of activities and levels of MMP-2 and -9 by gelatin zymography and ELISA

Activities of MMP-2 and -9 were determined by gelatin zymography according to a protocol developed by Kleiner et al. [23]. Of each plasma sample, 20 μl was loaded onto an electrophoresis with a precast sodium dodecyl sulfate–polyacrylamide gel containing 0.1% gelatin. After electrophoresis, gels were processed as described by Yang et al. [24] and nonstaining bands represented the levels of latent form of MMP-2 and -9 activities were quantitatively measured by spot density measurement using a digital imaging analysis system (Alpha Innotech, Mt. Prospect, IL, USA) [25].

MMP-2 and MMP-9 levels in plasma samples were analyzed by human MMP-2 and MMP-9 ELISA kits (R&D Systems, Abingdon, UK) and each sample was assayed in duplicate and samples with a reading higher than the linear range of the standard curve will be appropriately diluted and tested again.

2.3. Statistical analysis

Values were expressed as means±S.E.M. The statistical significance of difference between groups was determined by Mann–Whitney Rank sum test. A linear regression analysis was employed to determine the relation between values of MMPs and serum creatinine. A p value of <0.05 was considered statistically significant.

3. Results

3.1. Characteristics of CKD patients

The clinical characteristics of studied CKD patients (including 29 males and 31 females) were summarized in Table 1. These patients had a median age of 60.5 y. Serum

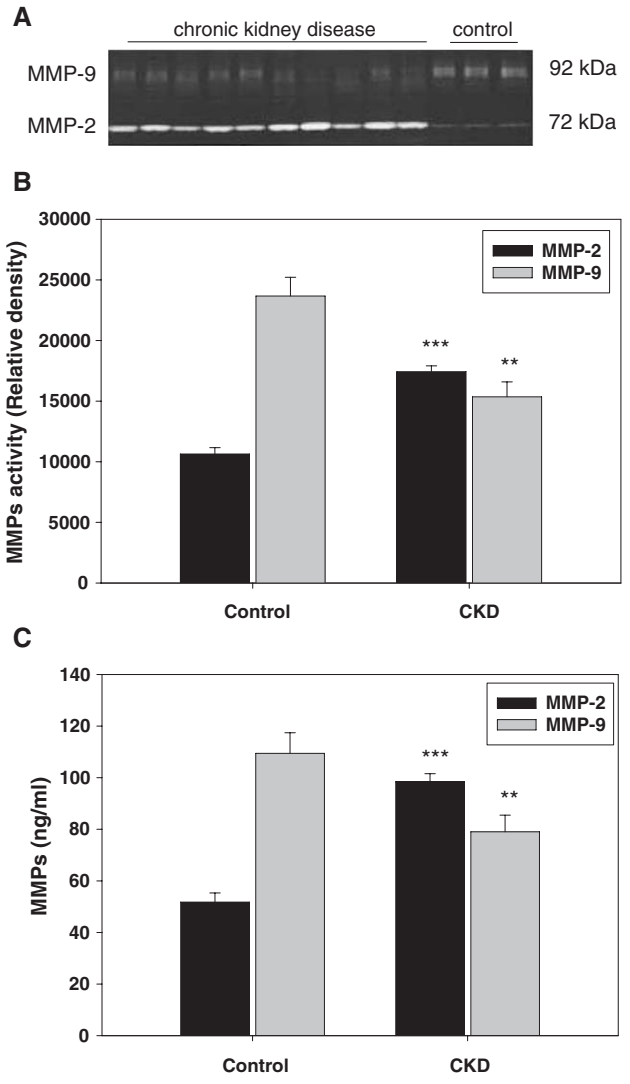

Fig. 1. MMP-2 and MMP-9 levels analyzed by gelatin zymography and ELISA. (A) Representative gelatin zymography of plasma samples from CKD patients and control subjects. (B) While average of quantitated MMP-2 was higher in CKD patients than control subjects, MMP-9 activities were significant lower. (C) MMP-2 and MMP-9 levels in 60 CKD patients and 40 control subjects determined by an ELISA kit following the manufacturer's instruction. MMP-2 levels were significantly higher in CKD patients than control subjects while MMP-9 levels were significant lower. $^{**}p<0.05$, $^{***}p<0.001$.

creatinine concentration of CKD patients (6.58±0.38 mg/dl) were significantly higher, as expected, than that of controls (0.77±0.03 mg/dl) ($p<0.0001$). Furthermore, CCr of CKD patients (11.28±0.75 ml/min) were significantly lower than that of control subjects (105.1±5.66 ml/min) ($p<0.0001$).

3.2. Gelatin zymographic and ELISA analysis for MMP-2 and MMP-9

Plasma activities of MMP-2 and MMP-9 of control subjects and CKD patients were assayed by gelatin zymography. The presence of MMP-2 and MMP-9 was indicated as a band of 72 kDa and 92 kDa, respectively, as

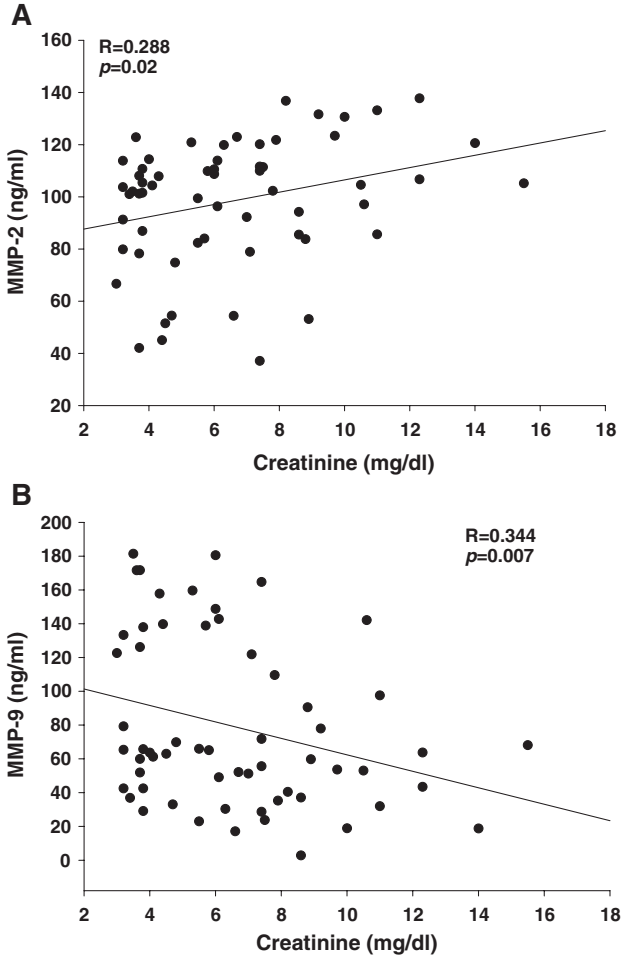

Fig. 2. (A) Linear regression analysis of the relationship between serum creatinine concentrations and MMP-2 activity ($Y=2.35X+82.925$, $R=0.288$, $p<0.05$). (B) Linear regression analysis of the relationship between serum creatinine concentrations and MMP-9 activity ($Y=-4.873X+111.1034$, $R=0.344$, $p=0.007$).

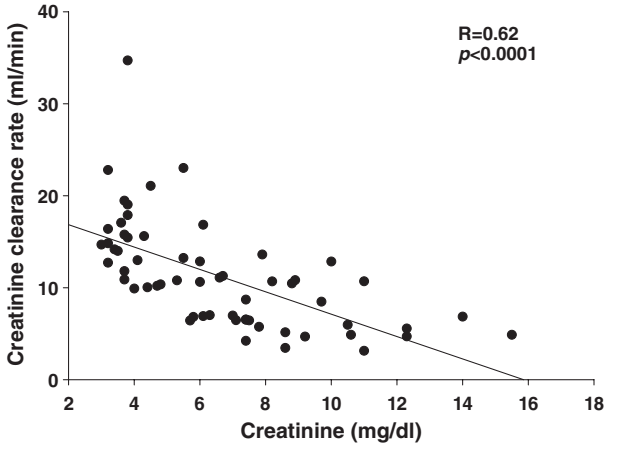

Fig. 3. Linear regression analysis of the correlation between the concentrations of serum creatinine and creatinine clearance ($Y=-1.2159X+19.2845$, $R=0.62$, $p<0.0001$).

shown in Fig. 1A, indicating that a higher MMP-2 activity and a lower MMP-9 activity were observed in CKD patients compared with those of the control subjects (Fig. 1B).

In order to quantitatively determine the MMP-2 and MMP-9 levels, ELISA kits were used and results, as shown in Fig. 1C, indicated that average MMP-2 level of CKD patients (98.43±3.13 ng/ml) was significantly higher than that of control subjects (51.56±3.54 ng/ml; $p<0.001$), while average MMP-9 level was significantly lower in CKD patients (79.04±6.41 vs. 109.41±8.03 ng/ml of control subjects ; $p<0.005$).

3.3. The correlation between serum creatinine concentrations and MMPs levels in CKD patients

Based on a linear regression analysis, we found that serum creatinine concentration in CKD patients was significantly correlated with MMP-2 level ($Y=2.35X+82.925$, $R=0.288$, $p<0.05$) (Fig. 2A) and inversely correlated with MMP-9 level ($Y=-4.873X+111.1034$, $R=0.344$, $p<0.01$) (Fig. 2B).

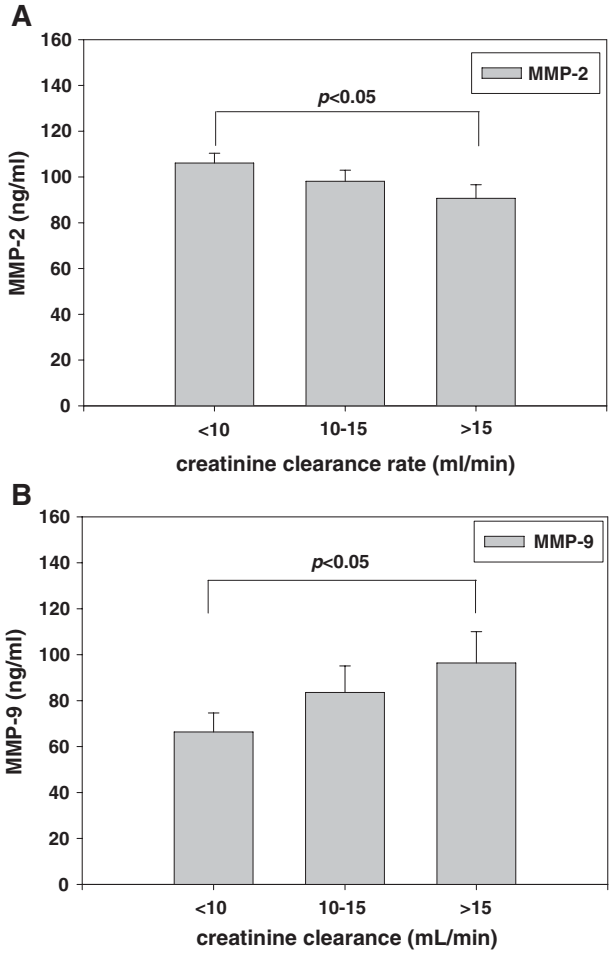

Fig. 4. ELISA-determined plasma MMP-2 levels (A) and MMP-9 levels (B) of 60 CKD patients with various CCr values. Results showed that MMP-2 was significantly lower in patients with a CCr>15, while their MMP-9 was significantly higher.

3.4. The relationship between the CCr and MMPs

Using linear regression analysis, the correlation between CCr and serum creatinine concentrations was initially inspected and, as expected, results showed a significant relationship between creatinine and CCr in CKD patients ($Y=-1.2159X+19.2845$, $R=0.62$, $p<0.0001$; Fig. 3). Furthermore, CKD patients were divided into 3 groups based on CCr levels: CCr<10 ml/min, $10\leq CCr\leq 15$, and CCr>15. The results showed that MMP-2 was significantly lower in patients with a CCr>15 ($p<0.05$) (Fig. 4A), while their average level of MMP-9 was significantly higher ($p<0.05$) (Fig. 4B).

4. Discussion

This study showed that significantly increased levels of circulating MMP-2 and serum creatinine were detected in 60 CKD patients, as compared to that of 40 control subjects while significantly lower MMP-9 and CCr levels were also detected. These results suggested that higher serum creatinine concentration and lower CCr was a consistent pattern in CKD patients, which has been shown in some previous studies [22,26]. Furthermore, serum creatinine concentration was positively correlated with MMP-2 level and inversely correlated with that of MMP-9 level in CKD patients (Fig. 2A and B). To our knowledge, this is the first report to demonstrate the association of MMP-2, -9 levels with serum creatinine concentration in CKD patients.

MMPs play a role in the glomerular sclerosis [12,27] and interstitial kidney fibrosis [17]. Although ECM changes have major contributions in the pathogenesis of CKD, only very few studies have measured the circulating levels of MMP-2 and -9 in CKD patients with hemodialysis [28,29], Since MMPs are rapidly released from cells after biosynthesis in the matrix, MMP-2 and MMP-9 may easily diffuse into the blood and serve an indicator of collagen turnover. Thus, circulating levels of MMP-2 and MMP-9 could be altered in hepatic disease [30,31], non-small cell lung cancer [24], community-acquired pneumonia [32], and renal diseases such as lupus nephritis, glomerulonephritis [27], chronic transplant nephropathy [33] or hemodialysis patients [28]. Although circulating levels of MMP-2 and MMP-9 in various diseases have been determined in numerous studies, the situation in CKD was still unclear. Therefore, this study was aimed to investigate the clinical implications of circulating MMP-2 and MMP-9 plasma levels in patients with CKD.

In the glomerulus, epithelial cells could produce both MMP-2 and MMP-9 [34,35], whereas mesangial cells only produce MMP-2 [36]. Changes in MMP-2 and MMP-9 expression or activity in the glomerulus will directly translate into an altered ECM turnover, which may lead to glomerular scarring and a decline in renal function. Our present study has revealed a significant change in the MMP-9 and MMP-2 activities in CKD patients with the highest

activity of MMP-2 and the lowest activity of MMP-9. Furthermore, linear regression analyses have revealed a direct association of MMP-2 with serum creatinine, a well-known predictor for renal function. MMP-2 levels were closely involved in the formation of glomerular fibrosis [7] and the expressions of MMP-2 were also significantly increased in cases with kidney damage and fibrosis [17,33]. Based on these, we suggested that a higher creatinine may be indicative of a higher extent of fibrosis, leading to a higher level of MMP-2 in CKD patients. In the other hand, reduced MMP-9 levels may lead to a decrease in the anti-fibrosis potential of CDK patients [11].

The diagnosis of CKD relies on the determination of serum creatinine, CCr and/or glomerular filtration rate (GFR) [26]. In a previous study, CCr was estimated by the Cockcroft–Gault equation [22], and in further analysis this clearance estimate was used to place patients within stages of kidney disease as described by Kidney Disease Outcomes Quality Initiative (K/DOQI) [37]. Although many centers employ the Modification of Diet in Renal Disease (MDRD) equation, a GFR calculator is also recommended in the guidelines [38]. Since Cockcroft–Gault is commonly used in clinical purposes in Taiwan, we use this equation, which is permitted by the K/DOQI guidelines, to categorize the stage of kidney disease in this study. Furthermore, our findings have revealed a significant change in the MMP-2 and MMP-9 activities in different CCr group (Fig. 4). To the best of our knowledge, this was also the first report to demonstrate the association of circulating MMP-2, -9 levels with different CCr group of CKD patients and implicated that circulating MMP-2 and MMP-9 levels are deeply involved in CKD.

In conclusion, this study has revealed a correlation between MMP-2, -9 and serum creatinine concentration, as well as between both MMP-2 and MMP-9 and the severity of CKD. Such correlations may have significant benefits in clinical interpretation. The detailed mechanism underlying the correlation of MMPs and serum creatinine in CKD was still unclear and further studies are needed to assess the exact role of MMPs in CKD, which may be of value for understanding the pathogenic mechanism of renal disease, as well as providing an early detection and effective therapy for CKD.

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