European Review for Medical and Pharmacological Sciences 2014; 18: 861-868

Role of matrix metalloproteinases and tissue inhibitor of matrix metalloproteinases-1 in Crimean-Congo hemorrhagic fever disease

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Abstract. – AIM: Crimean-Congo hemorrhagic fever is a potentially fatal viral disease in humans caused by CCHF virus. We aimed to demonstrate change in serum levels of matrix metallopeinase/tissue matrix metalloproteinase inhibitor (MMP/TIMP) associated with CCHF.

PATIENTS AND METHODS: Blood specimens were collected in acute and convalescence periods from the patients presented to Cumhuriyet University Hospital, Department of Emergency and Infection Diseases with presumed as CCHF between May 2010 and September 2010. Forty-one age and gender matched healthy individuals had not any viral, bacterial, acute or chronic disease were enrolled as the controls. Blood specimens were centrifuged at 4000 rpm for 5 min with in "Hettich universal 32" centrifuge. Serum samples obtained were kept at –80°C. All the specimens were brought to room temperature during the study and MMP-1, 2, 7, 9, 10 and TIMP-1 tests were studied at one time using "RayBiotech" brand kit in "Grifols" brand "Triturus" model ELISA device. Acute, convalescence and control groups were compared in terms of the serum levels of MMP-1, 2, 7, 9, 10 and TIMP-1.

RESULTS: There was a statistically significant increase in serum levels of MMP-1, 7, 9, 10 and TIMP-1 in the convalescence period ($p < 0.05$) compared to the controls, while the increase in levels of MMP-2 was not statistically significant ($p > 0.05$). In acute period of CCHF, mean TIMP-1 levels of severe patients was significantly lower than that of the non-severe patients (207913 ± 31051 versus 231300 ± 13267, respectively, $p = 0.023$).

CONCLUSIONS: High serum levels of MMP and TIMP in CCHF disease were found to decrease as progressed to convalescence from the acute period. It is thought MMP and TIMP plays a significant role in pathogenesis of CCHF.

Key Words:
Crimean Congo hemorrhagic fever disease, Matrix metalloproteinase, Tissue matrix metalloproteinase inhibitors.

Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a potentially fatal viral disease in humans caused by CCHF virus. The disease has been reported in Africa, Asia, eastern Europe, and the Middle East. Transmission to humans is resulted from tick bites, contact with a patient with CCHF during the acute stage, or contact with blood or tissue from contamined livestock[1].

The disease was encountered in Turkey for the first time around the province of Tokat in spring and summer of 2001, and it was proven to be CCHF in 2003[2,3]. Epidemic which began in 2002 is still continued, and the number of proven CCHF cases about 5000 as of 2010, according to the recordings from the Ministry of Health[4]. The typical course of CCHF has four separate phases: incubation, pre-hemorrhagic, hemorrhagic, and convalescence[5].

Despite pathogeneses of viral hemorrhagic fevers are similar, it is important to understand the pathogenesis and to plan the treatment. Although pathogenesis may present pathophysiological differences, basic characteristics such as microvascular damage and deterioration of hemostasis are important.

Matrix metalloproteinases (MMPs) are the proteases which break extracellular matrix, and the proteins lay out of the extracellular matrix. They have been found to have an important role in many physiological and pathological events. These enzymes have a very essential position in turnover, tissue remodeling, angiogenesis and morphogenesis of the extracellular matrix (ECM). MMPs are accounted for the destruction of the extracellular matrix, while tissue matrix metalloproteinase inhibitors (TIMPs) block the

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activity of metalloproteinases and prevent the destruction of the extracellular matrix. This keeps the production and destruction of the extracellular matrix in a state of continuous balance. Several studies report that increase in the activity of MMPs and decrease in the activity of TIMPs is associated with pathogenesis of many acute and chronic diseases (cardiac diseases, atherosclerosis, periodontal disease, tumor cell metastasis, arthritis, etc.)[6-10].

MMPs are known to take part in the destruction of ECM as well as play a role as bioactive molecules. In addition, they enable the breakdown of the receptors on the cell surfaces through release of apoptotic ligands and activation of chemokines and cytokines. MMPs also take part in the proliferation, migration and differentiation of cells and in angiogenesis, apoptosis and cell defense[11-13]. Thus, MMPs are an enzyme groups taking part in many pathological and physiological events.

Studies conducted so far have reported that matrix metalloproteinases are found in many species from bacteria to humans, and they have more than 200 members[14] with 25 in vertebrates; 24 in mammals and 23 of these are found in humans[7]. MMPs have been divided into 5 groups as follows, according to their primary structure, substrate specificity and cellular location:

1. Collagenases MMP-1, MMP-8, MMP-13 and MMP-18
2. Stromelysins: Stromelysin -1 (MMP-3) and Stromelysin -2 (MMP-10)
3. Gelatinases: Gelatinase A (MMP-2) and Gelatinase B (MMP-9)
4. Matrilysins: Matrilysin -1 (MMP-7) and Matrilysin -2 (MMP-26)
5. Membrane-type MMPs (Mt-MMP)[7,15,16].

Tissue matrix metalloproteinase inhibitors are natural inhibitors of matrix metalloproteinases such as α-2 macroglobulin and ovostatin. TIMPs obtained from the rabbit bone for the first time in recent studies are glycoproteins containing 184 amino acids, weighed 28.5 kDa and found in many tissues and body fluids. TIMPs are 4 types; TIMP-1, 2, 3, 4. TIMP-1 and TIMP-2 are the best described among these. TIMP-1 inhibits MMPs out of Mt-1-MMP and MMP-2. Many stimuli such as growth factor, platelet derived growth factor, phorbol esters and interleukin-1 increase the expression of TIMP-1 in fibroblasts. Actual role of TIMPs is to regulate the activity of the matrix[16-20].

MMP/TIMP balance is believed to be an important factor in regulation of the proteolytic activity. TIMPs contain two structural domains. N-terminal domain of the inhibitor inhibits the substrate by binding in the stoichiometric and non-covalent form to its binding site in the active center of the enzyme[17,21]. Despite of the pathogenic involvement of MMPs has been described in several infectious diseases such as dengue fever and Hanta virus infections[22], no reports were found in the literature regarding to MMP role in CCHF. The aim of this investigation is to detect circulating metalloproteinases and TIMP-1 levels during acute CCHF and associate them with disease severity.

Patients and Methods

Blood specimens were collected in acute and convalescence periods from the patients presented to Cumhuriyet University Hospital, Department of Emergency and Infection Diseases with presumed as CCHF between May 2010 and September 2010. Blood specimens from all the acute cases were sent to the Refik Saydam Hygiene Center of Ankara, Turkey for ELISA and PCR tests. PCR and/or ELISA yielded positive (+) in 41 of them. Blood specimens of the convalescence period were collected from these patients. Blood specimens were collected from age and gender matched 41 healthy individuals those had not any viral, bacterial, acute or chronic disease, enrolled as controls. These specimens were taken into anticoagulant-free, gel containing tubes and after kept the in-room temperature for 10 minutes, they were centrifuged at 4000 rpm for 5 min with in "Hettich universal 32" (Tuttingen, Germany) centrifuge. Serum specimens obtained were taken into Eppendorf of 1,5 ml and kept at –80°C. All the specimens were brought to room temperature during the study and MMP-1, 2, 7, 9, 10 and TIMP-1 tests were studied at once using "RayBiotech" brand kit in "Grifols" brand "Triturus" model ELISA device (Norcross, GA, USA).

All CCHF patients were classified into two groups in terms of disease severity ("severe" and "non-severe"), according to the predictive factors for fatal outcome reported by Swanepoel et al[4]. Patients in the acute infection period were assigned into Group 1, those in the convalescence period into Group 2 and the controls into Group 3. Group 1, Group 2 and Group 3 were compared in terms of the serum levels of MMP-1, 2, 7, 9, 10 and TIMP-1.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 14 for Windows (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. Data were analyzed with independent-samples *t*- test and MannñWhitney U-test, as appropriate values of $p < 0.05$ were considered statistically significant. All the variables were expressed as mean ± standard deviation.

Ethical approval was taken as 2010-03/09.

Table I. Demographic characteristics of the groups.

	Control (n = 41) $\bar{x}$ ± SD	CCHF* patients (n = 41) $\bar{x}$ ± SD	p
Mean age (year)	43.9 ± 17.9 (18-74)	44.4 ± 19.9 (18-78)	0.903
Gender			
Male	26 (63.4%)	28 (68.3%)	0.641
Female	15 (36.6%)	13 (31.7%)	

*CCHF: Crimean-Congo Hemorrhagic Fever.

Results

Clinical characteristics of the patients: of 41 cases with positive (+) PCR and/or ELISA test, 28 (68.29%) were male and 13 (31.71%) female patients. Mean age of the patients was found as 44.41±19.9 (range 18-78). Of 41 cases in the control group, 26 (63.41%) were male and 15 (36.59%) female patients with a mean age of 43.9±17.9 (range 18-74). There was not significant difference between the groups in age and gender ($p > 0.05$) (Table I).

According to Swanepoel criteria, 28 (68.3%) of the patients were classified as "non-severe", and the remaining 13 (31.7%) were classified as "severe" in our study. A statistically significant increase was seen between the control and patient groups in terms of the levels of MMP-1, 2, 7, 9, 10 and ($p < 0.05$) (Table II).

Serum levels of MMP-1, -2, -7, -9, -10 and TIMP-1 were found statistically significantly lower in convalescence than in the acute period ($p < 0.05$) (Table III). There was a statistically significant increase in serum levels of MMP-1,-7,-9,-10 and TIMP-1 in the convalescence period ($p < 0.05$) compared to the controls, while the increase in levels of MMP-2 was not statistically significant ($p > 0.05$) (Table IV). No significant difference was found in serum MMP1, 2, 7, 9, and 10 levels between patients with severe versus non-severe forms of the disease in acute or convelescence period ($p > 0.05$). Also, there was no significant difference between the TIMP-1 levels in severe or non-severe patients with CCHF in convalescence period. However, in the acute period of CCHF, there was a significant difference in serum TIMP-1 levels between the patients with severe versus non-severe forms of the disease (p = 0.023). In acute period of CCHF, mean TIMP-1 levels of severe patients was significantly lower than that of the non-severe patients (207913 ± 31051 versus 231300 ± 13267, respectively, $p = 0.023$).

Discussion

The present report is the first investigation of serum MMP and TIMP levels in patients with CCHF. In this study, serum enzyme levels of MMP-1, 2, 7, 9, 10 and TIMP-1 were found significantly higher in the acute period of CCHF disease than in convalescence period and controls ($p < 0.05$). There was a statistically significant increase in serum levels of MMP-1, -7, -9, -10 and TIMP-1 in the convalescence period ($p < 0.05$) compared to the controls, while the increase in

Table II. Serum levels of MMP/TIMP in the Control and CCHF patients with acute period.

	Control (n = 41) $\bar{x}$ ± SD	CCHF patients (n = 41) $\bar{x}$ ± SD	p
$MMP\text{-}1_{pg/ml}$	15.8 ± 2.6	67.0 ± 31.0	0.000
$MMP\text{-}2_{ng/ml}$	478.3 ± 71.5	702.7 ± 217.1	0.000
$MMP\text{-}7_{ng/ml}$	10.2 ± 3.1	29.2 ± 5.8	0.000
$MMP\text{-}9_{pg/ml}$	588.5 ± 98.3	1272.9 ± 204.9	0.000
$MMP\text{-}10_{pg/ml}$	255.4 ± 51.8	397.1 ± 90.7	0.000
$TIMP\text{-}1_{pg/ml}$	145427 ± 23133	223884 ± 23009	0.000

CCHF: Crimean-Congo hemorrhagic fever, MMP: Matrix Metalloproteinase, TIMP1: Tissue matrix metalloproteinase inhibitor type.

Table III. Serum levels of MMP/TIMP in CCHF patients with acute and convalescence periods.

Measurements	CCHF patients with acute period (n = 41) $\bar{x} \pm SD$	CCHF patients with convalescence period (n = 41) $\bar{x} \pm SD$	p
MMP-1 $_{pg/ml}$	67.0 ± 31.0	43.2 ± 16.3	0.000
MMP-2 $_{ng/ml}$	702.7 ± 217.1	500.5 ± 182.9	0.000
MMP-7 $_{ng/ml}$	29.2 ± 5.84	17.6 ± 4.2	0.000
MMP-9 $_{pg/ml}$	1272± 204	1091 ± 172	0.000
MMP-10 $_{pg/ml}$	397.1 ± 90.7	333.8 ± 80.1	0.001
TIMP-1 $_{pg/ml}$	223884 ± 23009	210268 ± 23920	0.010

CCHF: Crimean-Congo hemorrhagic fever, MMP: Matrix Metalloproteinase, TIMP1: Tissue matrix metalloproteinase inhibitor type 1.

levels of MMP-2 was not statistically significant ($p > 0.05$). In the acute period of CCHF, there was a significant difference in serum TIMP-1 levels between the patients with severe versus non-severe forms of the disease; the mean TIMP-1 levels of severe patients was significantly lower than that of the non-severe patients.

MMPs are synthesized in many cells, including epithelial, endothelial, liver, fibroblast and inflammatory cells in a latent form. Their activity and synthesis increase in many conditions (diseases, tumor tissues, inflammation, etc.). MMPs are proteases, which break extracellular proteins such as collagens, elastins, proteoglycans and laminins. Levels of MMP/TIMP differ in various tissues and cells, and active cells are accounted for these differences[15,23]. Infection of mononuclear phagocyte system cells, liver and endothelial cells is known to play a crucial role in pathogenesis of CCHF[24,25]. We aimed to demonstrate change in serum levels of MMP/TIMP, which are synthesized in many cells, including epithelial, liver, fibroblast and inflammatory cells associated with CCHF. It was observed in the literature that serum levels of MMP/TIMP have been studies in viral infections, viral hemorrhagic fever and sepsis cases, but there is not any study conducted on CCHF disease.

Collagenases (MMP-1, MMP-8, MMP-13) are endopeptidases, which can break extracellular proteins in addition to main collagens. These are synthesized in a latent form and once activated they are inactivated only by several special inhibitors[26,27]. MMP-1 are primarily released as proMMP-1 from Kupffer cells and hepatic stellate cells. They are activated by proteolytic enzymes and take part in destruction of type I, II and III collagens, which constitute the basic structure of extracellular matrix[28,29]. Continuity of the structure and homeostasis of the tissues are provided depending on the balance between synthesis and destruction of the extracellular matrix proteins (fibronectin, laminin, tenascin-C, proteoglycan, core protein)[30]. Studies with chronic viral hepatitis reported low MMP-1 and increased TIMP-1 levels[31,32]. In our study, levels of MMP-1/TIMP-1 were found higher in the acute period than in the control group and convalescence period. In addition, levels of MMP-1/TIMP-1 were found statistically significantly increased in a statistically significant increase was found in the convalescence period compared to the control group ($p < 0.05$). These two studies were parallel to our studies in terms of TIMP-1 level, while they were not parallel in terms of

Table IV. Serum levels of MMP/TIMP in CCHF patients with acute and convalescence periods.

Measurements	Control (n = 41) $\bar{x} \pm SD$	CCHF patients with convalescence period (n = 41) $\bar{x} \pm SD$	p
MMP-1$_{pg/ml}$	15.8 ± 2.6	43.2 ± 16.3	0.000
MMP-2$_{ng/ml}$	478.3 ± 71.5	500.5 ± 182.9	0.470
MMP-7$_{pg/ml}$	10.2 ± 3.1	7.6 ± 4.2	0.000
MMP-9$_{pg/ml}$	588.5 ± 98.3	1091 ± 172	0.000
MMP-10$_{pg/ml}$	255.4 ± 51.8	333.8 ± 80.1	0.000
TIMP-1$_{pg/ml}$	145427 ± 23133	210268 ± 23920	0.000

CCHF: Crimean-Congo hemorrhagic fever, MMP: Matrix Metalloproteinase, TIMP1: Tissue matrix metalloproteinase inhibitor type 1.

MMP-1 level. We believe this difference was resulted from the studies being conducted with chronic viral diseases, while our study included acute viral disease and convalescence period.

Green et al[33]. reported that serum levels of MMP-1 and 3 are correlated with joint damage and clinical picture in rheumatoid arthritis and increase of these markers may be helpful in follow-up of the disease. Myers et al[34] reported that levels of MMP-1 and TIMP-1 significantly increase in psoriatic arthritis and other arthritis. Therefore, it may be interpreted as the increase in MMP-1 level influences extracellular matrix. High levels of MMP-1 also in CCHF patients may be associated with joint pain. Collagenase levels are recommended to be studied in the cases with and without joint pain in order to investigate the role of MMP-1 in joint pain in CCHF patients.

Gelatinases (MMP-2 and -9) have an important activity against the basal membrane components, including type IV collagen, laminin and elastin[35]. Grote et al[36] reported that reactive oxygen species (ROS) produced by membrane dependent NADPH oxidase increase expression of MMP-2, while similarly, Hanemaaijer et al[37] reported that expression of MMP-1, 3, 8 and 9 from macrophages increase through the mediators like IL-1α and TNF-α. A correlation has been found between the increase in expression of MMP-2 and MMP-9 and increase seen in vascular endothelial permeability in dengue hemorrhagic fever[22,38]. It has been demonstrated in diabetics[39] that hyperglycemia causes to destruction in vascular basal membrane and interstitial matrix, leading to increase in production of MMP-1 and 2, in turn MMP-2 greatly increases levels of many MMPs, especially MMP-9. In another study, MMP-2, MMP-3 and MMP-9 are stated to be accounted for impaired basal lamina and increased vascular permeability[22,38-40].

MMPs are stated to have an important role in liver damage and, especially MMP-2 and 9 to influence basal membrane by breaking the collagens and other matrix proteins. It was reported that levels of MMP-2 increase in liver diseases, while MMP-9 levels donít change in chronic liver diseases[41,42]. Kuyvenhoven et al[43] reported levels of MMP-2 and 9 were high pre-transplantation in patients with chronic liver disease and undergone transplantation, while this level significantly decreased post-transplantation. Hoffmann et al[44] reported that MMP-9, TIMP-2 and TIMP-1 are elevated in severe sepsis. As can be seen from the studies levels of MMP 1, 2 and 9 increase in patients with increased serum ROT, IL-1α, TNF-α levels, hyperglycemia, dengue fever and liver damage. In a work conducted with CCHF patients, it has been reported that reactive oxygen species (ROS) increased[45]. Increased serum TNF-α and IL-6 levels have been associated with CCHF severity[46]. In our study, we found levels of MMP-1, 2 and 9 higher in the acute period than in controls and convalescence period. Levels of MMP-1 and 9 were found significantly high in the convalescence period compared to the controls, while the increase in the levels of MMP-2 was not statistically significant. Therefore, ROT, IL-1α, TNF-α and viral infection contribute to increase in levels of MMP-1, 2 and 9 in CCHF patients may be considered. Increased levels of MMP-1, 2 and 9 is thought to be caused increased vascular permeability via the damage of basement membrane, interstitial matrix and liver. In this respect, we recommend more extensive researches on this subject.

MMP-7 which has been first isolated from tumoral cells has a high affinity for elastin. MMP-7 is expressed by gastrointestinal system, prostate and breast tumors and by malignant epithelial cells. Inflammatory cells, including vascular cells, polymorphonuclear leukocytes and macrophages produce collagenases (MMP-1) and elastases (MMP-7)[47,48]. Conant et al[49] found levels of MMP-2, 7 and 9 higher in cerebrospinal fluid (CSF) of patients infected with HIV virus than the control group, and attributed this to high serum level and increased synthesis of metalloproteinases from the cerebral cells stimulated by TNF-α. In patients having sepsis[50], plasma levels of MMP-7 and 9 found significantly increased compared to the control group, and a negative correlation was defined between this increase and multi organ dysfunction.

In addition, in the conducted studies MMP-7 which is synthesized in neutrophils and alveolar macrophages and takes part in destruction of elastin has been demonstrated to play a role in activation of antimicrobial proteins[51,52]. MMP-7 levels found in our paper were parallel with the reports conducted with patients having sepsis and HIV. High MMP-7 level in CCHF patients may be attributed to viral infection, increased TNF-α and activation of polymorphonuclear leukocytes and macrophages. Further comprehensive researches should be conducted to clarify the relationship between viral load and MMP-7 level in order to show antimicrobial effects of MMP-7 in CCHF patients.

In patients having sepsis, levels of MMP-10 and TIMP-1 were found significantly higher compared to the controls[53,54]. High plasma levels of TIMP-1 have been demonstrated to be correlated with the increased mortality rate in the patients with severe sepsis[44]. In addition, another work[55] reported that levels of MMP-9, 10 and TIMP-1 can be conveniently used as noninvasive measurement methods in order to show prognosis of sepsis. In our investigation, levels of MMP-10 and TIMP-1 were found high in CCHF patients who present a pathogenesis similar to sepsis. Results from the above-mentioned studies were parallel with our findings. Further reports are needed related to the predictivity of levels of MMP-9, 10 and TIMP-1 for prognosis CCHF patients. However, in the acute period of CCHF, the mean TIMP-1 levels of severe patients was significantly lower than that of the non-severe patients. Pathogenesis of CCHF is similar to sepsis. The reason of high TIMP level in sepsis to be low in CCHF patients in the acute period is yet to be clarified. According to us, low acute serum level of TIMP in CCHF patients in the acute period could be explained in two ways. First, TIMP might be used excessively in order to reduce effect of MMPs since tissue destruction and level of MMPs were high. Second, release of TIMP might be less in some patients with genetic and other reasons. This condition may contribute severe course of the disease in these patients. Further genetic researches are needed to clarify this condition.

In the viral infection models, due to MMP/TIMP imbalance, increased inflammatory process in the brain was determined[56]. MMP/TIMP imbalance in severe CCHF patients in the acut period might be the cause of clinical findings to be high.

Kenedy et al[57] showed that ribavirin and pegylated IFN-α therapy decreased level of MMP 9 in the patients HIV HCV co-infection. Altough controversial, ribavirin therapy is used in CCHF patients. Whether ribavirin would decrease the elevated MMP level, especially in the early period and/or severe CCHF patient and whether this would lead to a reduction in the clinical symptoms is a subject of a further research.

Results of our work indicated serum levels of collagenases (MMP-1), gelatinases (MMP-2 and MMP-9), matrilysin (MMP-7) stromelysin (MMP-10) and TIMP increase in CCHF patients. These data suggest extracellular matrix components may be broken and influence pathogenesis of the disease depending on the levels of enzymes. Tetracycline analogs such as minocycline and doxycycline have been used as MMP inhibitors in treatment of the diseases such as periodontal disease and arthritis[58-60]. Recent studies report that specific inhibitors may be a new approach in treatment of vascular, acute and chronic inflammatory diseases61. Because of the increased and activated MMPs damage to many organs and tissues, further comprehensive reports on this topic are recommended, considering MMP inhibitors may contribute to treatment of CCHF patients.

Conclusions

This is the first study to show elevated levels of MMPs in the serum of patients with CCHF. High serum levels of MMP and TIMP in CCHF disease were found to decrease as progressed to convalescence from the acute period. Elevated serum MMP may be contributed to clinical symptoms of patients with CCHF by tissue damage. This indicates these markers can be used follow-up methods in the management of CCHF disease. Further studies are required to understand the ultimate role of MMPs and TIMP-1 in the pathogenesis of CCHF.

Acknowledgements

The Authors thank Refik Saydam Hygiene Center of Ankara, Turkey for testing the serum samples and our colleagues from the Turkish Ministry of Health for their contributions.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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