Serum levels of MMP-9 in children and young adults with chronic kidney disease treated conservatively and undergoing hemodialysis

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Abstract

End-stage renal disease is characterized by chronic inflammatory state resulting in initiate reaction of interstitial fibrosis. The matrix metalloproteinase-9 (MMP-9) is the zinc–dependent proteolytic enzyme which has the ability to degrade the components of the extracellular matrix and plays an important role in the physiological and pathological tissue remodeling. The data suggest that MMP-9 may take part in various pathologic disorders in uremic and hemodialyzed (HD) patients. The aim of the study was to determine the level of MMP-9 (type IV collagenases/gelatinases), in peripheral blood samples taken from children and young adults with end-stage renal disease and undergo renal replacement therapy.

We investigated 21 patients (9 female, 12 male) suffering from chronic kidney disease (CKD) on maintenance hemodialysis (HD) (group A) aged from 12 to 48 years, and 13 (6 female, 7 male) with CKD treated conservatively, aged from 4 to 17.5 years (group B). In group A the analysis was performed before (A1) and after (A2) a single of the HD session. Also 20 healthy subjects (C) served as a control group was tested. Concentration of MMP-9 in serum was determined by the ELISA method.

Non significant differences between levels of MMP-9 in group A1 (median: 189.69 ng/mL, range: 31.13--673.3 ng/mL), A2 (median: 142.63 ng/mL, range: 35.42-313 ng/mL) and B (median:173.6 ng/mL, range: 30.4 -325.8 ng/mL) in comparison with group C (median: 194.6 ng/mL, range: 73-322 ng/mL) were found. Although MMP-9 levels in patients at the end of HD were not significantly lower than those in the beginning of HD but in 11/21 (52%) individual cases concentrations of MMP-9 were smaller after HD than before HD. There were no significant differences in serum MMP-9 concentrations between group A1, A2 and B.

These results indicate that serum concentration of MMP-9 in patients after HD showed a tendency to a decrease compared to the levels before HD. The increased serum concentrations of MMP-9 in some individual cases both in hemodialyzed patients and those who were treated conservatively may contribute to the high risk for complication e.g. cardiovascular diseases.

A better understanding of the inflammatory reactions in chronic kidney disease may lead to new treatment strategies.

Key words: *chronic kidney disease, hemodialysis, matrix metalloproteinase-9, children.*

Introduction

End-stage renal disease (ESRD) is a major health problem worldwide. In this disorder depressed immune responses have been observed frequently but some immunological factors are also activated and overproduced. ESRD is associated with chronic inflammatory reaction. However, the mechanisms involved in causing this injury still remain unknown.

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Chronic inflammation and complications occurring in the end-stage renal disease patients may lead to significant morbidity and mortality. Many studies documented various complications observed in uremic and hemodialyzed adults. But in the past years much more studies involved also pediatric patients. The important complications are e.g. infections, osteodystrophy, malnutrition, vascular and heart failure, hemorrhagic stroke, cerebrovascular accidents [1-4]. These disorders have a special significance when are concerned in the period of development child organism. Chronic inflammation in ESRD patients may be partly due to the uremic intoxication and also is increased by hemodialysis treatment with frequent contact between patients' blood and dialyzer membranes. The types of artificial membranes play a significant role as well. Moreover, the types of fistulae and the catheter-related bloodstream infection are very important for the health status of hemodialyzed patients.

Many cells and mediators are involved in the acute and chronic inflammatory process. It may be associated with the overproduction of proinflammatory cytokines, oxygen radicals and enzymes [5, 6]. One of the most important proteolytic enzymes is zinc-dependent endopeptidase - matrix metalloproteinase-9 (MMP-9) (gelatinaseB/92kDa type IV collagenase). It is secreted as inactive proenzyme mainly from neutrophils, macrophages, eosinophils, T lymphocytes, osteoclast and endothelial cells in response to various stimuli, and can be activated extracellulary by several factors. MMP-9 plays an important role in both normal and pathological tissue remodeling, angiogenesis, tissue repair, apoptosis [7]. It is a factor of cells migration across basement membrane by the ability to degrade type IV and V collagen, type I gelatin, elastin fibres [8]. This enzyme may also influence on cytokine release and is implicated in chronic inflammatory conditions that occurred in some pathological disorders in experimental and clinical studies, e.g. arthritis, cardiovascular disease, nephritis, chronic lung diseases, systemic sclerosis, cancer and other [9-13]. Various studies have shown that elevated levels and mRNA expression of MMP-9 in chronic kidney disease (CKD) and in dialysis patients may contribute to the pathogenesis of some complications by enhancing inflammation [14].

In the available literature we have not found the study on the role of MMP-9 in children with chronic kidney disease. The aim of this study was to assess whether serum concentrations of MMP-9 differed between patients suffering from chronic kidney disease treated conservatively and undergoing hemodialysis therapy. In order to evaluate the influence of hemodialysis on levels of MMP-9, we studied serum before and after the single hemodialysis session.

Material and methods

Subjects

The study was carried out on the group of 34 patients with chronic kidney disease (table 1). Among them 21 were on regular hemodialysis treatment (group A), aged 12 to 48 years, from both sexes and 13 treated conservatively, aged 4 to 17.5 years, from both sexes (group B). None of these patients had acute diseases in the course of the investigation. Patients of both groups were admitted (and treated) to the Department of Pediatric Nephrology in Wroclaw, Poland.

The patients were hemodialyzed from 0.5-11 years, 2 or 3 times weekly, with cuprophan (n=16) or polysulfone (n=5) dialyzers membrane and a routine used heparin as anticoagulants and dialysate with bicarbonate and low calcium content. The time of a single session of hemodialysis (HD) was 4-6 hours. The causes of CKD in group A were as follows: chronic glomerular nephritis (n= 9), hydronephrosis (n=4), neurogenic bladder (n=1), polycystic kidney disease (n=1), hypoplasia renum (n=1), systemic lupus erythomatosus (n=3), rheumatoid arthritis (n=2). The causes of CKD in group B were as follows: chronic glomerular nephritis (n=4), hydronephrosis (n=1), polycystic kidney disease (n=4), hypoplasia renum (n=1), chronic interstitial nephritis (n=1), urethrae valves (n=2).

Control group (C) consisted of 20 healthy subjects, both sexes, without chronic diseases and without treatment in anamnesis.

Blood samples were taken from the peripheral vein in all subjects. Venous blood was allowed to clot at room temperature for 60 min before centrifugation to obtain

Table 1. Characteristics of the study groups

Group	A	B	C
Number (n)	21	13	20
Sex			
F (%)	9 (43)	6 (46)	12 (60)
M (%)	12 (57)	7 (54)	8 (40)
Age (years, range; mean ±SD)	12-48 (25.5±10)	4-17.5 (12±4.5)	1-23 (8±6.5)
Duration of HD therapy (months) range (mean ±SD)	6-132 (31.3±33.47)	–	–

A – chronic hemodialysis patients; B – patients with chronic kidney disease treated conservatively; C – healthy subjects; F – female; HD – hemodialysis; M – male; n – number of subjects; SD – standard deviation.

Table 2. Serum concentration of MMP-9 in patients and in healthy control group

Group	MMP-9 [ng/mL]
A1	189.69 (133.22-217.2)
A2	142.63 (110.05-217.8)
B	173.6 (105.8-325.8)
C	194.6 (121.13-231.54)

A1 – patients with chronic kidney disease before hemodialysis; A2 – patients with chronic kidney disease after hemodialysis; B – patients with chronic kidney disease treated conservatively; C – healthy subject; MMP-9 – matrix metalloproteinase-9. The results are presented as median and interquartile range (25%-75%).
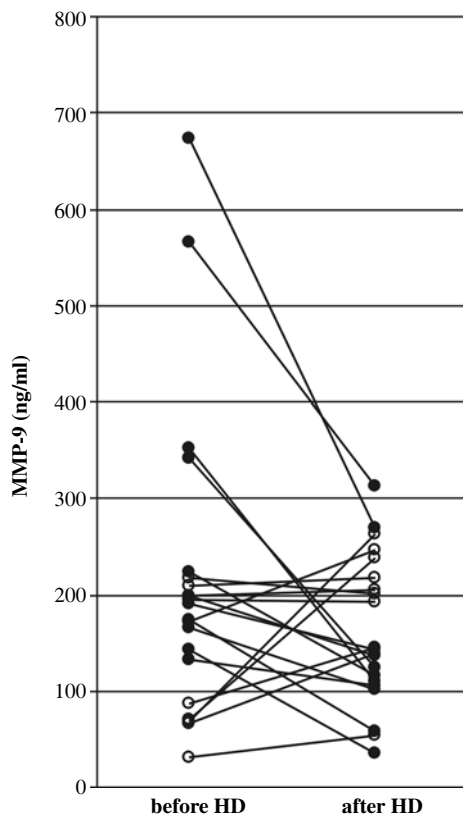

Fig. 1. Changes of serum concentration of MMP-9 in patients before and after HD session. MMP-9 – matrix metalloproteinase-9; HD – hemodialysis

serum. Serum samples were stored in aliquotes at –80°C until assayed. In group A the analysis was performed before (A1) and after (A2) a single of the HD session.

MMP-9 assay

The total serum concentrations (free proMMP-9 and free MMP-9) of MMP-9 were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Inc, Minneapolis, MN, USA). The detection limits of the MMP-9 concentration was 0.156 ng/mL. The analyses were performed according to the manufacturers' recommendations.

Statistical analysis

Statistical analysis was preceded by using the Walda-Wolfowitza and Manna-Whitneya test for independent and Wilcoxon test for dependent variables. Correlations between parameters were tested with the Spearman test. The level of statistical significance was assumed to be $p < 0.05$.

Statistical analysis was performed using the Statistica PL computer program.

Results

Circulating MMP-9 values

Serum MMP-9 levels are shown in table 2. In the group of patients before HD (A1) serum levels of MMP-9 ranged from 31.13 to 673.3 ng/mL with a median of 189.69 ng/mL and a mean of 31.13±673.3 ng/mL, after HD (A2) ranged from 35.42 to 313 ng/mL with a median of 142.63 ng/mL and a mean of 35.42±313.49 ng/mL, and in not undergoing dialysis group (B) ranged from 30.4 to 325.8 ng/mL with a median of 173.6 ng/mL and a mean of 239.65±186.39 ng/mL.

There was no difference between serum MMP-9 concentration in the patients treated conservatively and undergoing dialysis and those found in the controls which ranged from 73 to 322 ng/mL with a median of 194.6 ng/mL and a mean of 188.63±73.28 ng/mL.

Although MMP-9 levels in patients at the end of HD were not significantly lower than those at the start of HD, nevertheless in 11/21 (52%) individual cases concentrations of MMP-9 were lower after HD than before HD (figure 1). We found that 3/21 (14%) sera from A1, 3/21 (14%) from A2 group and 6/13 (46%) sera of patients from group B had increased levels above normal range. No significant differences were identified in serum MMP-9 concentrations between group A1, A2 and B.

Discussion

It is difficult to understand the inflammatory process occurring in the end-stage renal disease because of variety cells and metabolites playing a pivotal role in the connective tissue destruction.

MMP-9 is one of the major proteolytic enzymes capable of degrading the extracellular matrix (ECM) and basement membrane compounds increasing the migration of pro- and anti-inflammatory cells and has immunomodulatory properties. In this reason MMP-9 is implicated in tissue remodeling processes and takes part in the development of the inflammatory state. To the tissue damage may contribute:

68   Central European Journal of Immunology 2007; 32(2)

the imbalance between the MMP-9 and endogenous tissue inhibitors of metalloproteinase (TIMP-1), regulation its activity by transcription of the gene some cytokines (e.g. IL-8) or several serine proteases (trypsin, cathepsin G, elastase), and also other MMPs [10, 15].

A recent study showed that MMP-9 plays multifunctional significant roles in pathogenesis of some disorders occurring in uremic and hemodialyzed patients.

In the present paper, we demonstrated that the serum concentration of MMP-9 did not statistically differ in children and young adults suffering from uremia undergoing dialysis and treated conservatively compared to healthy donors. But we also have shown that in individual cases the level of MMP-9 was much more above normal range in both groups especially in group treated conservatively. In our opinion the rise in MMP-9 production could be due to injury processes and may contribute to the development of some complications. Concentration changes occurred in the course of HD treatment may be of prognostic importance. Thus, in these cases children should be covered by a special prevention and even more intense treatment. Our data also showed that in 52% of sera samples the decreased concentrations of MMP-9 were the result of HD treatment. Chou et al. in similar studies demonstrated that hemodialysis process tends to decrease plasma concentration of MMP-9 (from 187±148 ng/mL before to 124±72 ng/mL after HD) but TIMP-1 was not affected by HD [16].

The lower levels of MMP-9 may contribute to an increased extracellular matrix deposition and to irreversible fibrosis. Tissue remodeling by initiating the degradation of the ECM by MMP-9 can make the migration of host defense cells to inflammatory foci easier. These activated neutrophils, macrophages, lymphocytes, fibroblast as well as their products can also cause and support an inflammatory reaction, and additionally may be a source of the MMP-9 secreted into the circulation, fueling the vicious circle of inflammation.

All forms of ESRD are associated with chronic inflammatory reaction resulting in interstitial fibrosis. Various immune effectors cells and mediators take part in these processes. The inflammatory cascade is initiated in several situations – infection, trauma, surgery. The frequent contact of blood and dialytic membrane in patients requiring HD additionally results in a wide range of abnormalities of the immune system.

Neutrophils and other activated inflammatory cells produce a wide array of proteases including matrix metalloproteinase, elastase, cathepsin G, reactive oxygen species, cytokines (e.g. IL-8, IL-1, TNF-alpha), adhesion molecules. These products are not only involved in defense against a broad spectrum of microorganisms but upon release may also cause tissue injury and may also be involved in the subsequent repair processes.

Cells activation occurs during hemodialysis, and its intensity depends on the type of dialyzer and whether it is new or reused. Ebihara et al. reported that in HD patients MMP-9 mRNA levels did not differ among the types of membranes [14].

Patients with chronic renal failure have a disturbed defense, which causes infections (bacteremia, metastatic infection, sepsis) often leading to death of the patients. The role of MMP-9 in the innate immune response to serious infectious is also discussed in the literature [17]. Data in animals' models suggest that MMP-9 may be an essential component of an effective host response to *E. coli* peritonitis. Mice with MMP-9 gene deficient neutrophils showed a reduced phagocytosis of *E. coli* [18]. On the other hand, MMP-9 may serve as sensitive and early markers for cell activation in acute inflammatory responses. The significantly elevated levels of MMP-9 in severe sepsis were found [19].

Matrix metalloproteinases play an important role in the degradation of extracellular matrix and basement membranes by destroying the elastic lamina. It is also well known that oxidative stress has been identified as one of the most important causes of vascular injury also in renal failure. On the other hand, Tepel et al. described that regular hemodialysis sessions using biocompatible membranes have no effect on the elevated intracellular reactive oxygen species in patients with end-stage renal failure [20].

It is well known that patients with CKD are at increased risk for cardiovascular morbidity and mortality. The data indicate that in HD patients cardiovascular diseases are associated with oxidative stress and with an imbalance in antioxidant status. Moreover, reactive oxygen species production was significantly positive correlated with MMP-9/TIMP-1 system. In contrast, the levels of MMP-9 in HD patients (investigated in pre-dialysis period) with and without cardiovascular disease were similar to the controls [21]. The results published by Nakamura et al. indicate that when low density lipoprotein (LDH) was removed from blood of the hemodialysis patients with arteriosclerosis obliterans, in the process of apheresis, plasma levels of MMP-9 and serum levels of inhibitor TIMP-1 decreased significantly compared to those before LDH apheresis [22]. The data demonstrated that inflammation and increased oxidant stress together with early cardiovascular damage were found in non dialysis, peritoneal dialysis and on HD children with chronic renal disease [23, 24]. Also in children with ESRD treated conservatively lipid metabolism disturbances were found [25].

Vascular inflammation of arteriovenous fistulas is very important for the later complication (e.g. the thrombotic process). Chang et al. basing on the obtained results concluded that elevated expression of MMP-9 by macrophages at luminal edge may cause disruption of the anticoagulant endothelial barrier and may contribute to the luminal thrombosis of arteriovenous fistulas [26]. Elliot at al. demonstrated that treatment with pentosan polysulfate in hemodialysis patients inhibited smooth muscle cells proliferation and reduced the accumulation of type I and IV collagens in stenosis vascular, and they suggested that this effect was associated with increase of MMP-9 in the tissue [27].

Malnutrition, systemic inflammation, and atherosclerosis, known as MIA syndrome [28, 29], is associated with high cardiovascular mortality rate in dialysis patients [30].

It is very essential to prevent of renal osteodystrophy for the development of the young organism in children [31]. Also MMP-9 may play a pathogenic role in cartilage degradation and in the regulation of angiogenesis near the epiphyseal growth plate. The data indicate that growth plates from MMP-9-null mice in culture show a delayed release of an angiogenic activator [32].

Early diagnosis is very important for health condition of children and young adults with ESRD and long-term dialysis in order to prevent from the occurrence of any complications. For this reason the use of new biochemical indices of inflammatory stage should be crucial.

Better understanding of the biology of MMP-9 in modulation of inflammatory processes in children with ESRD is needed for more effective therapies. Based on knowledge of the MMP-9 actions the objective of many investigations is the search of the MMP-9 antagonists as new therapeutic targets for many disorders [33]. The use of the new treatment preventing from early and future complications will improve the quality of life and will lower the cost of treatment.

Conclusions

Obtained results indicate that elevated levels of MMP-9 only in small part of patients from both investigated group were found. We conclude that concentration changes occurring in the course of HD treatment may be of prognostic importance. In these cases increased levels of MMP-9 may be serving as marker of risk for complication. A better understanding of the inflammatory reactions involved in end-stage renal disease will enable the development of new treatment strategies. Usefulness of determination of serum MMP9 in pediatric patients with end-stage renal disease on hemodialysis or treated conservatively should be verified by further study.

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