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REGULAR ARTICLE

Transcriptional expression and gelatinolytic activity of matrix metalloproteinases in Henoch–Schonlein purpura

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Abstract

Aim: Accelerated extracellular matrix breakdown caused by the increased activity of matrix metalloproteinases (MMPs) has been implicated in several rheumatological disorders and systemic vasculitides, especially Takayasu's arteritis and Kawasaki disease. Therefore, the aim of the present study was to investigate the potential role of MMPs in Henoch–Schonlein purpura (HSP), an acute type of systemic vasculitis in children.

Methods: We studied the activity of MMP-2 and MMP-9 in the sera using gelatin zymography and the transcriptional expression in peripheral blood mononuclear cells using semi-quantitative RT-PCR in 20 patients with HSP in acute and convalescent phase and in 20 healthy children, who were siblings of the subjects with same age group.

Results: All 20 children with HSP showed increased levels of serum activity of MMP-2 and MMP-9 in acute phase as compared with their convalescent phase [MMP-2 ($p > 0.05$); MMP-9 ($p > 0.05$)] and their control counterparts [MMP-2 ($p < 0.001$); MMP-9 ($p < 0.001$)]. Similarly, transcriptional expression of MMPs was found to be higher in the acute phase of HSP than in convalescent phase [MMP-2 ($p < 0.05$); MMP-9 ($p < 0.001$)] and in their healthy controls [MMP-2 ($p < 0.001$); MMP-9 ($p < 0.01$)].

Conclusion: The presence of excessive transcriptional expression and gelatinolytic activity of MMPs may be downstream to the actual aetiopathogenetic factors.

INTRODUCTION

Henoch–Schonlein purpura (HSP) is the most common cause of non-thrombocytopaenic purpura in children and is an autoimmune acute leucocytoclastic, small vessel vasculitis. It is initiated by deposition of immunoglobulin A containing immune complexes in the skin and in certain internal organs notably the kidney and the gastrointestinal tract (1,2). Indian data on HSP are scanty and the condition is thought to be less frequent than other rheumatological conditions like juvenile idiopathic arthritis and systemic lupus erythematosus (3). Bagga et al. (4) reported a series of 47 patients with HSP from Delhi observed over a period of 17 years. However, we have earlier reported 45 cases of HSP over a short period of 4 years, implying that the condition is perhaps as common in our country as it is in the west (3). A number of pathogenetic mechanisms have been hypothesized to act as intermediates, which include the role of reactive nitrogen intermediates, free oxygen radicals and more recently, antiphospholipid antibodies (2). Genetic factors like familial Mediterranean fever, HLA-B35 associations with nephritis, PAX2 and vascular endothelial growth factor polymorphisms, also appear to have a role in the pathogenesis of the disease (2). However, the evidence for or against these hypotheses is not conclusive.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases produced by a variety of cell types and have the ability to degrade the extracellular matrix (5). In the MMP family, two gelatinases, MMP-2 (72-kDa form) and MMP-9 (92-kDa form), have a unique elastinolytic activity. In vascular lesions, the disintegration of arterial elastic lamella and basement membranes by proteolytic enzymes may play an important role in arterial stability, excessive cell migration and proliferation (5). These two members of MMPs have been shown to be involved in the pathogenesis of inflammatory vascular diseases, such as giant cell arteritis, kawasaki disease, temporal arteritis, Takayasu's arteritis by degrading the internal elastic lamina (6–10) and therefore play a pivotal role in facilitating the extravasation and migration of leucocytes by breaking down the basement membrane and therefore suggested to contribute to the pathogenesis of HSP. However, there is a paucity of literature relating to the expression and activity of MMPs

Abbreviations
FMF, familial mediterranean fever; HSP, Henoch–Schonlein purpura; MMPs, matrix metalloproteinases; PBMCs, peripheral blood mononuclear cells.

in HSP disease. Therefore, against this background, we in the present study, for the first time, have studied the mRNA expression and serum activity of MMP-2 and MMP-9 in subjects with HSP.

PATIENTS AND METHODS

Study subjects

Subjects diagnosed to have HSP in the Pediatric Rheumatology and Immunology Clinic of our institute were enrolled. The diagnosis of HSP was made if a child fulfilled the following criteria: non-thrombocytopaenic palpable purpura on extensor aspects of legs or on buttocks with demonstration of either leucocytoclostic vasculitis on skin biopsies or Immunoglbulin A deposits on immunofluorescence (2,11).

Children who required corticosteroids or cytotoxic agents as part of clinical management and those with urinary tract or systemic infections were excluded from the study. A child was said to be in remission when the rash had disappeared; there were no arthritis or gastrointestinal complaints and the urine examination was normal.

The present study included three groups: group-1: 20 children with HSP; group-2: 20 cases included in group-1, in their follow-up (4–6 months later); group-3: 20 controls, siblings of the subjects with HSP, not having the disease and nearest in age to the index patients. The controls were age- and gender-matched to the study group patients. The study protocol was approved by the Institute Ethics Committee.

Sampling

After obtaining informed consent from parents of all the children, venous blood sample was collected after an overnight fast at the time of enrolment during the acute phase of the illness and subsequently during remission at the time of follow-up visit after 4–6 months into plain sterile tube for serum and in ethylenediaminetetraacetic acid (EDTA) for isolation of peripheral blood mononuclear cells (PBMCs). The control population was studied only once at the time of enrolment in the study. Serum was separated and stored at −80°C for further analysis. Quantification of proteins in serum samples was performed by the method of Bradford (12) using bovine serum albumin as standard.

RNA extraction and RT-PCR

Total cellular RNA was isolated from PBMCS, reverse transcribed to cDNA using random hexamers (K1162; Fermentas Inc., St. Leon-Rot, Germany) and was amplified by polymerase chain reaction using MMP-9, MMP-2 and ß-actin specific primer pairs. The levels of mRNA expression of each gene were calculated as described previously (13).

Gelatin zymography of MMP-2 and MMP-9

Gelatin zymography is one of the most common methods to assess the levels of active and latent (pro) forms of MMP-2 and MMP-9. The enzymes are separated on the basis of their molecular weights after gel electrophoresis under denaturing conditions. Thereafter, the enzymes are refolded and the proteolytic activity of each form is visualized in the zymograms. Zymography was performed as described previously (10). Briefly, 25 μg of protein/lane of each serum sample was diluted in sample buffer (2×) (2% SDS, 125 mM Tris–HCl; pH 6.8, 10% glycerol, and 0.001% bromophenol blue) and subjected to electrophoresis on 7.5% SDS–PAGE co-polymerized with gelatin (2%). After electrophoresis, the gels were washed thrice at room temperature in a 2% Triton X-100 and incubated at 37°C for 48 h in Tris-HCl buffer, pH 7.4, containing 10 mmol/L $CaCl_2$ and Brij 35. The gels were stained with 0.05% Coomassie Brilliant Blue R-250, and then destained with 30% methanol and 10% acetic acid. Enzyme activity was assayed by densitometry using Scion Image Software, (Scion Inc., Maryland, USA), and were semi-quantified with reference to a MMP-9 standard (R&D, USA) to allow inter-gel analysis and comparisons.

Statistical methods

Statistical analysis was performed using software SPSS-13 (SPSS Inc., Chicago, IL, USA). As our data were skewed, Mann–Whitney test and Wilcoxon signed rank test were used to compare the difference between two groups.

RESULTS

Median age of the children with HSP was 5 years (range 3–12 years) and the male–female ratio was 1.5:1. Arthritis was present in 15 (75%) and abdominal pain in 12 (60%). One patient (5%) had a transient scrotal swelling. Transient nephritis with non-nephrotic range proteinurea was seen in two (10%). None of the patients had any significant gastrointestinal complications.

Skin biopsies were performed in 17 of 20 cases in acute stage from the site of lesion. Histopathology showed leucocytoclostic vasculitis in 11 out of 17 cases (65%). Immunofluoresence showed IgA 2+ to 3+ in 15 out of 17 cases (88%), IgM 1+ to 2+ in 11 out of 17 cases (65%), IgG and C3 were found in 3 out of 17 cases (18%).

Transcriptional (mRNA) expression of MMP-2 and MMP-9

We observed significantly higher mRNA expression of both MMP-2 (40.9 ± 16.7 AU; $p < 0.001$) and MMP-9 (57.6 ± 43.6 AU; $p < 0.01$) in PBMCs derived from HSP subjects in acute phase when compared with the levels in PBMCs obtained from healthy control subjects (MMP-2: 13.65 ± 1.78 AU; MMP-9: 21.95 ± 14.7 AU). On the contrary, we observed a significant decrease in the mRNA expression of both MMPs in PBMCs of the patients who were in convalescent phase (MMP-2: 20.45 ± 10.2 AU; MMP-9: 32.45 ± 34.5 AU) as compared with the subjects in acute phase (group-2 vs. group-1; MMP-2: $p < 0.05$; MMP-9: $p < 0.001$). When mRNA expression was compared between healthy controls (group-3) and the subjects in convalescent phase (group-2), expression of MMP-2 was significantly increased in PBMCs obtained from HSP subjects in

convalescent phase ($p < 0.01$), whereas MMP-9 expression remained insignificant ($p > 0.05$; Fig. 1).

Gelatin zymography

Gelatinolytic activities of MMP-9 and MMP-2 were observed as bands of clear zones in gelatin impregnated gels (Fig. 2). Gelatinolytic activity levels were found to be statistically higher in HSP subjects with acute phase (MMP-2: 41.15 ± 16.7 AU; MMP-9: 121.45 ± 42.1 AU) as compared with the healthy controls (MMP-2: 6.95 ± 3.2 AU; MMP-9: 40.95 ± 16.8 AU) (group-1 vs. group-3; MMP-2: $p < 0.001$; MMP-9: $p < 0.001$). However, no significant difference was observed in the levels of activity of both the MMPs when compared between HSP subjects in acute phase and in convalescent phase (MMP-2: 31.80 ± 15.8 AU; MMP-9: 103.75 ± 36.9 AU) (group-1 vs. group-2; $p > 0.05$). When the data were compared between healthy controls and HSP subjects in convalescent phase, significantly low MMP-2 and MMP-9 activities were observed in the control group (group-2 vs. group-3; $p < 0.001$).

DISCUSSION

We enrolled 20 children who were diagnosed to have HSP on clinical grounds. This clinical diagnosis was further confirmed by histopathological examination and immunofluorescence studies performed on the skin biopsy (14). It may be noted that the rash of HSP is not always pathognomonic and the cutaneous lesions may, at times, mimic other rheumatological and non-rheumatological disorders especially in the initial phase of the illness (11,15). In our experience, a skin biopsy is often helpful in such situations (14). All children included in the study group met the classification criteria for the disease (2,11).

We observed augmented mRNA expression of MMP-2 and MMP-9 in acute phase of the disease, which declined in convalescent phase. To the best of our knowledge, ours is the first study to demonstrate the transcriptional expression of MMP-2 and MMP-9 in subjects with HSP. Upregulated expression of MMPs in PBMCs thus serves as important source of elevated production of MMPs in serum of these subjects with HSP.

To confirm whether the activities of MMPs are also augmented in these subjects, we further determined the gelatinolytic activity of MMP-2 and MMP-9 in serum of study subjects using zymography. Activities of both the MMPs were also significantly higher in acute phase of HSP when compared with the activities observed in controls. Our data are further supported by an isolated report of Zou et al. (16), who reported increased plasma levels of MMP-9 in children in acute phase of HSP when compared with their controls. Their study also demonstrated presence of increased MMP-9 positive cells in circulating white blood cells in patients as determined by immunocytochemistry. However, when the serum activities of MMP-2 and MMP-9 were compared between HSP subjects who were in acute phase of the disease with those who were in convalescent phase, we observed no significant difference. Zou et al. (16) have reported lack of significant difference in the plasma levels of MMP-9 in HSP subjects who were in acute phase when compared with their levels in the convalescent phase.

Another important finding in our study was that the transcriptional expressions as well as serum activities of both MMP-2 and MMP-9 were significantly higher in HSP subjects in convalescent phase than in healthy controls. Our observations thus strongly suggest that determining levels of MMPs could be useful in the long-term monitoring of HSP disease.

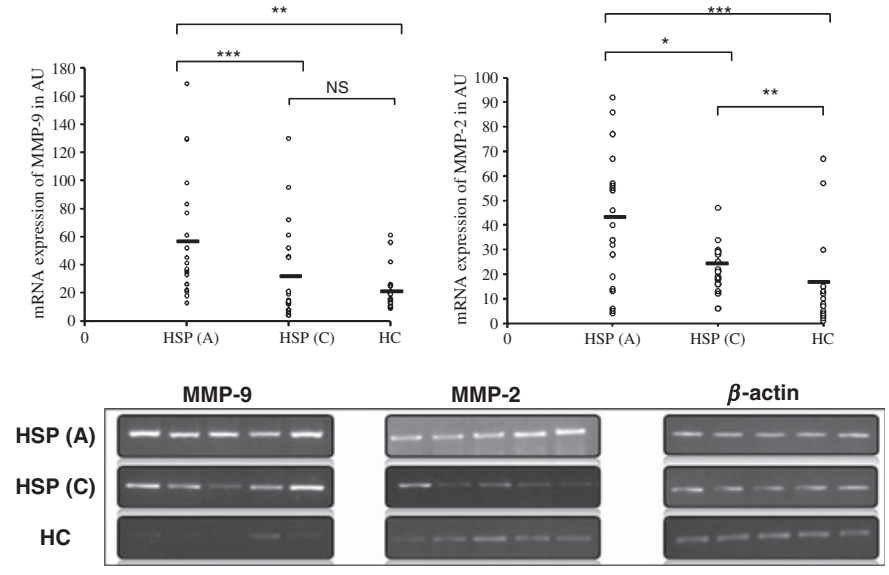

Figure 1 mRNA expression of MMP-9 and MMP-2 in peripheral blood mononuclear cells isolated from subjects with Henoch–Schonlein purpura (HSP) in acute phase [HSP (A)], in subjects with HSP in convalescent phase [HSP (C)] and in normal healthy controls (HC). NS = Not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$. Horizontal lines in the two upper figures indicate mean values.

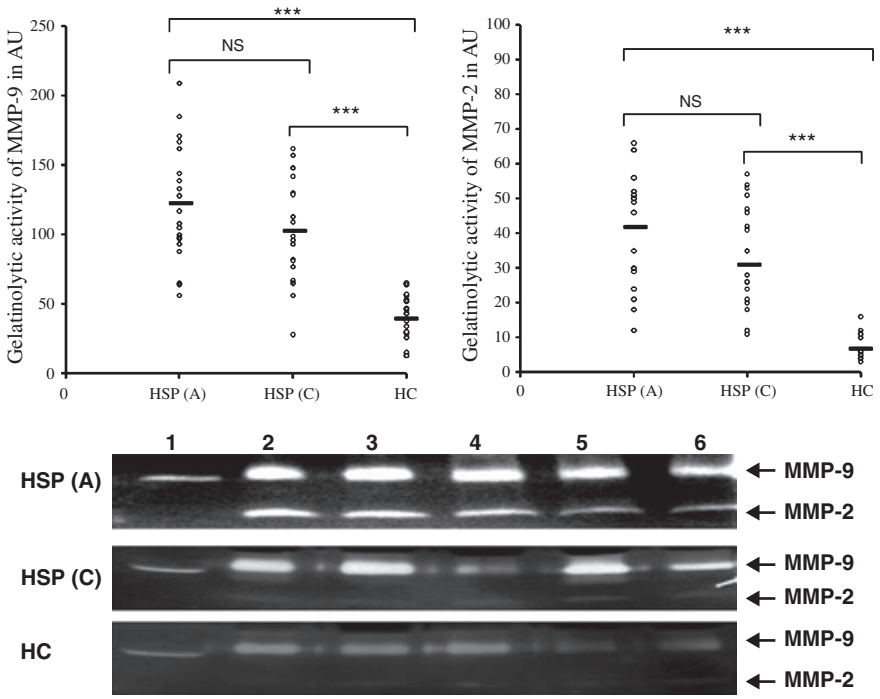

Figure 2 Gelatinolytic activity of MMP-9 and MMP-2 in serum of subjects with Henoch–Schonlein purpura (HSP) in acute phase [HSP (A)], subjects with HSP in convalescent phase [HSP (C)] and in normal healthy controls (HC). First lane contains MMP-9 standard. NS = Not significant; ***$p < 0.001$. Horizontal lines in the two upper figures indicate mean values.

In our study finding increased expression and activity of MMPs in subjects with HSP probably suggests that these molecules may have a role to play in the pathophysiology of this disease. However, as our numbers are small, it would be presumptuous on our part to state that there is a direct causal relationship.

Several studies have reported that MMP-9 is associated with pathogenesis of IgA nephropathy and other types of nephritis (17,18). It may be noted that the pathogenesis of HSP nephritis is similar to that of IgA nephropathy. Also, it is possible that our observations of finding elevated MMP levels in HSP patients were a result of the ongoing inflammation occurring in these subjects. Increased MMPs have also been detected in aortic aneurysms in adult humans (19), suggesting their important role in arterial wall destruction and resultant aneurysm formation (20). Therefore, prolonged MMP activity could be related to the long-term outcome, such as nephritis and recurrence of the disease. A long-term follow-up study in larger number of subjects in near future could fill this lacuna of knowledge in the pathophysiology of the disease.

The studies undertaken at the mRNA level are largely based on a key assumption that mRNA expression is informative in predicting protein expression levels in relation to gene functions. Therefore, most current studies use mRNA expression levels as a proxy for estimating functional aspects that occur at the protein level. Biologically, the discrepancies between mRNA and protein expression can be caused by post-transcriptional regulation, as well as differences in mRNA and protein turnover rates. So far, only few studies have investigated the correlation of mRNA and protein expression levels in humans and the results have been relatively inconsistent (21). However, the degree of validity of this key assumption has seldom been answered with certainty.

We observed that mRNA expression of MMPs is not reflective of the disease status when the data were compared with the serum activity of MMPs in HSP subjects in acute phase versus convalescent phase (Figs 1 and 2). This discrepancy could be explained further on the basis that we have studied PBMCs as cellular model for gene expression; besides these, MMPs are expressed by a variety of other cells e.g. fibroblasts, endothelial cells and smooth muscle cells, which may further add MMPs in the pool. The relative abundance of different pro-forms may vary accordingly among different cells and tissues and among individuals and/or conditions and could be another explanation for our observations and therefore should be exploited in the near future.

Our findings suggest that increased expression of these MMPs in HSP subjects may or may not be exclusively associated with vascular lesion, but may, in part, be attributed to the increased rate of matrix turnover and tissue remodelling as MMP-2 and MMP-9 have been shown to play a pivotal role in facilitating the extravasation and migration of leucocytes by breaking down the basement membrane. Therefore, higher levels of MMP activity in combination with increased transcriptional expression of these enzymes in PBMCs may injure vasculature and thus may initiate inflammation by releasing a cascade of inflammatory mediators

such as adhesion molecules, cytokines, chemokines, integrins, matrix degrading enzymes etc., which could therefore contribute to the pathogenesis of HSP vasculitis. Our data suggest that monitoring levels of MMP-2 and MMP-9 as well as their activity in the serum of HSP patients may be helpful in predicting the disease status.

COMPETING INTERESTS

The authors declare that they have no competing interests.

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