The gene expression profile of matrix metalloproteinases and their inhibitors in children with Henoch-Schönlein purpura

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Directed by Professor Jae Seung Lee

The Doctoral Dissertation submitted to the Department of Medicine, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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June 2009

Jae Il Shin, M.D.

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ABSTRACT

The gene expression profile of matrix metalloproteinases and their inhibitors in children with Henoch-Schönlein purpura

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(Directed by Professor Jae Seung Lee)

To investigate the gene expression profile of all known matrix metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) in children with Henoch-Schönlein purpura (HSP) and to

examine the role, if any, of MMP in the pathogenesis of HSP, peripheral blood samples were obtained from 10 patients with HSP (nine were in the acute stage of HSP, one had HSP nephritis) and 4 healthy controls. Peripheral blood samples were also taken from the nine HSP patients when they reached the convalescent stage of the disease. From these samples, total RNA was purified and gene expressions were measured using real-time polymerase chain reactions.

MMP-8 expression was decreased in patients with arthralgia ($p = 0.038$), and MMP-3 ($p = 0.03$) and TIMP-4 expressions ($p = 0.016$) were elevated in HSP patients with nephritis. Soft tissue edema was associated with decreased expression of MMP-26 ($p = 0.038$) and MMP-28 ($p = 0.038$). MMP-1, MMP-8, MMP-9, MMP-10, MMP-13, MMP-16 and MMP-26 levels were significantly higher in patients in the acute stage of HSP than in normal controls ($p < 0.05$), and MMP-9 ($p = 0.097$) and MMP-19 ($p = 0.054$) levels decreased to borderline

significance in patients in the convalescent stage compared to patients in the acute stage. The duration of steroids administered was negatively correlated with MMP-1, MMP-2, MMP-7, MMP-10, MMP-12, MMP-19, MMP-23, and TIMP-1 levels ($p < 0.05$), suggesting a suppressive effect of steroids on the expressions of MMP and TIMP.

This was the first study to describe the expression profile of all known MMPs and TIMPs in children with HSP, and our results suggested that abnormal levels of MMP and TIMP activity may have a role in the pathogenesis of HSP.

Key words: Henoch-Schönlein purpura, matrix metalloproteinases, tissue inhibitors of metalloproteinases, children, gene expression

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I. INTRODUCTION

Henoch-Schönlein purpura (HSP) is a systemic vasculitis associated with IgA-mediated immune deposits that predominantly affect the skin, gastrointestinal tract, joints, and kidneys.[1] The long-term prognosis of

HSP is determined by the severity of renal involvement.[2] Although the pathogenesis of HSP has not yet been fully elucidated, endothelial injury by IgA-mediated immune complexes and pro-inflammatory cytokines, including interleukin (IL)-1, IL-6, and tumor necrosis factor-α (TNF-α), might be involved in the pathogenesis.[3]

Matrix metalloproteinases (MMPs) are a family of enzymes, which were discovered due to their ability to degrade extracellular matrix (ECM) components and their important role in many physiological and pathological processes.[4,5] The balance between the synthesis and breakdown of the ECM is controlled by MMP and their endogenous inhibitors (tissue inhibitors of MMP: TIMP).[4,5]

Because inflammatory cytokines have been known to be potent inducers of MMP, and MMP itself can promote inflammation,[6] we speculated that MMP activation might be involved in the pathogenesis of HSP vasculitis.

Recent reports have hypothesized the pathogenic role of MMP-9 in patients with HSP.[7-9] However, no data has yet been described regarding the expression profile of all known MMPs and TIMPs in children with HSP. Therefore, we performed this study to examine the role, if any, of MMP in the pathogenesis of HSP and to characterize the gene expression profile of MMP and TIMP in relation to disease activity in HSP.

II. PATIENTS AND METHODS

1. Patients and clinical characteristics

In this study, we recruited 10 patients (4 female, 6 male) with HSP (nine in the acute stage, one with HSP nephritis) and 4 healthy controls from the years 2007 to 2008. All of the studied HSP patients fulfilled both the ACR (American College of Rheumatology) and the recently revised the EULAR/PreS (European League Against Rheumatism/Paediatric Rheumatology European Society) criteria for HSP[10,11.] Four patients had arthralgia, 5 patients had abdominal pain, 4 patients had soft tissue edema and 3 patients developed nephritis (Table 1).

Peripheral blood samples were obtained upon presentation and when the nine HSP patients reached the convalescent stage of the disease. The convalescent stage was defined as when the skin rash, arthritic and

abdominal pain had resolved. Nephritis was defined by the presence of macroscopic or microscopic haematuria with or without proteinuria. This study was approved by the institutional review board and the research ethics committee of Yonsei Severance Hospital.

Table 1. Characteristics of the patients with HSP

Patient No.	Age (years)	Sex	Abdominal pain	Arthralgia	Nephritis	Soft tissue edema
1	5.4	F	+*	-	-	+
2	6.0	F	+	-	+	-
3	2.9	M	+	+	-	-
4	4.5	F	-	-	-	+
5	7.0	F	+	-	-	-
6	6.0	M	+	+	+	-
7	4.6	M	-	+	-	+
8	3.4	M	-	-	-	-
9	8.7	M	-	+	-	+
10	11.7	M	-	-	+	-

*: Present

2. Primer design and RNA extraction

Oligonucleotide primers were designed using the Primer3plus interface v.0.4.0. Primers were selected to span at least one intron of the genomic

sequence to minimize DNA contamination.

Peripheral venous blood was drawn into EDTA tubes, and they were immediately immersed in ice. Total RNA was extracted from the blood samples using a commercial kit (QIAamp RNA blood Mini Kit, Qiagen, Chatsworth, CA) according to the manufacturer's instructions. The quantity of purified RNA was then measured spectrophotometrically. Total RNA was reverse-transcribed before real-time PCR.

3. Synthesis of cDNA

Complementary DNA (cDNA) was synthesized from 1 μg of total RNA, using the Power cDNA Synthesis Kit (First-strand cDNA Synthesis) (iNtRON Biotechnology, Kyunggi, South Korea). cDNA was stored at -20°C until it was used.

4. Real-time PCR

Real-time PCR reactions were performed using the ABI Prism 7300 sequence detection system and according to the manufacturer's protocol (PE Applied Biosystems, Foster City, CA). To quantify mRNA expressions, real-time RT-PCR using SYBR green I dye was performed with KAPA^{TM} SYBR$^{®}$ FAST qPCR Kit; Master Mix (2X) Universal (KAPABIOSYSTEMS, Boston, Massachusetts, U.S.A). The GAPDH gene was used as an endogenous control to normalize for differences in the amount of total RNA present in each sample.

The thermal cycling conditions included an initial denaturation step at 95 °C for 10 min and 40 cycles at 95 °C for 15 sec, annealing at 56 °C for 1 min, and extension at 72 °C for 40 sec. All measurements were triplicated.

To determine the relative mRNA levels within the samples, standard curves for each gene were generated using the cDNA from 1 sample and making 10-fold serial dilutions across an appropriate range. Values

were calculated based on the standard curves generated for each gene. Normalization of samples was determined by dividing the number of copies of MMPs and TIMPs by the number of copies of GAPDH, and all statistical tests were performed using these ratios.

5. Statistical analysis

The Mann-Whitney, Kruskal-Wallis, Wilcoxon signed rank test and Spearman correlation analysis were performed with SAS software. Each value was presented as the mean ± standard errors of the mean (SEM). P-values of less than 0.05 were regarded as statistically significant.

III. RESULTS

1. Differential gene expression in relation to the symptoms of HSP (Table 2-5, Fig. 1-3)

MMP-8 expression was decreased in patients with arthralgia ($p = 0.038$) (Table 2, Fig. 1), and MMP-3 ($p = 0.03$) and TIMP-4 expressions ($p = 0.016$) were elevated in HSP patients with nephritis than those without (Table 3, Fig. 2). Soft tissue edema was associated with decreased expressions of MMP-26 ($p = 0.038$) and MMP-28 ($p = 0.038$). MMP-21 was elevated in HSP patients with abdominal pain with a borderline significance ($p = 0.055$) (Table 4, Fig. 3). Positive occult blood in the stool was associated with an increased WBC count ($p = 0.033$).

Table 2. Differential gene expression in relation to arthralgia

MMPs	With arthralgia (n = 4)	Without arthralgia (n = 6)
MMP-1	203.76 ± 81.39	130.8 ± 36.42
MMP-2	75.45 ± 56.42	1.58 ± 0.52
MMP-3	3.83 ± 1.83	3.24 ± 1.10
MMP-7	1.81 ± 0.73	6.00 ± 3.33
MMP-8	1.54 ± 0.94	100.66 ± 74.89*
MMP-9	575 ± 2.47	15.17 ± 11.46
MMP-10	37.01 ± 16.57	17.86 ± 7.26
MMP-11	5.73 ± 2.15	2.91 ± 1.17
MMP-12	2503 ± 1346	8597 ± 7984
MMP-13	19.35 ± 13.63	20.61 ± 11.98
MMP-14	206.6 ± 93.59	403.74 ± 154.08
MMP-15	2.33 ± 1.90	3.09 ± 1.06
MMP-16	37.64 ± 29.49	18.84 ± 10.24
MMP-17	0.26 ± 0.07	0.33 ± 0.17
MMP-19	8.22 ± 6.92	2.06 ± 0.59
MMP-21	123.1 ± 64.57	4577 ± 3660
MMP-23	0.93 ± 0.15	1.30 ± 0.36
MMP-24	3.33 ± 2.80	3.88 ± 2.88
MMP-25	5.54 ± 2.72	5.10 ± 2.23
MMP-26	243.3 ± 201.7	135.6 ± 42.72
MMP-27	0.11 ± 0.03	64.99 ± 49.32
MMP-28	24.14 ± 13.75	83.97 ± 62.36
TIMP-1	2.12 ± 0.30	1.79 ± 0.20
TIMP-2	2.02 ± 1.02	1.81 ± 0.63
TIMP-3	6.30 ± 3.51	39.07 ± 32.58
TIMP-4	5160 ± 5095	19179 ± 18531

* $p < 0.05$

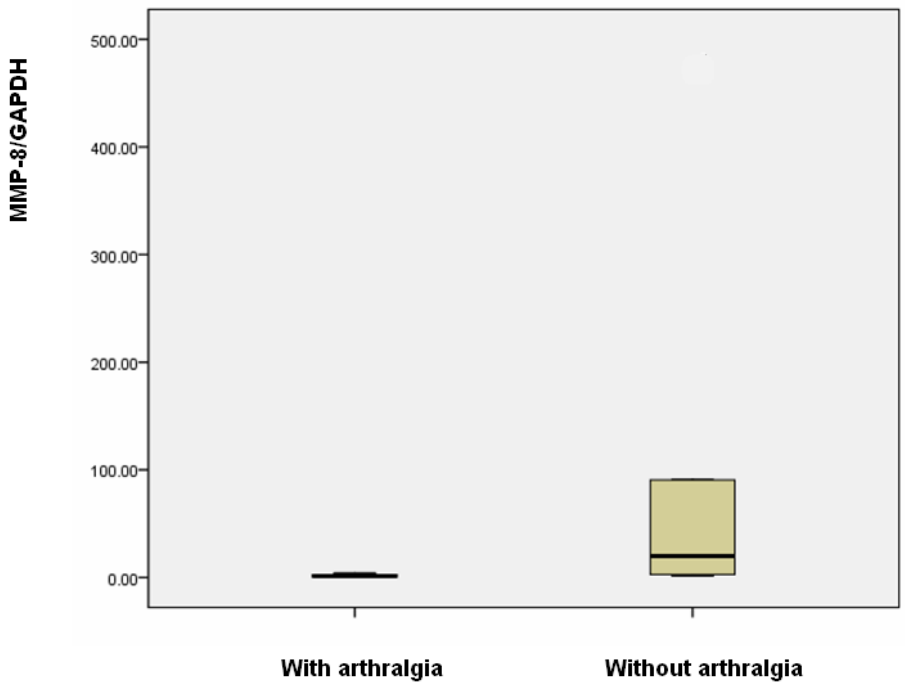

Figure 1. MMP-8/GAPDH ratio was significantly higher in HSP patients without arthralgia than in those with.

Table 3. Differential gene expression in relation to nephritis

MMPs	With nephritis (n = 3)	Without nephritis (n =7)
MMP-1	107.19 ± 70.97	182.61 ± 46.26
MMP-2	3.04 ± 0.92	43.17 ± 33.77
MMP-3	6.81 ± 1.43	2.06 ± 0.65*
MMP-7	8.25 ± 6.23	2.64 ± 1.41
MMP-8	19.9 ± 6.79	11.07 ± 2.71
MMP-9	163.5 ± 117.8	36.52 ± 15.89
MMP-10	29.28 ± 10.07	23.90 ± 11.01
MMP-11	5.06 ± 2.44	3.60 ± 1.34
MMP-12	16395 ± 16054	1773 ± 817
MMP-13	22.00 ± 20.38	19.29 ± 9.80
MMP-14	468.11 ± 240.76	263.39 ± 106.32
MMP-15	3.40 ± 1.51	2.52 ± 1.23
MMP-16	22.60 ± 19.67	27.97 ± 16.93
MMP-17	0.58 ± 0.31	0.18 ± 0.02
MMP-19	2.42 ± 1.10	5.43 ± 3.93
MMP-21	7576 ± 7499	746 ± 581
MMP-23	1.48 ± 0.25	1.01 ± 0.30
MMP-24	1.48 ± 1.21	4.59 ± 2.74
MMP-25	10.60 ± 3.60	2.98 ± 0.95
MMP-26	359.7 ± 243.9	101.1 ± 41.8
MMP-27	116.16 ± 97.28	5.99 ± 4.45
MMP-28	151.59 ± 119.3	20.80 ± 12.19
TIMP-1	1.92 ± 0.12	1.93 ± 0.24
TIMP-2	3.72 ± 1.14	1.11 ± 0.24
TIMP-3	12.78 ± 10.24	31.61 ± 28.11
TIMP-4	44995 ± 33797	104.25 ± 36.57*

* $p < 0.05$

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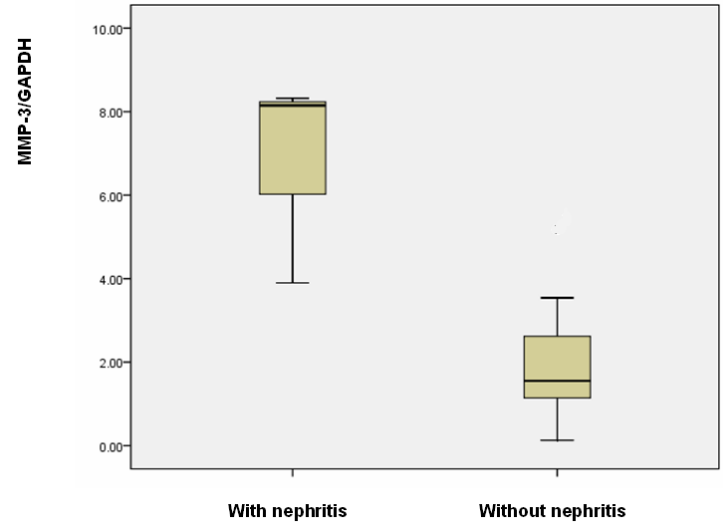

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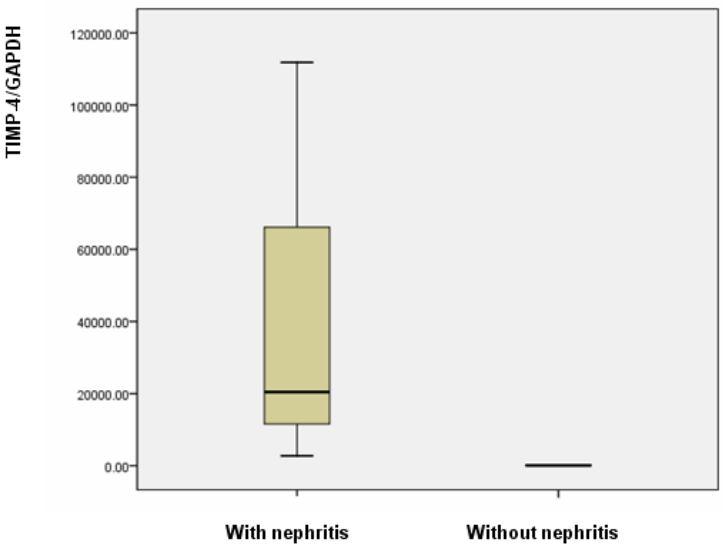

Figure 2. MMP-3/GAPDH (A) and TIMP-4/GAPDH ratios (B) were significantly higher in HSP patients with nephritis than in those without.

Table 4. Differential gene expression in relation to abdominal pain

MMPs	With abdominal pain (n = 5)	Without abdominal pain (n = 5)
MMP-1	174.04 ± 72.1	145.93 ± 36.14
MMP-2	12.54 ± 11.05	49.72 ± 47.64
MMP-3	3.25 ± 1.37	3.71 ± 1.40
MMP-7	7.04 ± 3.88	1.60 ± 0.56
MMP-8	16.25 ± 4.22	11.18 ± 4.05
MMP-9	117.37 ± 71.17	31.87 ± 20.21
MMP-10	28.23 ± 11.72	22.80 ± 12.12
MMP-11	4.34 ± 1.57	3.73 ± 1.81
MMP-12	1248 ± 830	11072 ± 9403
MMP-13	35.62 ± 14.22	4.58 ± 2.11
MMP-14	250.0 ± 116.86	399.76 ± 170.49
MMP-15	3.11 ± 1.47	2.45 ± 1.31
MMP-16	45.64 ± 23.02	7.08 ± 2.32
MMP-17	0.42 ± 0.2	0.18 ± 0.02
MMP-19	7.36 ± 5.45	1.68 ± 0.31
MMP-21	5460 ± 4349	130.31 ± 90.71*
MMP-23	1.19 ± 0.83	1.08 ± 0.50
MMP-24	1.24 ± 0.69	6.08 ± 3.71
MMP-25	6.87 ± 3.02	3.68 ± 1.22
MMP-26	281.30 ± 150.59	76.06 ± 24.67
MMP-27	68.80 ± 60.46	9.28 ± 7.52
MMP-28	113.06 ± 70.47	7.02 ± 2.25
TIMP-1	2.10 ± 0.32	1.75 ± 0.12
TIMP-2	2.62 ± 0.92	1.16 ± 0.33
TIMP-3	11.11 ± 6.20	40.82 ± 39.74
TIMP-4	26572 ± 21667	570.4 ± 539.2

* $p < 0.05$

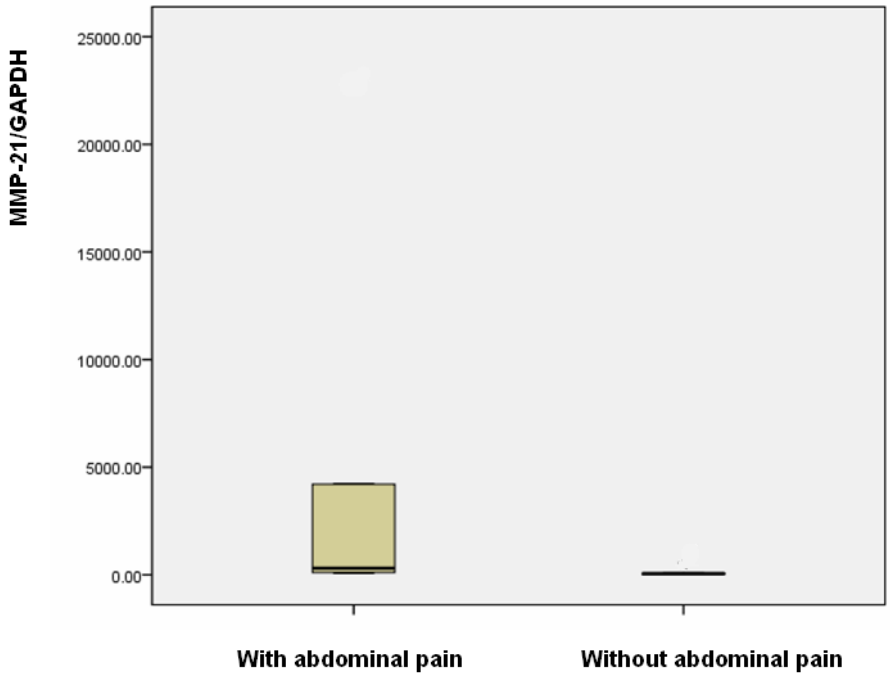

Figure 3. MMP-21/GAPDH ratio was significantly higher in HSP patients with abdominal pain than in those without.

Table 5. Summary of differential gene expression in relation to the symptoms of HSP patients

Symptoms	MMP	Expression	With symptom	Without symptom	p-value
Arthralgia	MMP-8	Decreased	1.54 ± 0.94	100.66 ± 74.89	0.038
Nephritis	MMP-3	Increased	6.81 ± 1.43	2.06 ± 0.65	0.030
	TIMP-4	Increased	44,995 ± 33,797	104.25 ± 36.57	0.016
Soft tissue edema	MMP-26	Decreased	40.64 ± 26.00	270.71 ± 120.54	0.038
	MMP-28	Decreased	5.00 ± 2.61	96.74 ± 59.80	0.038
Abdominal pain	MMP-21	Increased	5460 ± 4349	130.31 ± 90.71	0.055

2. Differential gene expression in the acute and convalescent stages of HSP, and normal controls (Table 6)

MMP-1, MMP-8, MMP-9, MMP-10, MMP-13, MMP-16 and MMP-26 were significantly higher in patients in the acute stage of HSP than in normal controls ($p < 0.05$), and MMP-9 (Fig. 4, $p = 0.097$) and MMP-19 ($p = 0.054$) decreased with a borderline significance in the convalescent stage compared to the acute stage of HSP. MMP-1 ($p = 0.006$) and MMP-10 ($p = 0.034$) levels in patients in the convalescent stage were significantly higher than in normal controls.

Table 6. Differential gene expression in the acute, convalescent stages of HSP and normal controls

MMPs	Acute stage (n = 9)	Convalescent stage (n = 9)	Normal children (n = 4)
MMP-1	171.39 ± 40.88*	87.48 ± 27.25***	5.57 ± 3.75
MMP-2	34.19 ± 26.47	0.91 ± 0.4	4.24 ± 2.33
MMP-3	2.96 ± 0.86	4.86 ± 3.02	2.34 ± 0.99
MMP-7	4.65 ± 2.27	2.8 ± 2.48**	2.0 ± 0.99
MMP-8	13.28 ± 3.19*	11.08 ± 4.72	1.17 ± 0.44
MMP-9	81.73 ± 41.36*	9.59 ± 2.72	3.58 ± 2.27
MMP-10	25.18 ± 8.93*	5.45 ± 1.48***	1.31 ± 0.60
MMP-11	4.46 ± 1.17	4.22 ± 1.86	3.65 ± 1.94
MMP-12	1455 ± 659	822 ± 392	586 ± 560
MMP-13	22.19 ± 9.24	7.14 ± 5.25	0.46 ± 0.17
MMP-14	271.0 ± 94.96	164.43 ± 68.32	36.22 ± 32.51
MMP-15	2.54 ± 1.01	5.54 ± 3.0	2.75 ± 2.19
MMP-16	29.04 ± 13.83*	5.58 ± 3.74	1.06 ± 0.57
MMP-17	0.32 ± 0.11	0.34 ± 0.11	0.15 ± 0.07
MMP-19	4.85 ± 3.04	1.05 ± 0.37**	1.23 ± 0.63
MMP-21	3100 ± 2475	225.92 ± 152.97	30.00 ± 18.67
MMP-23	1.12 ± 0.25	0.76 ± 0.15	0.54 ± 0.13
MMP-24	4.03 ± 2.14	0.66 ± 0.47	0.63 ± 0.40
MMP-25	5.47 ± 1.8	3.74 ± 0.75	2.53 ± 1.42
MMP-26	188.88 ± 88.32*	167.38 ± 135.46	19.72 ± 17.81
MMP-27	39.06 ± 33.97	8.62 ± 6.84	2.33 ± 1.20
MMP-28	66.3 ± 41.5	25.58 ± 20.24	5.27 ± 1.93
TIMP-1	1.95 ± 0.19	1.48 ± 0.4	1.39 ± 0.79
TIMP-2	1.94 ± 0.58	1.41 ± 0.25	0.96 ± 0.54
TIMP-3	28.82 ± 21.67	3.00 ± 0.88	1.25 ± 0.58
TIMP-4	14776 ± 1.335	326 ± 230	84.12 ± 33.78

* $p < 0.05$ in comparison to normal controls

** $p < 0.05$ in comparison to the acute stage of HSP

*** $p < 0.05$ in comparison to normal controls

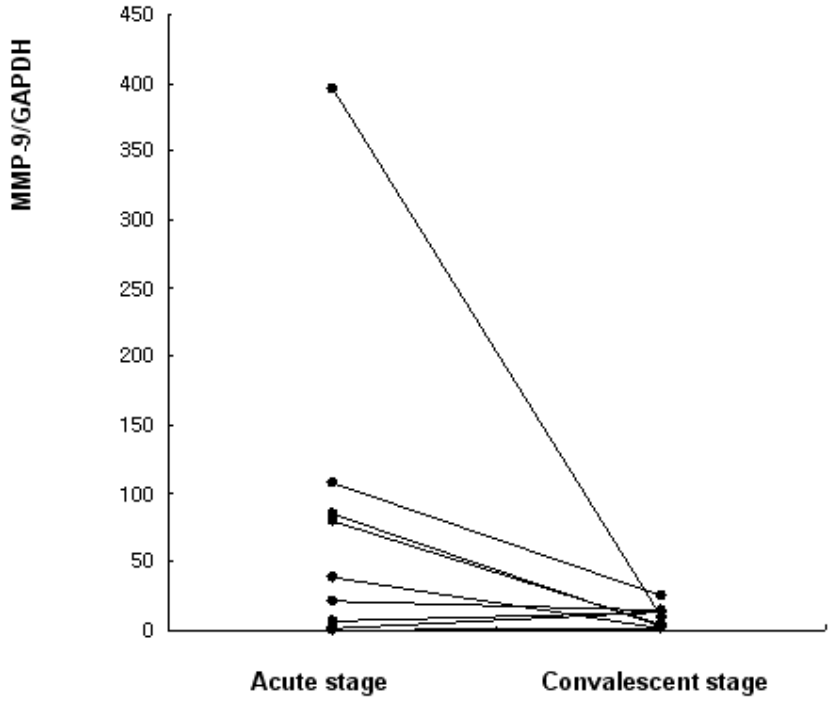

Figure 4. Serial changes of MMP-9/GAPDH ratio at the acute and convalescent stages of HSP.

3. Correlations among the laboratory findings of HSP (Table 7)

The white blood cell (WBC) count correlated positively with the neutrophil count, monocyte count, basophil count, D-dimer and serum IgG levels. The neutrophil count correlated positively with the monocyte count, basophil count, platelet count, erythrocyte sedimentation rate (ESR) and serum IgG levels. The lymphocyte count correlated negatively with complement 4 (C4) and fibrinogen levels. The monocyte count correlated positively with the basophil count, ESR, and serum IgA levels. The platelet count correlated positively with the ESR, and high-sensitivity C-reactive protein (hsCRP) levels. hsCRP correlated positively with C3, C4, fibrinogen, and D-dimer levels. Serum IgA levels correlated positively with serum IgM levels and C3 did positively with C4 levels. D-dimer levels correlated positively with eosinophil cationic protein (ECP) levels.

Table 7. Correlations among the laboratory findings of HSP

	WBC	Neut	Lym	Mono	Baso	Plt	ESR	hsCRP	ECP	IgG	IgA	IgM	C3	C4	Fib	D-dim
WBC		<0.0001		<0.0001	0.002					0.015						0.029
Neut	<0.0001			0.0001	0.038	0.02	0.03			0.029						
Lym														0.003(-)	0.018(-)	
Mono	<0.0001	0.0001			0.04		0.038				0.01					
Baso	0.002	0.038		0.04												
Plt		0.02					0.016	0.036								
ESR		0.03		0.038		0.016										
hsCRP						0.036							0.02	0.004	0.037	0.0035
ECP																0.037
IgG	0.015	0.029														
IgA				0.01								0.044				
IgM											0.044					
C3								0.02						0.035		
C4			0.003(-)*					0.004					0.035		0.018	
Fib			0.018(-)					0.037						0.018		
D-dim	0.029							0.0035	0.037							

WBC = white blood cell count; Neut = neutrophil count; Lym = lymphocyte count; Mono = monocyte count; Baso = basophil count; Plt = platelet count; ESR = erythrocyte sedimentation rate; hsCRP = high sensitivity C-reactive protein; ECP = eosinophil cationic protein; Fib = fibrinogen; D-dim = D-dimer

*: negative correlation

4. Correlations between the laboratory findings of HSP and expressions of MMP and TIMP (Table 8)

MMP-8 levels correlated positively with the WBC count (Fig. 5), neutrophil count, monocyte count and basophil count. MMP-19 levels correlated positively with the WBC count, hsCRP and D-dimer levels. MMP-12 and MMP-14 levels correlated negatively with the WBC count, neutrophil count, monocyte count and serum IgG levels. The monocyte count correlated negatively with MMP-10 and MMP-23 levels. Hemoglobin levels correlated negatively with MMP-2 and MMP-25 levels. ESR levels correlated negatively with MMP-2 and MMP-3 levels. ECP levels correlated positively with MMP-7 and MMP-21 levels. Serum IgG levels correlated positively with MMP-11 levels and negatively with MMP-12, MMP-14, and MMP-15 levels. Serum IgA levels correlated negatively with MMP-2 levels and C3 and C4 levels correlated negatively with MMP-8 levels. von Willebrand

factor antigen (vWF) levels correlated positively with TIMP-2 levels (Fig. 6). IgA rheumatoid factor (RF) levels correlated positively with MMP-25 and TIMP-2 levels, but correlated negatively with MMP-19 levels.

Table 8. Correlations between the laboratory findings of HSP and expressions of MMP and TIMP

	M2	M3	M7	M8	M10	M11	M12	M14	M15	M19	M21	M23	M25	T2
WBC				0.01			0.01(-)	0.044(-)		0.03				
Neut				0.046			0.014(-)	0.013(-)						
Mono				0.035	0.036(-)*		0.002(-)	0.03(-)				0.039(-)		
Baso				0.005										
Hb	0.011(-)												0.009(-)	
ESR	0.002(-)	0.012(-)												
hsCRP										0.005				
ECP			0.002								0.013			
IgG						0.049	0.015(-)	0.035(-)	0.035(-)					
IgA	0.032(-)													
C3				0.029(-)										
C4				0.009(-)										
D-dim										<0.0001				
vWF														0.048
RF										0.049(-)			0.007	0.007

M = matrix metalloproteinases; T = tissue inhibitors of metalloproteinases; WBC = white blood cell count; Neut = neutrophil count; Mono = monocyte count; Baso = basophil count; Hb =

hemoglobin; ESR = erythrocyte sedimentation rate; hsCRP = high sensitivity C-reactive protein; ECP = eosinophil cationic protein; D-dim = D-dimer; vWF = von Willebrand factor antigen; RF = IgA rheumatoid factor

*: negative correlation

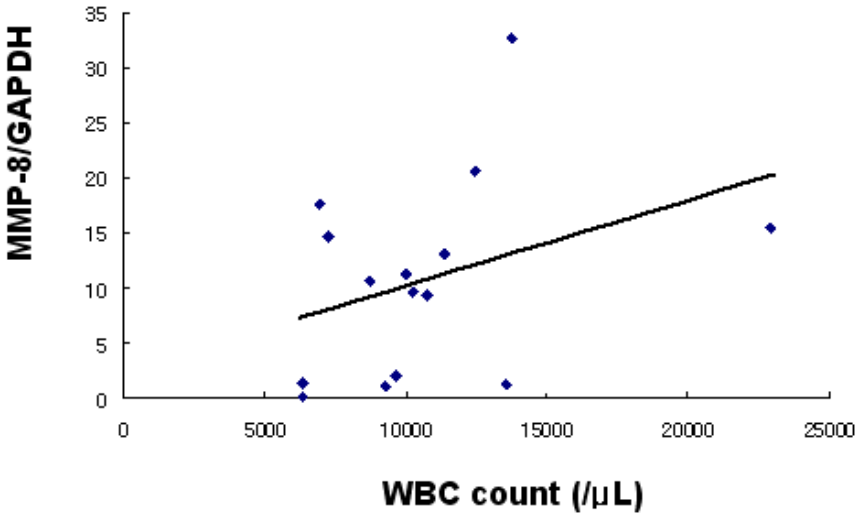

Figure 5. MMP-8/GAPDH correlated positively with the WBC count.

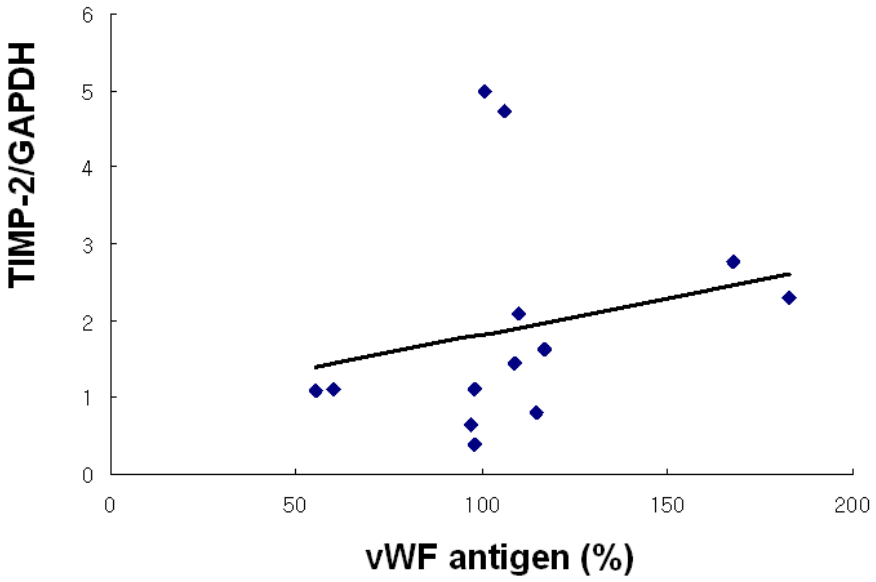

Figure 6. TIMP-2/GAPDH correlated positively with von Willebrand factor antigen.

5. Correlations among expressions of all MMP and TIMP genes (Table 9)

Table 9 shows correlations for all MMP and TIMP genes. Of note, MMP-1, MMP-10, MMP-16, MMP-19, MMP-28 and TIMP-1 were predominantly coexpressed with various MMPs and TIMPs ($p < 0.0001$).

Table 9. Correlations of expression among all MMP and TIMP genes

	M1	M2	M3	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M19	M21	M23	M24	M25	M26	M27	M28	T1	T2	T3	T4
M1							●			●	●	*	●	*	●	*	*	*		*		*	●			
M2			*	*									*		*			*					*	*		
M3		*		*	*			*							*	*						*				*
M7		*	*			*		*								*	*	*		*		●	*			*
M8			*			*													*					*		*
M9				*	*		*			*				*		*			*	*		*	*	●		*
M10	●					*			*	*	●		●	*	●	*	●	●		*		*	*			*
M11			*	*																		*				
M12							*				*				*											
M13	●					*	*				*		●	*	*	*	*	*		*		*	●	*		
M14	●						●		*	*		*	*		●		*	*		*			*			
M15	*										*						*	*							*	
M16	●	*					●			●	*			*	●	*	*	*		*		●	●	*		
M17	*					*	*			*			*		*	*	*		*	*		●	*	*		
M19	●	*	*				●		*	*	●		●	*		*	●	*		*		*	*		*	
M21	*		*	*		*	*			*			*	*	*					●	*	●	*			*
M23	*			*			●			*	*	*	*	*	●			*		*		*	*		*	
M24	*	*		*			●			*	*	*	*		*		*					*	*		*	
M25					*	*								*										●		
M26	*			*		*	*			*	*		*	*	*	●	*					●	●	*		
M27																*										*
M28	*		*	●		*	*	*		*			●	●	*	●	*	*		●			●	*		*
T1	●	*		*		*	*			●	*		●	*	*	*	*	*		●		●		*		*
T2		*			*	●				*			*	*					●	*		*	*			*
T3												*			*		*	*								
T4			*	*	*	*	*									*					*	*	*	*		

M = matrix metalloproteinases; T = tissue inhibitors of metalloproteinases, * $p < 0.05$, ● $p < 0.0001$

6. Correlations between the duration of steroids and expressions of MMP and TIMP (Table 10)

The duration of steroids administered was negatively correlated with MMP-1, MMP-2, MMP-7, MMP-10 (Fig. 7), MMP-12, MMP-19, MMP-23 and TIMP-1 levels, suggesting the suppressive effect of steroids on the expressions of MMP and TIMP.

Table 10. Correlations between the duration of steroids and expressions of MMP and TIMP

MMPs	Correlation coefficient	p-value
MMP-1	-0.481	0.037
MMP-2	-0.541	0.017
MMP-7	-0.525	0.021
MMP-10	-0.513	0.025
MMP-12	-0.480	0.037
MMP-19	-0.513	0.025
MMP-23	-0.485	0.035
TIMP-1	-0.586	0.008

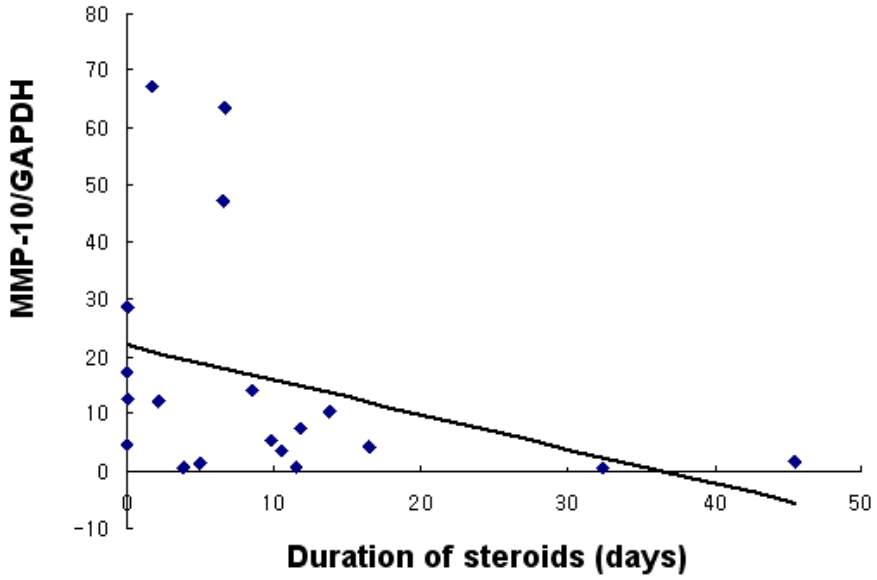

Figure 7. MMP-10/GAPDH correlated negatively with the duration of steroids.

IV. DISCUSSION

MMPs are known to be a family of 24 human zinc-dependent proteases that can degrade the ECM and basement membrane components.[4] They are divided into six subgroups according to the structure and substrate specificity; collagenases, stromelysins, gelatinases, matrilysins, membrane-type MMPs and others.[12] They have important roles in apoptosis, morphogenesis, wound healing, tissue repair and remodeling, angiogenesis, inflammation and immunity.[4,5]

Some reports have shown that MMPs might play a pathogenic role in vasculitides, such as Kawasaki disease, ANCA-associated vasculitis, giant cell arteritis or Takayasu arteritis.[13-16] However, there have been few reports on the role of MMPs in the pathogenesis of childhood HSP.[7-9]

Kobayashi et al. described a HSP patient with multiple blisters and,

using zymography, demonstrated that MMP-9 (gelatinase B) was elevated in the blister.[7] They hypothesized that MMP-9, which is secreted by polymorphonuclear leukocytes (PMNs) that migrated from the site of intensive vasculitis to the dermal side of the dermoepidermal junction could cause blister formation by degrading basement membrane components such as type VII collagen.[7] Urushihara et al. showed that MMP-9 staining, which was almost negative in normal glomeruli, was increased in the mesangial area and correlated with the degree of glomerular cell proliferative changes in various mesangial proliferative glomerulonephritides, including HSP nephritis.[8] Furthermore, Zou et al. recently reported that plasma MMP-9 levels in patients in the acute phase of HSP were significantly higher than in healthy controls and speculated that MMP-9 might play an important role in the vascular destruction of HSP.[9]

Taken together,[7-9] it was suggested that MMP-9 might be involved in

the pathogenesis of HSP. Nevertheless, there has been no report on the expression of various other MMPs in HSP. Therefore, we firstly examined all known MMPs and TIMPs in childhood HSP. Our studies revealed that MMPs might have an important role in the pathogenesis of HSP and the expression of some MMPs were correlated to the disease activity of HSP.

Regarding MMP-9, our study also showed that it was significantly higher in patients in the acute stage of HSP than in normal controls, which was consistent with the results of a previous report.[9] Besides MMP-9, three collagenases (MMP-1, MMP-8, MMP-13), MMP-10 (stromelysin 2), MMP-16 (MT3-MMP), and MMP-26 (matrilysin 2) levels were also elevated in patients in the acute stage of HSP. Although not studied in HSP, expression of these MMPs has also been documented in various skin diseases.[17-20]

Rijken et al. showed that staining of MMP-1, MMP-8 and MMP-9

was present following exposure to solar-simulated radiation and skin-infiltrating neutrophils could be the major source of these MMPs.[17] Regarding MMP-13, Niimi et al. reported that the number of cells expressing MMP-13 was significantly increased in the skin lesions of bullous pemphigoid patients as compared to that of patients with normal skin.[18] This finding suggests that MMP-13 might have a role in blister formation in bullous pemphigoid.[18] Also, Hattori et al. showed that MMP-13 was detected in the vasculature in 17 out of 20 human basal cell carcinoma samples and speculated that endothelial cells in the skin might be a source of MMP-13.[19]

Järvinen et al. demonstrated that MMP-10 and MMP-26 were abundantly expressed by keratinocytes in skin samples of systemic lupus erythematosus (LE), discoid LE and subacute cutaneous LE.[20] Also, Seandel et al. found that multiple MMPs including MMP-13 and MMP-16 were detected in angiogenic tissue.[21] They speculated that the

angiogenic effect by vascular endothelial growth factor (VEGF) might be dependent on MMP activity, because new vessel growth was inhibited by MMP inhibitors.[21] Because it was reported that VEGF might also play a role in the morphological and functional changes of the vascular bed and in the inflammatory reaction in HSP,[22] we speculate that MMP-13 and MMP-16 might be associated with this angiogenic effect in HSP.

In our study, the expression patterns of MMP differed according to the symptoms of HSP. Firstly, we found that MMP-8 was decreased in HSP patients with joint involvement compared to those without. Although not studied in HSP, that serum levels of proMMP-3, -8 and -9 were reported by Tchetverikov et al. to be higher in patients with rheumatoid arthritis than in patients with osteoarthritis or in healthy controls.[23] The authors also reported a strong correlation was seen between serum and synovial fluid levels of MMP-8 and -9 in rheumatoid arthritis

patients.[23] Also, Rajasekhar et al. showed that immune complexes elicited significant MMP-8 secretion from peripheral blood PMNs, and serum MMP-8 levels correlated positively with serum CRP levels, suggesting that serum MMP-8 might be an indicator of acute inflammatory activity.[24] In our study, MMP-8 levels correlated positively with the WBC count, but HSP patients with arthralgia had a significantly lower expression of MMP-8. Furthermore, inflammatory markers such as the WBC count, ESR and hsCRP were lower in patients with arthralgia than in those without, although this finding was not statistically significant.

Secondly, we found that MMP-3 and TIMP-4 levels were significantly higher in HSP patients with nephritis than in those without. With the same comparison, TIMP-2 levels were close to significance ($p = 0.066$). Although not studied in HSP, Akiyama et al. demonstrated that the levels of MMP-3 and TIMP-2 were increased in patients with IgA

nephropathy and lupus nephritis, which involve mesangial proliferative glomerulonephritis.[25] Because the renal involvement in HSP also shows a pattern of mesangial proliferative glomerulonephritis,[26] MMP-3 and TIMP-2 might therefore also be involved in the pathogenesis of HSP nephritis. Regarding TIMP-4, our study demonstrated that MMP-3 levels correlated positively with TIMP-4 levels. Camp et al. showed that increases in the levels of TIMP-4 in the medullae of 6-week-old spontaneously hypertensive rats as compared with 2-week-old spontaneously hypertensive rats.[27] This finding suggests that TIMP-4 might inhibit the collagenolytic activity of MMP associated with glomerular injury, matrix accumulation and glomerulosclerosis in the medulla.[27]

Thirdly, our results showed that MMP-21 was elevated in HSP patients with abdominal pain with a borderline significance ($p = 0.055$), and MMP-21 levels correlated positively with TIMP-4 ($p = 0.023$) and

ECP levels ($p = 0.013$), suggesting a possible association between eosinophil activation and gastrointestinal involvement in the pathogenesis of HSP. In both humans and mice, MMP-21 has been detected in the epithelial cells of the developing kidneys, intestine, neuroectoderm, and skin.[28] Also, positive occult blood in the stool was associated with an increased WBC count ($p = 0.033$), which also correlated with MMP-8 levels with a borderline significance ($p = 0.066$), suggesting that severe inflammation might cause gastrointestinal bleeding in HSP.

In this study, we also analyzed the correlations between the inflammatory markers and the expressions of MMPs and TIMPs in HSP. The WBC count and neutrophil count correlated positively with MMP-8 levels, but correlated negatively with MMP-12 and MMP-14 levels, suggesting a differential expression within the MMP family in HSP. Also, MMP-7 and MMP-21 levels correlated positively with ECP,

which is an important marker of eosinophil activation in the pathogenesis of HSP.[29] It has been reported that D-dimers (a marker of thrombogenesis) and vWF antigens (a marker of endothelial injury) are increased in the acute stage of HSP.[30] In our study, D-dimer levels correlated positively with MMP-19 levels and vWF antigen levels correlated positively with TIMP-2 levels.

We also demonstrated that the duration of steroids administered was negatively correlated with MMP-1, MMP-2, MMP-7, MMP-10, MMP-12, MMP-19, MMP-23, TIMP-1 levels, suggesting that the steroids suppressed the expression of these MMPs and TIMPs. Although not studied in HSP, some investigators have suggested that corticosteroids might down-regulate the expression of MMP-9.[31] Because MMP-9 is one of the important MMPs in the pathogenesis of HSP and MMP-9 levels are positively correlated with MMP-7 and MMP-10 levels in our study, the use of steroids would be beneficial by inhibiting MMPs

associated with matrix degradation.

V. CONCLUSION

This was the first study to characterize the expression profile of all known MMPs and TIMPs in children with HSP, and our results suggested that abnormal expressions of MMP and TIMP activity might have a role in the pathogenesis of HSP.

However, there are some limitations in our study: (1) a small number of patients were studied, (2) only mRNA expressions of MMP were measured, and (3) the exact role of each MMP gene in the pathogenesis of HSP was not fully elucidated. Nevertheless, we found that expression patterns of MMP were different according to the symptoms of HSP, and correlations among MMP and TIMP levels provided important insights regarding the co-regulation of these genes. Because neutrophil elastase is known to activate MMPs, and it might also suppress TIMP activity,[13] it would be interesting to see the relationship

between the expressions of MMP and neutrophil elastase in the future. Also, further studies are necessary to examine whether MMPs could predict the subsequent course and long-term outcome of HSP and to evaluate inhibiting pathogenic MMPs would be useful in the treatment of HSP in the future.

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metalloproteinase-1 expression in asthma. J Allergy Clin Immunol 1999;104:356-63.

ABSTRACT (IN KOREAN)

헤노흐 쇤라인 자반증 소아에서 기질금속단백분해효소 및 그 억제제의 유전자 발현 프로파일

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신 재 일

목적: 헤노흐 쇤라인 자반증 환아에서 모든 알려진 기질금속단백분해효소 및 금속단백분해효소의 조직 억제제의 유전자 발현 프로파일을 조사하여 자반증의 병인에 역할을 하는지 알아보기 위해 본 연구를 진행하였다.

방법: 헤노흐 쇤라인 자반증 환아 10 명 (9 명은 자반증의 급성기, 1 명은 자반증 신장염시)과 4 명의 정상 대조군 소아에서 말초 혈액을 얻었고, 9 명의 자반증 환아가 회복기에 들어갔을 때 또한 혈액을 채혈하였다. 혈액에서 총 RNA 를 추출하여 순화시키고 cDNA 를 합성하여 real-time polymerase chain reaction 으로 기질금속단백분해효소 및 금속단백분해효소의 조직 억제제의 mRNA 값을 측정하였다.

결과: MMP-8 mRNA 발현량은 관절증상을 동반한 자반증 환아에서 유의하게 감소되었고 ($p = 0.038$), MMP-3 ($p = 0.03$) 과 TIMP-4 ($p = 0.016$) mRNA 발현량은 신장염을 동반한 환아에서 유의하게 증가되어 있었다. 연부조직 부종은 MMP-26 ($p = 0.038$) 과 MMP-28 ($p = 0.038$)의 감소된 mRNA 발현량과 연관되어 있었다. MMP-1, MMP-8, MMP-9, MMP-10, MMP-13, MMP-16, MMP-26의 mRNA 발현량은 자반증의 급성기에 정상 대조군보다 유의하게

증가된 소견을 보였고 ($p < 0.05$), MMP-9 ($p = 0.097$)와 MMP-19의 mRNA 발현량 ($p = 0.054$)은 회복기에 감소되는 경향을 보였다. 스테로이드의 사용기간은 MMP-1, MMP-2, MMP-7, MMP-10, MMP-12, MMP-19, MMP-23, TIMP-1의 mRNA 발현량과 유의한 음의 상관관계를 보여($p < 0.05$), 스테로이드가 MMP 와 TIMP의 발현을 억제하는 것으로 생각되었다.

결론: 본 연구는 헤노흐 쇤라인 자반증 환아에서 모든 알려진 기질금속단백분해효소의 유전자 발현 프로파일을 조사한 최초의 연구이고, 기질금속단백분해효소 및 금속단백분해효소의 조직억제제의 비정상적인 유전자 발현은 자반증의 병인에 역할을 하는 것으로 사료된다.

핵심되는 말: 헤노흐 쇤라인 자반증, 기질금속단백분해효소, 금속단백분해효소의 조직억제제, 소아, 유전자 발현