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Tissue Inhibitor of Metalloproteinase-1 Is Responsible for Residual Pleural Thickening in Pleural Tuberculosis

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Residual pleural thickening (RPT) is the most frequent complication associated with pleural tuberculosis, and may occur even after successful anti-tuberculosis medications. Matrix metalloproteinases (MMPs) are zinc-dependent proteinases capable of degrading all components of the extracellular matrix. The proteolytic action of MMPs may be involved in the pathogenesis of tuberculosis. MMP-9, secreted by monocytes and lymphocyte, may lead to long-term fibrosis. The aim of the present study was to determine whether MMP-2 and/or MMP-9 and their specific inhibitors, tissue inhibitors of metalloproteinase 1 (TIMP-1) and TIMP-2, could be used to predict RPT. This retrospective study enrolled 52 patients diagnosed with pleural tuberculosis. Levels of MMP-2, MMP-9, TIMP-1, and TIM-2 were determined in the pleural fluid by ELISA. The RPT was measured on chest X-ray at the completion of treatment and the final follow-up. The average periods of anti-tuberculosis medication and the follow-up after completion of treatment were 6.7 and 7.6 months, respectively. MMP-2 or MMP-9 levels had no significant correlation to RPT. The patients with RPT > 2 mm at the completion of anti-tuberculosis medication and the final follow-up had higher TIMP-1 levels (p = 0.00 and p = 0.001, respectively). However, patients with RPT > 2 mm at the completion of anti-tuberculosis medication had lower TIMP-2 levels (p = 0.005). In a logistic regression model, elevated TIMP-1 levels at the completion of anti-tuberculosis medications were associated with RPT. In conclusion, higher TIMP-1 levels are responsible for the development of RPT and may be helpful for predicting RPT in pleural tuberculosis.

Keywords: matrix metalloproteinase; pleural fluid; pleural tuberculosis; residual pleural thickening; tissue inhibitors of metalloproteinase
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Introduction

Pleural tuberculosis is one of the most common forms of extrapulmonary tuberculosis. The immediate cause of the effusion is a delayed hypersensitivity response to mycobacterial antigens in the pleural space. Anti-tuberculosis medication may hasten the resolution of pleural effusion and reduce the incidence of residual pleural thickening (RPT). Nevertheless, RPT still occurs in most patients with pleural tuberculosis despite advances in the treatment of tuberculosis. The prevalence of RPT varies from 10% (Lee et al. 1988) to 78% (Lee et al. 2001) because of lack of a uniform concept for RPT. Several studies have tried to identify the predictive factors for the development of RPT in pleural tuberculosis; however, there were no definitive predictive factors so far.

The matrix metalloproteinases (MMPs) are potentially important mediators of inflammatory response and tissue destruction in tuberculosis. The MMPs are classified functionally according to their relative substrate specificity. For example, collagenases (MMP-1, MMP-8 and MMP-13) degrade type I collagen, whereas gelatinases (MMP-2 and MMP-9) degrade gelatin (denatured collagen) and type IV collagen, a major component of basement membranes. In humans, MMPs levels were higher in exudates compared to transudates and additionally increased in pleural tuberculosis compared to non-tuberculosis effusion (Eickelberg et al. 1997; Hoheisel et al. 2001; Vatansever et al. 2009; Hsieh et al. 2012). Furthermore, MMP-9 may be key in tissue remodeling and scaring that lead to long-term fibrosis (Woessner 1991; Kotyza et al. 2004). However, there was no report as to definitive relation between RPT and MMPs.

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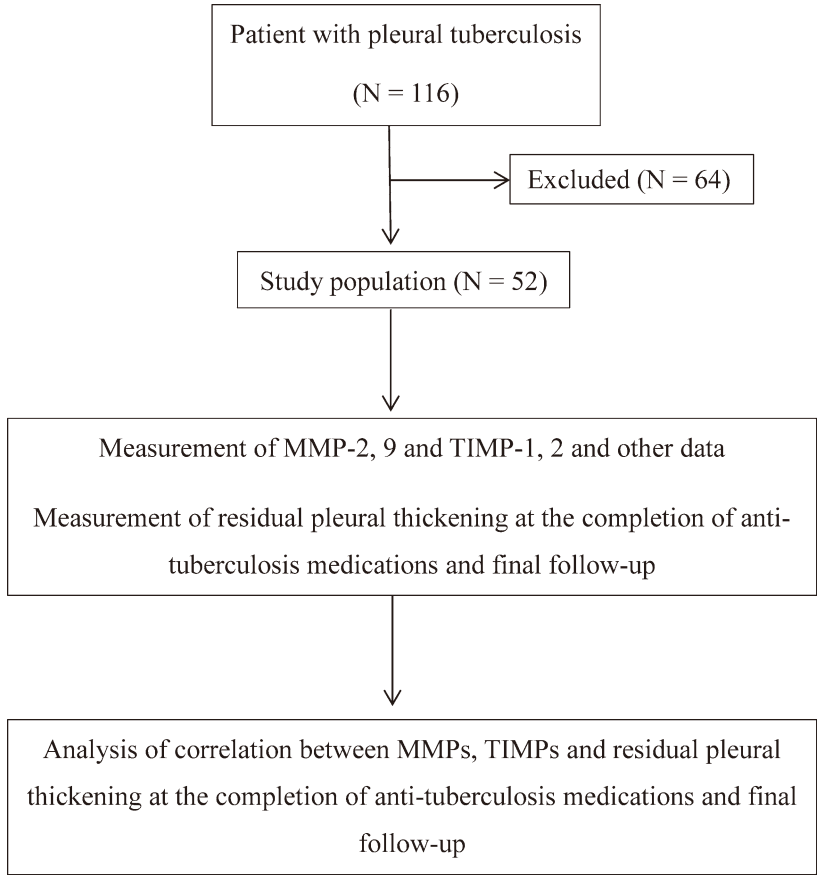

Fig. 1. Flow chart of study design.
MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase.

Therefore, the aim of this study was to examine MMPs and the tissue inhibitors of metalloproteinases (TIMPs) and to identify that these factors could be predictive of the occurrence of RPT.

Materials and Methods

At the pulmonary department of the Wonkwang University Hospital, 116 patients were diagnosed with pleural tuberculosis between January 2009 and December 2012. Of these, 92 patients completed anti-tuberculosis medications of more than six months. Of 92 patients, 52 patients had a chest radiograph three months after completion of treatment (Fig. 1). The pleural effusion was considered an exudate if it met the criteria described by Light et al. (1972). Pleural tuberculosis was diagnosed as follows: (1) a positive stain or culture of *Mycobacterium tuberculosis* from pleural fluid, (2) pleural biopsy collected in cases with caseous granulomas, after exclusion of other granulomatous disease, (3) lymphocyte-predominant pleural effusion and an adenosine deaminase (ADA) level > 45 IU/L (Ocaña et al. 1983) if there was a good therapeutic response to anti-tuberculosis medications. Except for patients with at least once before pleural disease or thoracentesis, thoracic surgery, after admission to the first thoracentesis that is collected analyzed.

Pleural effusion quantities at diagnosis of pleural tuberculosis were categorized into three groups; small (the costophrenic angle was obliterated but the hemi-diaphragm was not covered); medium (less than two-thirds of the space between the mediastinum and chest wall at the height of the hilum); and large (greater than medium). Between days 1 and 20 after completion of anti-tuberculosis medications, the RPT was measured in the lower lateral hemi-thorax on postero-anterior chest radiograph at the level of an imaginary line intersecting the diaphragmatic dome. The RPT was also measured on chest radiograph at the final follow-up. Two levels of pleural thickness were used in order to classify patients by the presence or absence of significant RPT. First, RPT was defined as a pleural thickness > 2 mm, a value that is generally considered as radiologically abnormal (Light 1990). Analyses were repeated using RPT defined as a pleural thickness ≥ 10 mm because this level of thickness has been shown to be a more specific marker for the development of restrictive functional sequela in patients with tuberculous effusions, compared to 2 mm. However, of 52 patients, 6 patients (11.5%) had an RPT ≥ 10 mm at the completion of anti-tuberculosis medication and 4 patients (7.6%) had an RPT ≥ 10 mm at the final follow-up. For statistical analysis, 2 mm of RPT was used as a cutoff value to distinguish RPT with RPT (+) or RPT (−).

We obtained information regarding the patients' age, gender, and laboratory data at diagnosis of pleural tuberculosis. Patients with radiographic evidence of pleural effusion underwent diagnostic thoracentesis with pleural biopsy if clinically indicated. As part of the routine diagnostic work-up, the following tests were performed on pleural fluid: biochemistry (total protein, lactate dehydrogenase, and ADA); cytological examination; differential leukocyte count; and microbial culture, including culture for *Mycobacterium tuberculosis*.

Pleural effusions were immediately centrifuged at 1,500 rpm for 7 min at 4°C and the supernatant was stored at −70°C pending analysis of MMPs and TIMPs. Metalloproteinases (MMP-2 and MMP-9) and tissue inhibitors of metalloproteinases (TIMP-1 and TIMP-2) were estimated in pleural fluids by a commercially available Multiplex ELISA kit (R&D system).

Qualitative data were expressed as numbers (percentages), and quantitative data as the median (range). The chi-square test was used to compare the distribution of patients' characteristics, and an independent t-test was used to analyze continuous variables. Univariate analysis first assessed the association between each variables and RPT. Variables selected by univariate analysis ($p < 0.05$) and those considered as clinically relevant were entered in a logistic regression model. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 21.0 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

The study included 52 patients with pleural tuberculosis with a median age of 45.25 ± 22.0 years selected according to the criteria described above. The average period of anti-tuberculosis medication and the follow-up period after completion of treatment were 6.7 months and 7.6 months, respectively. Patients in the study were classified into two groups according to presence of RPT at the completion of anti-tuberculosis medication and at the point of the final follow-up.

Classification of patients according to RPT

Twenty-eight patients (54%) had an RPT > 2 mm at the completion of anti-tuberculosis medication and twenty-five patients (48%) had an RPT > 2 mm at the final follow-up. The characteristics of the study subjects according to their classification group are presented in Tables 1, 2 and 3. There were no age or gender differences between the two groups. Therapeutic thoracentesis was performed in most of the patients. The period of anti-tuberculosis medication and the pleural effusion volumes did not show significant differences between the two groups. The MMP and TIMP levels in the pleural fluid did not differ between patients with small, medium, or large effusion (data not shown).

The laboratory characteristics of serum and pleural effusion from these patients are shown in Tables 2 and 4. As shown in Tables 2 and 4, patients with RPT > 2 mm at the completion of anti-tuberculosis medications had a higher pleural fluid pH ($p = 0.051$), whereas patients with RPT > 2 mm at the final follow-up had higher pleural fluid cholesterol levels ($p = 0.023$). There were no significant differences in pleural fluid lactate dehydrogenase and glucose levels between the two groups.

Correlations of MMP and TIMP levels

To identify the relationship with RPT, we measured MMP-2, MMP-9, TIMP-1, and TIMP-2 in pleural fluid (Tables 5 and 6). There was no correlation between pleural fluid MMP levels and RPT, but differences were noted in TIMP-1 and TIMP-2. The patients with RPT > 2 mm at the completion of anti-tuberculosis medications had higher pleural fluid TIMP-1 levels ($p = 0.00$) and lower TIMP-2 levels ($p = 0.005$). However, patients with RPT > 2 mm at the final follow-up differed only in pleural fluid TIMP-1 levels ($p = 0.001$).

To determine the predictors of RPT, MMP-2, MMP-9, TIMP-1, and TIMP-2 were entered in a logistic regression model (Table 7). The amounts of pleural effusion and ADA levels were included in the regression analysis. The TIMP-1 levels at the completion of anti-tuberculosis medications predicted RPT (OR, 1.5; 95% CI, 0.94-1.86; p value < 0.05).

Table 1. Clinical and radiographic characteristics of patients with pleural tuberculosis according to the residual pleural thickening at the completion of anti-tuberculosis medication (N = 52).

Characteristics	RPT < 2 mm (N = 24)	RPT > 2 mm (N = 28)	*P*-value
Age (years)	47.0 ± 23.0	43.8 ± 21.6	NS
Gender (male/female)	18/6	20/8	NS
Diagnostic methods			NS
Pleural fluid laboratory test	16 (67%)	20 (72%)	
AFB stain & culture	3 (12%)	2 (7%)	
Biopsy	5 (21%)	6 (21%)	
Therapeutic thoracentesis	22 (92%)	23 (82%)	NS
Anti-tuberculosis medication (months)	6.62	6.89	NS
Amount of initial effusion			NS
Small amount	6 (25%)	6 (22%)	
Medium amount	16 (67%)	18 (64%)	
Large amount	2 (8%)	4 (14%)	

RPT, residual pleural thickening; AFB, acid fast bacillus.

Table 2. Laboratory characteristic of the patients with pleural tuberculosis according to the residual pleural thickening at the completion of anti-tuberculosis medication (N = 52).

Characteristics	RPT < 2 mm (N = 24)	RPT > 2 mm (N = 28)	*P*-value
Hemoglobin (g/dL)	12.45 ± 2.25	12.30 ± 2.16	NS
White blood cell (/μl)	7,160.00 ± 1,780.04	7,435.62 ± 2,307.05	NS
Neutrophil (/μl)	5,214.54 ± 1,563.56	5,163.13 ± 2,204.99	NS
Platelet (/μl)	319,181.81 ± 57,448.79	354,062.50 ± 84,987.43	NS
Protein (g/dL)	7.02 ± 0.81	6.94 ± 0.76	NS
LDH (IU/L)	379.08 ± 110.92	361.81 ± 63.70	NS
CRP (mg/L)	96.55 ± 51.47	95.13 ± 65.18	NS
Pleural fluid			
LDH (IU/L)	981.92 ± 631.51	958.19 ± 520.93	NS
ADA (IU/L)t	51.47 ± 18.80	46.10 ± 16.14	NS
Glucose (mg/dL)	89.50 ± 36.72	86.75 ± 28.72	NS
Cholesterol (mg/dL)	102.50 ± 17.93	86.33 ± 23.67	NS
Amylase (IU/L)	41.27 ± 10.37	39.0 ± 11.24	NS
CRP (mg/L)	41.48 ± 25.63	61.30 ± 37.52	NS
pH	7.41 ± 0.42	7.06 ± 0.48	0.051
White blood cell (/μl)	2,598.33 ± 1,569.78	2,941.50 ± 2,233.95	NS
Neutrophil (%)	14.41 ± 7.47	14.68 ± 7.63	NS
Lymphocyte (%)	85.58 ± 7.47	85.31 ± 7.63	NS

PRT, residual pleural thickening; LDH, lactate dehydrogenase; CRP, C-reactive protein; ADA, adenosine deaminase.

Table 3. Clinical and radiographic characteristics of patients with pleural tuberculosis according to the residual pleural thickening at the point of the final follow-up (N = 52).

Characteristics	RPT < 2 mm (N = 27)	RPT > 2 mm (N = 25)	*P*-value
Age (years)	47.2 ± 21.5	44.6 ± 23.1	NS
Gender (male/female)	19/8	19/6	NS
Diagnostic methods			NS
Pleural fluid laboratory test	19 (70%)	17 (68%)	
AFB stain & culture	3 (11%)	2 (8%)	
Biopsy	5 (19%)	6 (24%)	
Therapeutic thoracentesis	24 (91%)	21 (84%)	NS
Anti-tuberculosis medication (months)	6.72	6.81	NS
Amount of initial effusion			NS
Small amount	8 (30%)	4 (16%)	
Medium amount	18 (67%)	16 (64%)	
Large amount	1 (3%)	5 (20%)	

RPT, residual pleural thickening; AFB, acid fast bacillus.

Discussion

The aim of this study was to examine the roles of MMPs and TIMPs for RPT and to identify the factors that are predictive of the occurrence of RPT. MMPs, together with TIMPs, have been suggested toplay a role in the pathogenesis of pleural effusions, as in many other disease processes (Nguyen et al. 1994; Eickelberg et al. 1997; Hoheisel et al. 2001; Kotyza et al. 2004). The MMPs are potentially important mediators of inflammatory responses and tissue destruction in tuberculosis. The MMPs form a group of zinc-dependent proteases capable of degrading all components of the extracellular matrix (Visse and Nagase 2003). MMP secretion is tightly regulated by several mechanisms, including transcriptional regulation, compartmentalization and secretion of pro-forms that require activation.

Pleural tuberculosis is characterized by high monocyte and lymphocyte counts. MMP-9 is quantitatively the most important MMP secreted by monocytes and macrophages (Chang et al. 1996; Lee et al. 1999; Nagase and Woessner

Table 4. Laboratory characteristic of the patients with pleural tuberculosis according to the residual pleural thickening at the point of the final follow-up (N = 52).

Characteristics	RPT < 2 mm (N = 27)	RPT > 2 mm (N = 25)	*P*-value
Hemoglobin (g/dL)	13.21 ± 1.80	12.01 ± 2.21	NS
White blood cell (/μl)	8,116.00 ± 1,695.08	6,962.50 ± 2,208.39	NS
Neutrophil (/μl)	5,956.00 ± 1,651.08	4,778.75 ± 2,045.31	NS
Platelet (/μl)	344,000.00 ± 54,088.40	341,562.50 ± 88,689.69	NS
Protein (g/dL)	7.20 ± 0.44	6.75 ± 0.92	NS
LDH (IU/L)	355.72 ± 82.32	379.25 ± 91.91	NS
CRP (mg/L)	112.41 ± 63.95	84.08 ± 56.15	NS
Pleural fluid			
LDH (IU/L)	1,001.72 ± 656.15	949.81 ± 524.92	NS
ADA (IU/L)t	50.43 ± 18.33	47.79 ± 17.15	NS
Glucose (mg/dL)	82.81 ± 39.91	91.12 ± 26.89	NS
Cholesterol (mg/dL)	115.33 ± 23.86	83.14 ± 13.39	0.023
Amylase (IU/L)	38.90 ± 11.42	41.31 ± 10.48	NS
CRP (mg/L)	54.46 ± 33.33	56.71 ± 38.02	NS
pH	7.22 ± 0.41	7.18 ± 0.54	NS
White blood cell (/μl)	2,714.54 ± 1,958.81	2,904.00 ± 2,055.48	NS
Neutrophil (%)	15.43 ± 6.87	14.25 ± 8.10	NS
Lymphocyte (%)	84.54 ± 6.87	85.75 ± 8.10	NS

PRT, residual pleural thickening; LDH, lactate dehydrogenase; CRP, C-reactive protein; ADA, adenosine deaminase.

Table 5. Levels of metalloproteinase and tissue inhibitors of metalloproteinase according to the RPT at the completion of anti-tuberculosis medication (N = 52).

	RPT < 2 mm (N = 24)	RPT > 2 mm (N = 28)	*P*-value
MMP-2 (ng/mL)	116.34 ± 64.46	121.61 ± 29.22	0.76
MMP-9 (ng/mL)	5.58 ± 8.77	6.50 ± 5.59	0.74
TIMP-1 (ng/mL)	37.13 ± 12.06	95.98 ± 41.83	0.00
TIMP-2 (ng/mL)	65.77 ± 21.73	40.78 ± 22.89	0.005

RPT, residual pleural thickening; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase.

Table 6. Levels of metalloproteinase and tissue inhibitors of metalloproteinase according to the RPT at the point of the final follow-up (N = 52).

	RPT < 2 mm (N = 27)	RPT > 2 mm (N = 25)	*P*-value
MMP-2 (ng/mL)	108.79 ± 60.31	125.78 ± 36.24	0.34
MMP-9 (ng/mL)	4.69 ± 6.15	6.88 ± 7.69	0.41
TIMP-1 (ng/mL)	40.27 ± 19.11	92.09 ± 44.89	0.001
TIMP-2 (ng/mL)	59.85 ± 22.87	44.43 ± 25.91	0.09

RPT, residual pleural thickening; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase.

1999; Kyung et al. 2005; Park et al. 2005), and may also be secreted by activated lymphocytes (St-Pierre et al. 2003). MMP-9 secretion is of particular interest in pleural disease, since, as a gelatinase, it degrades type IV collagen and may contribute to loss of integrity of the basement membrane around blood vessels and under the mesothelial layer, leading to fluid accumulation in the pleural space (Sheen et al. 2009). MMPs are interesting candidate markers differentiating pleural tuberculosis from non-tuberculosis effusion. However, there was no report as to definitive relation between RPT and MMP. In this study, RPT in pleural tuberculosis was not related to the MMP-2 and MMP-9 lev-

Table 7. Variables predictive of the RPT at the completion of anti-tuberculosis medication on multivariate analysis.

	Odds ratio	95% confidence interval	*P*-value
MMP-2 (ng/mL)	1.0	0.9-1.1	0.65
MMP-9 (ng/mL)	0.8	0.4-1.5	0.40
TIMP-1 (ng/mL)	1.5	0.9-1.8	0.00
TIMP-2 (ng/mL)	0.9	0.7-1.1	0.67
ADA	0.9	0.8-1.0	0.12
Amount of pleural effusion	1.8	0.0-13.0	0.48

RPT, residual pleural thickening; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase; ADA, adenosine deaminase.

els in the pleural effusion.

MMPs are a class of proteolytic enzymes responsible for degradation of extracellular matrix components and are specifically inhibited by the TIMPs, TIMP-1 and TIMP-2 (Woessner 1991). However, in this study reciprocal to the high level in TIMP-1 there was low level of TIMP-2 in RPT. The mechanism underlying this result is unclear and is of great interest. TIMP-2 appears to be capable of inhibiting the growth of basic fibroblastic growth factor-induced human microvascular endothelial cells and independent biologic properties regardless of inhibiting MMPs (Moses et al. 1990; Murphy et al. 1993). There are possible hypotheses: independent biologic activation other than inhibitory effect of MMPs and MMP-2 activation, for small amount of TIMP-2 is an essential component for the activation of progelatinase A/proMMP-2, an unexplained mechanism about relation between MMPs and TIMPs. These mechanisms need further study.

The present study has several limitations, including the retrospective nature of the study. There is a possibility that selection bias influenced the significance of our findings. Our study is from a single institution and has a small sample size, which limits the extension of our findings to the general population. Further research using a larger sample size, prospective methodology and other estimation method, such as zymography is needed to confirm our results. MMPs were affected by the TMPs as well as other inflammatory mediators. Further research against these factors may be instrumental in elucidating a mechanism of RPT.

In summary, cases of pleural tuberculosis with high levels of TIMP-1 in pleural effusions have a high possibility of RPT after anti-tuberculosis medications.

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Conflict of Interest

The authors declare no conflict of interest.

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