

EXPRESSION OF MMP-2 AND 4 IN SPINAL NODULAR DISCS AND THEIR CLINICAL RELATIONSHIP

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Objective: To investigate the differences in the expression of matrix metalloproteinase-2 and -14 (MMP-2 and -14) in spinal tuberculosis intervertebral disc tissues, and their clinical relationship.

Methods: 42 patients in our hospital with spinal tuberculosis were selected for inclusion in the study between January 2014 and January 2015, as well as 42 patients with fractures. The mRNA expression levels of MMP-2 and -14 in their tissues were measured using real-time fluorescence quantitative PCR, and the relationship between the mRNA expression levels of MMP-2 and -14, along with the clinicopathological characteristics of the patients with spinal tuberculosis, were analyzed. The correlation between the mRNA expression levels of MMP-2 and -14 in the spinal tuberculosis intervertebral disc tissues was further analyzed. According to the median of the relative expression of MMP-2 and -14 in the spinal combined intervertebral disc tissues, all the spinal tuberculosis intervertebral disc tissues were divided into a relatively-high expression group and the relatively-low expression group. The relationship between the mRNA expression levels of MMP-2 and -14 was analyzed, along with the prognosis of patients.

Results: The mRNA expression levels of MMP-2 and -14 in the spinal tuberculosis intervertebral disc tissues were significantly higher than those in the normal intervertebral disc tissues ($P < 0.05$). The expression level of MMP-2 mRNA was correlated with the age and smoking status of patients with spinal tuberculosis ($P < 0.05$). The expression level of MMP-14 mRNA, meanwhile, was not associated with the age or smoking status of patients with spinal tuberculosis, nor was it associated with gender, work intensity, diseased intervertebral segments, or pain level ($P > 0.05$). The median postoperative survival time of patients with a high expression of MMP-2 mRNA was significantly reduced, at 18.71 months, compared with 41.21 months for patients with a low expression of MMP-2 mRNA ($P < 0.05$). The median survival time for patients with a high expression of MMP-14 mRNA was significantly lower, at 18.95 months, than the 42.00 months for patients with a low expression of MMP-2 mRNA ($P < 0.05$). The expression level of MMP-2 can be utilized as an independent risk factor that affects the prognosis of patients with spinal tuberculosis. After Spearman's correlation analysis, the expressions of MMP-2 and -14, in the intervertebral disc tissues of patients with spinal tuberculosis, showed a significant positive correlation ($r = 0.439$, $P < 0.01$).

Conclusion: The expressions of MMP-2 and -14 in patients with spinal tuberculosis are significantly increased, and the expressions of MMP-2 and -14 in the intervertebral disc tissues of patients with spinal tuberculosis are significantly positively correlated with each other. In addition, MMP-2 can independently indicate the prognosis of patients with spinal tuberculosis; measurement of its expression should play an important role in the treatment of patients with spinal tuberculosis.

Keywords: MMP-2, MMP-14, spinal tuberculosis, intervertebral disc tissue.

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Introduction

Tuberculosis is one of the most dangerous diseases in the world today. According to survey data, China is one of the most severely affected countries; the number of patients with spinal tuberculosis in China is the second greatest in the world⁽¹⁻²⁾. Extrapulmonary tuberculosis mostly involves other tissues and organs, especially the

spine, accounting for more than half of the cases of articular tuberculosis⁽³⁾. Spinal tuberculosis is caused by the invasion of tuberculous bacteria in the spine, resulting in damage to the vertebral body bone and intervertebral disc, which leads to a tuberculous abscess. The early inflammation usually manifests as exudation. During this period, the immune ability of patients decreases, the number of tuberculous bacteria is high, and the toxin is extremely strong;

as a result, most patients develop serous fibrinous inflammation⁽⁴⁻⁵⁾. In the later stage, the disease mainly manifests as hyperplasia, with the patients' immune ability increasing and the number of Mycobacterium tuberculosis decreasing; the patients present the characteristic tuberculous nodules⁽⁶⁾. Disc mutation is an important feature of this disease, which can lead to many complications and even a high disability rate due to lower limb paralysis⁽⁷⁾. Therefore, it is important to explore a mechanism for the destruction of the spinal tuberculosis intervertebral disc by tuberculosis bacillus, to prevent and treat this disease. Matrix metalloproteinase-2 (MMP-2) and -14 are important members of the matrix metalloproteinase family. It has been reported that MMP-14 can activate MMP-2, but the relationship between the two and spinal tuberculosis has not been clarified⁽⁸⁻⁹⁾. Therefore, this study will examine the expression of MMP-2 and -14 in spinal tuberculosis intervertebral disc tissue, and their relationship.

Materials and methods

General information

42 patients whose spinal tuberculosis intervertebral disc tissues were removed in our hospital between January 2014 and January 2015 were selected for the study.

The inclusion criteria were as follows:

- All patients met the diagnostic criteria for spinal tuberculosis after imaging and biochemical examinations;
- All patients' tissue was removed by surgery in our hospital;
- All patients signed their informed consent;
- The inclusion of all patients was approved by the ethics committee of our hospital.

The exclusion criteria, meanwhile, were as follows:

- Patients with other autoimmune diseases;
- Patients with tumors and immunodeficiency diseases.

At the same time, 42 cases of patients with fractures were selected. After the tissue was removed, normal saline could be used to wash the tissue, before it was placed in 10% formaldehyde for fixation after 5min. After 24h, the tissue was embedded in paraffin with a thickness of 4 μ m.

Sourcing the main reagents and instruments

Of the main reagents employed in this study, the mouse anti-MMP-2 and -14 monoclonal antibodies,

were purchased from Shanghai Yaji Biotechnology Co., Ltd. The DAB chromogenic agent was purchased from Beijing Fobo Biotechnology Co., Ltd. The reverse transcription kit was purchased from Harbin Xinhai Gene Testing Co., Ltd. The PBS powder was purchased from Sangon Bioengineering Co., Ltd.

Of the main instruments employed in this study, the multi-direction microscope was purchased from Shanghai Alighting Biochemical Technology Co., Ltd. The paraffin embedding machine was purchased from Dongguan Spectrum Standard Experimental Equipment Technology Co., Ltd. The high-speed centrifuge was purchased from Sichuan Shuke Instrument Co., Ltd. The medical microwave oven was purchased from Henan Zeyuan Medical Equipment Sales Co., Ltd. The slicer was purchased from Suzhou Ruinod Biotechnology Co., Ltd. Finally, the pathological tissue rinsing machine was purchased from Beijing Jiayuan Xingye Technology Co., Ltd.

Methods

- The mRNA levels of MMP-2 and -14 in the cylindrical tuberculous intervertebral disc tissues and the normal intervertebral disc tissues were measured using real-time fluorescent quantitative PCR. A total amount of RNA was extracted from the spinal tuberculous disc tissues and the normal disc tissues; this was then cut into small pieces and ground, while a little liquid nitrogen was continuously added. After that, RNAiso was added to mix the solution evenly, before trichloromethane was added. The solution was placed in a centrifuge for centrifugation. The supernatant was taken and left to stand for 15min. Centrifugation was continued, then the supernatant was discarded and air-dried naturally. The cDNA was obtained using a reverse transcription kit. According to the median of the relative expression of MMP-2 and -14 in the spinal combined intervertebral disc tissues, the tissues were divided into a relatively-high expression group and a relatively-low expression group.

- The study followed up on the 42 patients with spinal tuberculosis by telephone every 6 months.

- Spearman's non-parameter was adopted to analyze the correlation between the expressions of MMP-2 and -14 in the intervertebral disc tissues of patients with spinal tuberculosis.

Statistical methods

($\bar{x} \pm s$) was used to investigate the level of MMP-2 and -14 mRNA expression in each group, and t tests were carried out to compare the two groups. The correlation between the expression of MMP-2 and

-14 mRNA and the clinicopathological parameters of the patients with spinal tuberculosis was determined using a chi-squared test. The Kaplan-Meier method was utilized to analyze the survival rate of patients with spinal tuberculosis, and a log-rank test was employed to compare the survival rates of the two groups. A COX proportional risk regression model was adopted to analyze the independent prognostic factors for patients with spinal tuberculosis.

A Spearman's test analyzed the correlation between MMP-2 and -14 in the intervertebral disc tissues of patients with spinal tuberculosis. All data were analyzed using SPSS 23.0 software, with $P < 0.05$ considered to be statistically significant.

Results

The expression of MMP-2 and -14 in spinal tuberculosis intervertebral disc and normal intervertebral disc tissues

The mRNA expression levels of MMP-2 and -14 in spinal tuberculosis intervertebral disc tissues were significantly higher than those in normal intervertebral disc tissues ($P < 0.05$). See Table 1.

Group	n	MMP-2 mRNA	MMP-14 mRNA
Normal disc tissue	42	1.01±0.11	0.99±0.12
Spinal tuberculosis disc tissue	42	2.58±0.19	1.42±0.21
<i>t</i>		46.345	11.522
<i>P</i>		<0.001	<0.001

Table 1: The expression of MMP-2 and -14 in spinal tuberculosis intervertebral disc and normal intervertebral disc tissues.

The relationship between the mRNA expression levels of MMP-2 and -14 and the clinicopathological data for patients with spinal tuberculosis

The expression level of MMP-2 mRNA correlated with the age and smoking status of the patients with spinal tuberculosis ($P < 0.05$). MMP-14 was not associated with the age or smoking status of the patients with spinal tuberculosis, nor was it associated with their gender, work intensity, diseased intervertebral segments, or pain levels ($P > 0.05$). These findings are shown in Table 2.

The correlation between the expression of MMP-2 and -14 in the intervertebral disc tissues of patients with spinal tuberculosis

After conducting a Spearman's correlation analysis, the expressions of MMP-2 and -14 in the

intervertebral disc tissues of patients with spinal tuberculosis were found to exhibit a significant positive correlation ($r = 0.439, P < 0.01$).

Clinicopathological data	n	Percentage (%)	MMP-2 mRNA	<i>P</i>	MMP-14 mRNA	<i>P</i>
<i>Gender</i>				0.564		0.547
Man	17	40.48	2.70±0.24		1.46±0.24	
Woman	25	59.52	2.73±0.28		1.43±0.27	
<i>Age</i>				0.018		0.244
<45	19	45.24	2.68±0.24		1.50±0.26	
≥45	23	54.76	2.79±0.24		1.56±0.27	
<i>Work intensity</i>				0.306		0.693
Heavy	31	73.81	2.80±0.28		1.52±0.24	
Easy	11	26.19	2.74±0.11		1.54±0.25	
<i>Smoking status</i>				0.005		0.575
No	16	35.71	2.63±0.07		1.50±0.21	
Yes	26	59.52	2.87±0.22		1.59±0.27	
<i>Diseased intervertebral segments</i>				0.633		0.748
C2-T1	4	9.52	2.72±0.21		1.50±0.27	
T2-L1	22	52	2.78±0.26		1.56±0.25	
L1-S1	16	52.61			1.54±0.23	
<i>Pain level</i>				0.622		0.503
0-4	5	0.48	2.74±0.23		1.48±0.22	
5-7	17	40.48	2.76±0.26		1.52±0.24	
8-10	20	47.62	2.80±0.25		1.56±0.26	

Table 2: The relationship between the mRNA expression levels of MMP-2 and -14 and the clinicopathological data for patients with spinal tuberculosis.

The relationship between the mRNA expression levels of MMP-2 and -14 and the prognosis of the patients with spinal tuberculosis

The median postoperative survival time for patients with high expressions of the MMP-2 mRNA was significantly reduced, at 18.71 months, compared with 41.21 months for patients with low expressions of the MMP-2 mRNA ($P < 0.05$). The median survival time for patients with high expressions of the MMP-14 mRNA was significantly lower, at 18.95 months, compared with 42.00 months for patients with low expressions of the MMP-14 mRNA ($P < 0.05$). See Figures 1 and 2.

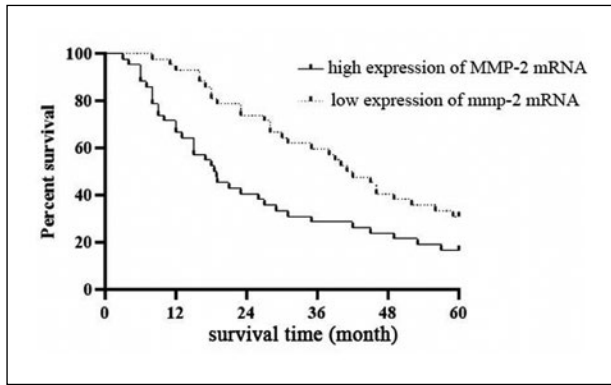


Figure 1: The relationship between the MMP-2 mRNA expression level and the prognosis of patients with spinal tuberculosis.

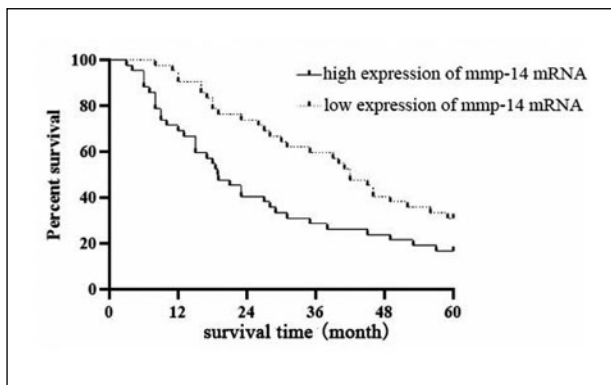


Figure 2: The relationship between the MMP-14 mRNA expression level and the prognosis of patients with spinal tuberculosis.

A COX proportional risk regression model analysis of patients with spinal tuberculosis

The expression level of MMP-2 can be utilized as an independent risk factor that affects the prognosis of patients with spinal tuberculosis.

Clinicopathological data	Single-factor analysis	P	Multivariate analysis	P
	95%CI		95%CI	
Gender	2.629 (1.296~5.348)	0.019	1.838 (0.776~3.375)	0.186
Age	1.104 (0.641~1.905)	0.763	1.554 (0.839~2.889)	0.183
Work intensity	0.816 (0.447~1.496)	0.498	1.374 (0.803~2.357)	0.275
Smoking status	1.685 (1.025~2.774)	0.039	0.734 (0.388~1.396)	0.339
Diseased intervertebral segments	1.082 (0.629~1.883)	0.741	1.624 (0.909~5.032)	0.098
Pain level	0.799 (0.425~1.474)	0.476	1.720 (0.939~3.085)	0.089
MMP-2 expression	2.529 (1.301~3.620)	0.004	1.806 (1.053~3.103)	0.046
MMP-14 expression	1.663 (1.003~2.752)	0.055	1.816 (0.754~4.353)	0.253

Table 3: A COX proportional risk regression model analysis of patients with spinal tuberculosis.

Discussion

Currently, tuberculosis of the bones and joints is more commonly occurring than that of spinal tuberculosis in clinical practice. However, due to the rapid increase in the number of multi-drug-resistant bacteria, the incidence of spinal tuberculosis is on the rise and seriously endangers people's quality of life and their health⁽¹⁰⁾. Therefore, there is an urgent need for us to explore methods for the treatment of spinal tuberculosis that reduce the occurrence of complications. After it is activated, MMP-2 functions to degrade components of the extracellular matrix. Some scholars have detected expressions of MMP-2 in the Type I collagen and the periosteum protein of the nucleus pulporeal tissue of the intervertebral disc, which has been surgically removed, finding that the expressions of all three were significantly increased⁽¹¹⁾. Some studies have utilized immunohistochemistry to detect MMP-2 and -3 in herniated disc tissues and normal disc tissues, finding that the expressions of MMP-2 and -3 in the herniated disc tissues are significantly higher than those in the normal disc tissues⁽¹²⁾.

In terms of the study on tuberculosis, some scholars have found that the expression of MMP-2 in the lung tissues of model mice has been significantly higher than those of normal mice; they have thereby established a mouse model for tuberculosis infection⁽¹³⁾. Other scholars have shown that the expression of MMP-2 in the serum and cerebrospinal fluid of patients with spinal tuberculosis is significantly increased⁽¹⁴⁾. All of the above experiments have proven that MMP-2 expression can be upregulated by the tuberculosis bacteria, though the mechanism for its destruction has not been elucidated. MMP-14 is a membrane collagenase that can degrade many extracellular matrix macromolecules; it participates in a variety of physiological and pathological processes in the human body. Currently, there are few studies on MMP-14 and spinal tuberculosis. It has been reported that MMP-14 plays an important role in the spinal union, through teaching collagen degradation and monocyte migration⁽¹⁵⁾. Some scholars have found the expression level of MMP-14 mRNA in the sputum of pulmonary tuberculosis patients to be around five times higher than a normal control group, while the expression level of this gene in the tuberculous granulation tissue is also significantly higher. However, the use of this gene inhibitor could significantly reduce the capacity of the tuberculosis

bacilli for collagen degradation and monocyte migration. Other scholars found an expression of MMP-14 in the tissues of model rabbits that is significantly higher than that of the rabbits in the normal control group, thereby establishing a hollow rabbit model.

This study collected 42 samples of spinal tuberculosis intervertebral disc tissue and 42 samples of normal intervertebral disc tissue. The PCR method was adopted to test the groups' MMP-2 and -14 mRNA expression levels, analysing the relationships between both and clinical-pathological features. The results demonstrate that the tuberculosis intervertebral disc tissue's MMP-2 and -14 mRNA expression levels are significantly higher than those of the normal intervertebral disc tissue ($P < 0.05$). This finding indicates that MMP-2 and -14 are involved in the process of spinal tuberculosis intervertebral disc destruction and play an important role. The mRNA expression level of MMP-2 is related to the age and smoking status of the patients with spinal tuberculosis ($P < 0.05$). MMP-14, meanwhile, is not associated with the age or smoking status of the patients with spinal tuberculosis, nor is it associated with their gender, work intensity, diseased intervertebral segments, or pain levels ($P > 0.05$).

The expression levels of MMP-2 and -14 could be affected by smoking status and age. The researchers then followed up with the 42 patients with spinal tuberculosis for 5 years and found that the median survival time for patients with high expressions of the MMP-2 mRNA was significantly lower, at 18.71 months after surgery, than that of patients with low expressions of the MMP-2 mRNA, at 41.21 months ($P < 0.05$). The median survival time of patients with high expressions of the MMP-14 mRNA was significantly lower, at 18.95 months, than that of patients with low expressions of the MMP-14 mRNA, at 42.00 months ($P < 0.05$). Using a COX proportional risk regression model, it was found that the expression level of MMP-2 represented an independent risk factor that affected the prognosis of patients with spinal tuberculosis. Finally, a Spearman's correlation analysis tested the correlation between MMP-2 and -14; the results demonstrated that the expressions of MMP-2 and -14 in the intervertebral disc tissues of patients with spinal tuberculosis were positively correlated with each other ($r = 0.439$, $P < 0.01$).

To conclude, the expressions of MMP-2 and -14 in patients with spinal tuberculosis are significantly

increased, as well as significantly positively correlated. In addition, MMP-2 can independently indicate the prognosis of patients with spinal tuberculosis; measurement of its expression should play an important role in the treatment of patients with spinal tuberculosis.

References

- 1) Dolla CK, Dhanraj B, Malaisamy M, Priyadarshini CP, Hissar SS, et al. Burden of pulmonary tuberculosis in modern prison: A cross sectional prevalence survey from south India. *Indian J Tuberc* 2019; 66: 189-192.
- 2) Cicchese JM, Dartois V, Kirschner DE, Linderman JJ. Both pharmacokinetic variability and granuloma heterogeneity impact the ability of the first-line antibiotics to sterilize tuberculosis granulomas. *Front Pharmacol* 2020; 11: 333.
- 3) Zhang J, Chen C, Yang J. Effectiveness of vitamin D supplementation on the outcome of pulmonary tuberculosis treatment in adults: a meta-analysis of randomized controlled trials. *Chin Med J (Engl)* 2019; 132: 2950-2959.
- 4) Saraceni V, Benzaken AS, Pereira GFM, Andrade KB, Oliveira PB, et al. Tuberculosis burden on AIDS in Brazil: A study using linked databases. *PLoS One* 2018; 13: 207859.
- 5) Ma AJ, Wang SF, Fan JL. Genetic diversity and drug susceptibility of mycobacterium tuberculosis isolates in a remote mountain area of China. *Biomed Environ Sci* 2018; 31: 351-362.
- 6) Hossen MJ, Yang WS, Kim D, Aravinthan A, Kim JH, et al. Thymoquinone: An IRAK1 inhibitor with in vivo and in vitro anti-inflammatory activities. *Sci Rep* 2017; 7: 42995.
- 7) Manyazewal T, Woldeamanuel Y, Holland DP, Fekadu A, Blumberg HM, et al. Electronic pillbox-enabled self-administered therapy versus standard directly observed therapy for tuberculosis medication adherence and treatment outcomes in Ethiopia (SELFTB): Protocol for a multicenter randomized controlled trial. *Trials* 2020; 21: 383.
- 8) Zhou YT, Qiu JJ, Wang Y, Liu PC, Lv Q, et al. Sperm protein antigen 17 expression correlates with lymph node metastasis and worse overall survival in patients with breast cancer. *Front Oncol* 2019; 9: 710.
- 9) Zandberga E, Zayakin P, Abols A, Pūpola D, Trapencieris P, et al. Depletion of carbonic anhydrase IX abrogates hypoxia-induced overexpression of stanniocalcin-1 in triple negative breast cancer cells. *Cancer Biol Ther* 2017; 18: 596-605.
- 10) Mukerji R, Turan JM. Exploring manifestations of TB-related stigma experienced by women in Kolkata, India. *Ann Glob Health* 2018; 84: 727-735.

- 11) Zhang W, Chen Q, Lei C. lncRNA MIAT promotes cell invasion and migration in esophageal cancer. *Exp Ther Med* 2020; 19: 3267-3274.
- 12) Lewinska A, Sodagam L, Bloniarz D, Siems K, Wnuk M, et al. Plant-derived molecules α -boswellic acid acetate, praeruptorin-A, and salvianolic acid-B have age-related differential effects in young and senescent human fibroblasts in vitro. *Molecules* 2019; 25: 141.
- 13) Yao X, Jiang W, Yu DH, Yan ZW. Luteolin inhibits proliferation and induces apoptosis of human melanoma cells in vivo and in vitro by suppressing MMP-2 and MMP-9 through the PI3K/AKT pathway. *Food Funct* 2019; 10: 703-712.
- 14) Fryczkowski M, Bułdak RJ, Hejmo T, Kukla M, Żwirska-Korczala K. Circulating levels of omentin, leptin, VEGF, and HGF and their clinical relevance with PSA marker in prostate cancer. *Dis Markers* 2018; 2018: 1-9.
- 15) Shang A, Wang W, Gu C, Chen W, Lu W, et al. Long non-coding RNA CCAT1 promotes colorectal cancer progression by regulating miR-181a-5p expression. *Aging (Albany NY)* 2020; 12: 7.

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