

Migration inhibitory factor in spinal tuberculosis: -173G/C polymorphisms, and transcript and protein levels in a northern province of China

Jun Wang, PhD^a, Bin Sheng, MD^b, XiaoPeng Li, MD^a, JianMin Sun, MD^a, Lin Shi, MD^a, WenTao Wei, MD^c, GuoWei Wang, MD^d, XueJun Cao, PhD^{a,*}

Abstract

The aim of this study was to elucidate the possible association between migration inhibitory factor (MIF)-173G/C gene polymorphisms and transcript and plasma levels of MIF in spinal tuberculosis (TB) patients. Clinical data were collected from 254 spinal TB patients and 262 healthy controls participating in the study. The genotype of the MIF-173G/C gene was amplified by polymerase chain reaction and genotyped by DNA sequencing technology. The level of mRNA expression was determined by real-time polymerase chain reaction and MIF plasma levels were measured by a solid-phase enzyme-linked immunosorbent assay. The frequency of the C allele and GC+CC genotype in MIF-173G/C was over-represented in spinal TB patients. The mean MIF mRNA level in spinal TB patients and patients with the GG and GC+CC genotype were significantly lower than controls; however, our study also indicated that the MIF concentration in spinal TB patients and patients with the GG and GC+CC genotypes were significantly higher than controls. Spinal TB patients with the GG genotype had higher MIF plasma levels than patients with the GC+CC genotype. The C-reactive protein level and erythrocyte sedimentation rate was correlated with the MIF plasma level. In summary, the association between the MIF-173G/C genetic polymorphism, reduced transcript and increased plasma levels of MIF in spinal TB patients, and MIF may play an important role in the occurrence, development, and damage of spinal TB in the northern Province population of China.

Abbreviations: CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, MIF = migration inhibitory factor, TB = tuberculosis, VAS = visual analog scale score.

Keywords: migration inhibitory factor, mRNA, polymorphisms, protein, tuberculosis

1. Introduction

Tuberculosis (TB) is a common disease in developing countries, and spinal TB is 1 of the most serious forms of extra-pulmonary TB, with its severe disability and high morbidity rate.^[1]

Macrophage migration inhibitory factor (MIF) is a soluble protein secreted by the activation of T cells, which was first described by Bloom in 1966.^[2] MIF regulates the balance of Th₁

and Th₂ cells during the host inflammatory reaction and immune responses, inhibits macrophage migration, and promotes accumulation of macrophages in areas of inflammation, infiltration, proliferation, activation, and secretion of IL-1, IL-2, IL-6, IL-8, TNF- α , and IFN- γ .^[3] MIF has a close relationship with macrophages and TB. MIF also aggravates inflammatory injuries through the interaction with inflammatory factors; spinal TB is 1 type of inflammation.

Editor: Gunjan Arora.

Competing interests: This study was funded by (1) the Natural Science Foundation of Shandong Province. Project No.: ZR2019PH026. Subject name: the mechanism and clinical application of miR-34a chondrocyte aging mediated by sestrin2/ mTORC1 in developmental dysplasia of the hip joint. (2) the Medical and health science and technology development plan of Shandong Province. Project No.: 2018WS082. Subject name: The mechanism of miR-146a regulating chondrocytes through TGF- β 1/ Smads pathway in osteoarthritis.

Availability of data and materials The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Weifang and Liaocheng Peoples Hospital in China.

Consent for publication was not applicable.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

^aWeifang People's Hospital, Weifang, ^bLiaocheng People's Hospital, Liaocheng, ^cWeifang Pozi Hospital Center, ^dChangle People's Hospital, Shandong, China.

*Correspondence: XueJun Cao, Department of Joint Surgery, Weifang People's Hospital, Weifang, Shandong 261041, China (e-mail: abcd747098875@126.com).

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc.

This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial License 4.0 (CCBY-NC), where it is permissible to download, share, remix, transform, and buildup the work provided it is properly cited. The work cannot be used commercially without permission from the journal.

How to cite this article: Wang J, Sheng B, Li X, Sun J, Shi L, Wei W, Wang G, Cao X. Migration inhibitory factor in spinal tuberculosis: -173G/C polymorphisms, and transcript and protein levels in a northern province of China. *Medicine* 2020;99:30(e21331).

Received: 8 November 2019 / Received in final form: 4 June 2020 / Accepted: 15 June 2020

<http://dx.doi.org/10.1097/MD.00000000000021331>

Two types of functional polymorphisms have been identified in the MIF promoter [-173G/C and -794CATT (5–8)] microsatellite polymorphism.^[4] Several recent reports have indicated that MIF is associated with the destruction of intervertebral discs and 2 kinds of polymorphisms are correlated with TB,^[5,6] while there is no research data reporting the correlation with spinal TB. The aim of this work was designed to elucidate whether or not there is an association between the MIF-173G/C genetic polymorphism, transcript and serum levels of expression, and spinal TB in Shandong, a northern province in China.

2. Materials and methods

2.1. Study population

This study was approved by the Ethics Committee of Weifang and Liaocheng in China. This study included 254 patients living in Shandong province, who were diagnosed with spinal TB and underwent surgery in the Weifang People's Hospital and Liaocheng People's Hospital between January 1, 2015 and April 30, 2020. The diagnosis of spinal TB was made based on post-operative histopathologic sections, and the etiologic examination showed that pus-positive, acid-fast staining or positive results of pus isolation and culture of *Mycobacterium TB*. Other diseases, such as acquired immune deficiency syndrome (AIDS), tumors, pulmonary TB, and ankylosing spondylitis, were excluded. The control group included 262 healthy subjects and subjects without pulmonary TB, spinal TB, and other forms of extra-pulmonary TB. The following data were collected for the 2 groups: gender; age; duration of symptoms; pain intensity (visual analog scale score); smoking habit; C-reactive protein (CRP) level; and erythrocyte sedimentation rate (ESR). All data for the 2 groups are listed in Table 1.

2.2. Genomic DNA and RNA extraction

Genomic DNA and total RNA were isolated from blood samples using TRIzol (Life Technologies, USA) according to the manufacturers' instructions. The extracted products were stored at -80°C. DNA and RNA qualities and concentrations were determined with a Nanodrop2000 micro-volume spectrophotometer (Thermo Scientific). RNA integrity was measured by 1% agarose gel electrophoresis and staining with ethidium bromide.

2.3. Polymerase chain reaction (PCR)

The primer sequences were designed using the UPL Assay Design Centre web service, as follows: forward primer, 5'-CTG ACTT CTC GGA CAC CAC T-3'; and reverse primer, 5'-AAG GGT AAG GGG CCA TCT TC-3'. Amplified fragment size was 352 bp. DNA was amplified in the PCR machine through PCR thermal cycling with the following conditions: initial denaturation at 95°C for 10 minute, followed by 30 amplification cycles of 95°C for 30 second; annealing temperature (given separately) and extension at 60°C for 30 s; 72°C for 1 minute; and a final extension at 72°C for 10 minute with annealing at 4°C. The PCR products were sent directly to the Sangon of Shanghai (China) for sequencing. The sequencing primers were the same as the sequencing primers of PCR.

2.4. Preparation of cDNA and real-time PCR

Reverse transcription was performed using the PrimeScript RT reagent kit with a gDNA Eraser (TaKaRa, Japan) with 1000 ng of

Table 1
Patient clinical characteristics.

Characteristic	Tuberculosis group		Control group	
		%		%
Sex	254		262	
Male	161	63.39	142	54.20
Female	93	36.61	120	45.80
Age (yr)				
<45	105	41.34	101	38.55
≥45	149	58.66	161	61.45
Smoking habits				
non-smokers	109	42.91	154	58.78
Smokers	145	57.09	108	41.22
Pain intensity (VAS)				
0-4	14	5.51	–	–
5-7	151	59.45	–	–
8-10	89	35.04	–	–
Duration of symptoms				
<3 mo	78	30.71	–	–
3-12 mo	104	40.94	–	–
>12 mo	72	28.35	–	–
CRP (mg/L)				
<10	36	14.17	–	–
10-30	102	40.16	–	–
>30	116	45.67	–	–
ESR (mm/h)				
<20	19	7.48	–	–
20-40	57	22.44	–	–
>40	178	70.08	–	–

CRP=C-reactive protein, ESR=erythrocyte sedimentation rate, VAS=visual analog scale score.

total RNA. The real-time PCR reaction used an ABI7500 real-time PCR system (Life Technologies). The reaction mixture consisted of 10 ml of Fast Start Universal SYBR Green Master (Roche, Germany), 0.6 ml of each upstream primer, 6.8 ml of *RNase*-free water, and 2 ml of cDNA. The RT-PCR reaction cycling conditions were as follows: 10 minute at 95°C; followed by 15 s at 95°C (40 cycles); and 1 minute at 60°C. To control the RT-PCR standard, beta actin was used as the internal control. The primer sequences were designed using the UPL Assay Design Centre web service, as follows: forward primer, 5'-AGA ACC GCT CCT ACA GCA AG-3'; and reverse primer, 5'-TAG GCG AAG GTG GAG TTG TT-3'. All RT-PCR products used ethidium bromide under a UV light trans-illuminator after electrophoresis on 1.5% agarose gels. MIF transcript levels were calculated using the $2^{-\Delta\Delta CT}$ method.^[7-9] All RT-PCR steps were repeated 3 times. The results of the analysis used ABI 7500 real-time PCR software (version 2.0.1).

2.5. Serum MIF levels

The MIF blood serum levels in all subjects were measured by testing with ELISA kits (R&D), according to the manufacturer's instructions. A standard curve was plotted, and the MIF concentration was calculated.

2.6. Statistical analysis

Statistical analysis was performed using SPSS 16.0 (SPSS, Inc., Chicago, IL). The general conditions of the 2 groups was compared using a *t*-test and chi-square test. The Kolmogorov-Smirnov 1-sample test was used to assess the normality of the

Table 2**Genotypic and allelic frequencies of MIF-173 in spinal tuberculosis patients and controls.**

Allele genotype	spinal tuberculosis (%) (n=254)	Control (%) (n=262)	P value	Odds ratio (95%CI)
rs755622 (G/C)				
GG	123 (48.43)	161 (61.45)	1	
GC+CC	131 (51.57)	101 (38.55)	.003	1.698 (1.196–2.409)
G	343 (67.52)	407 (77.67)	1	
C	165 (32.48)	117 (22.33)	<.001	1.673 (1.268–2.208)

distribution of the transcript levels and blood serum expression values for the genes studied. A *t*-test was used to compare the concentration of serum and mRNA expression of MIF between the 2 groups. Accordingly, a non-parametric test was used to compare the mRNA and blood serum expression of the MIF between groups and within the group in different genotypes and in spinal TB subjects with different clinicopathologic features. Hardy–Weinberg equilibrium was examined for each group. Data are expressed as the mean \pm SD. The odds ratio (OR) at the 95% confidence interval (CI) and Pearson *P*-value were calculated. A *P*-value < .05 was considered statistically significant.

3. Results

3.1. MIF-173 G/C polymorphism analysis

We evaluated the frequencies of MIF polymorphisms in spinal TB patients and controls, as shown in Table 2. The sequencing analysis results of the MIF-173GC polymorphism are shown in Figure 1. Both the genotype and allele frequency distributions were in agreement with the Hardy-Weinberg equilibrium. The C minor allele (32.48% vs 22.33%, $P < .001$, OR = 1.673, 95% CI = 1.268–2.208) and the GC + CC genotypes (51.57% vs 38.55%, $P = .003$, OR = 1.698, 95% CI = 1.196–2.409) of MIF-173GC were both significantly higher in spinal TB patients than controls.

3.2. Comparison of MIF transcript level and serum concentration in patients and controls

The MIF and beta-actin amplification curves showed that the cycle threshold values were within an acceptable range (Fig. 2). The specificity of the primers was verified through melting curves. The standard curves were plotted as the Ct versus the concentration of the total RNA from 10 times the gradient serial dilution.

The *t*-test showed that the transcript level of MIF in spinal TB patients was significantly lower than controls (0.36 ± 0.14 vs 1.22 ± 0.47 , $P < .001$); however, the serum concentration of MIF in spinal TB patients was significantly higher than controls, as shown in Table 3 (11.38 ± 3.79 ng/mL vs 5.76 ± 2.69 ng/mL, $P < .001$).

3.3. Comparison of MIF transcript level and serum concentration in patients and controls with different genotypes

The transcript level of MIF was significantly lower in spinal TB patients than in controls with GC+CC (0.36 ± 0.16 vs 1.17 ± 0.65 , $P < .001$) and GG genotypes (0.35 ± 0.13 vs 1.32 ± 0.41 ,

$P < .001$), and the serum MIF concentration was significantly higher in spinal TB patients than in controls with GC + CC (10.43 ± 3.29 ng/mL vs 5.82 ± 2.61 ng/mL, $P < .001$) and GG genotypes (11.89 ± 4.31 ng/mL vs 5.79 ± 2.75 ng/mL, $P < .001$). Our results also showed that the serum concentration of MIF in spinal TB patients with the GG genotype ($n = 123$) was significantly higher than spinal TB patients with the GC+CC genotype ($n = 131$; 11.89 ± 4.31 ng/mL vs 10.43 ± 3.29 ng/mL, $P < .001$; Table 3).

3.4. Comparison of MIF transcript and serum levels in patients with different clinicopathologic features

We further evaluated the association between stratification analysis of MIF with clinicopathologic factors in the spinal TB group. Patient clinical characteristics, such as gender, age, smoking habit, pain intensity, duration of symptoms, ESR, and CRP level, are shown in Table 1. The serum level of MIF was significantly higher in patients with high CRP levels (>30 mg/L) compared to patients with low CRP levels (<10 mg/L; $P < .001$), and was significantly higher in patients with a high ESR (>40 mm/h) than patients with low a ESR (<20 mm/h; $P < .001$; Fig. 3). Other patient characteristics did not significantly affect the serum levels of MIF in the present study. There was no significant difference in the MIF mRNA level based on clinicopathologic factors.

4. Discussion

TB is a curable infectious disease, but can result in death. The active TB prevalence rate in China was 5% in 2010, and every 3% to 5% of active TB cases involves dissolved bone lesions, most of which occur in the spine.^[10] Spinal lesions may lead to pain, nerve compression symptoms and paraplegia. Some studies have shown that spinal TB patients have different pathologic and predisposing factors.^[11] Further studies with spinal TB in other groups of patients will help clarify this question.

MIF is widely expressed in various cells, and is considered to be a versatile cytokine. MIF is not only a T cell-derived cytokine, but could also be released from other cells, such as monocyte-macrophage cells and anterior pituitary cells. MIF is an upstream inflammatory amplification factor which can amplify the inflammatory response and can counteract the effects of glucocorticoids, activate immune or inflammatory cells, and promote the expression of MMP-9, NO, IL-6, IL-8, TNF- α , IL-1 β , IFN- γ , and PGE2.^[3] Early reports indicate that MIF may be associated with susceptibility to TB and intervertebral disc lesions.^[5,6] In recent years, it has been reported that the MIF concentration may be associated with the severity of inflammatory damage and inhibition of biological function, and can also promote the repair and regeneration of the injured site.^[12] To

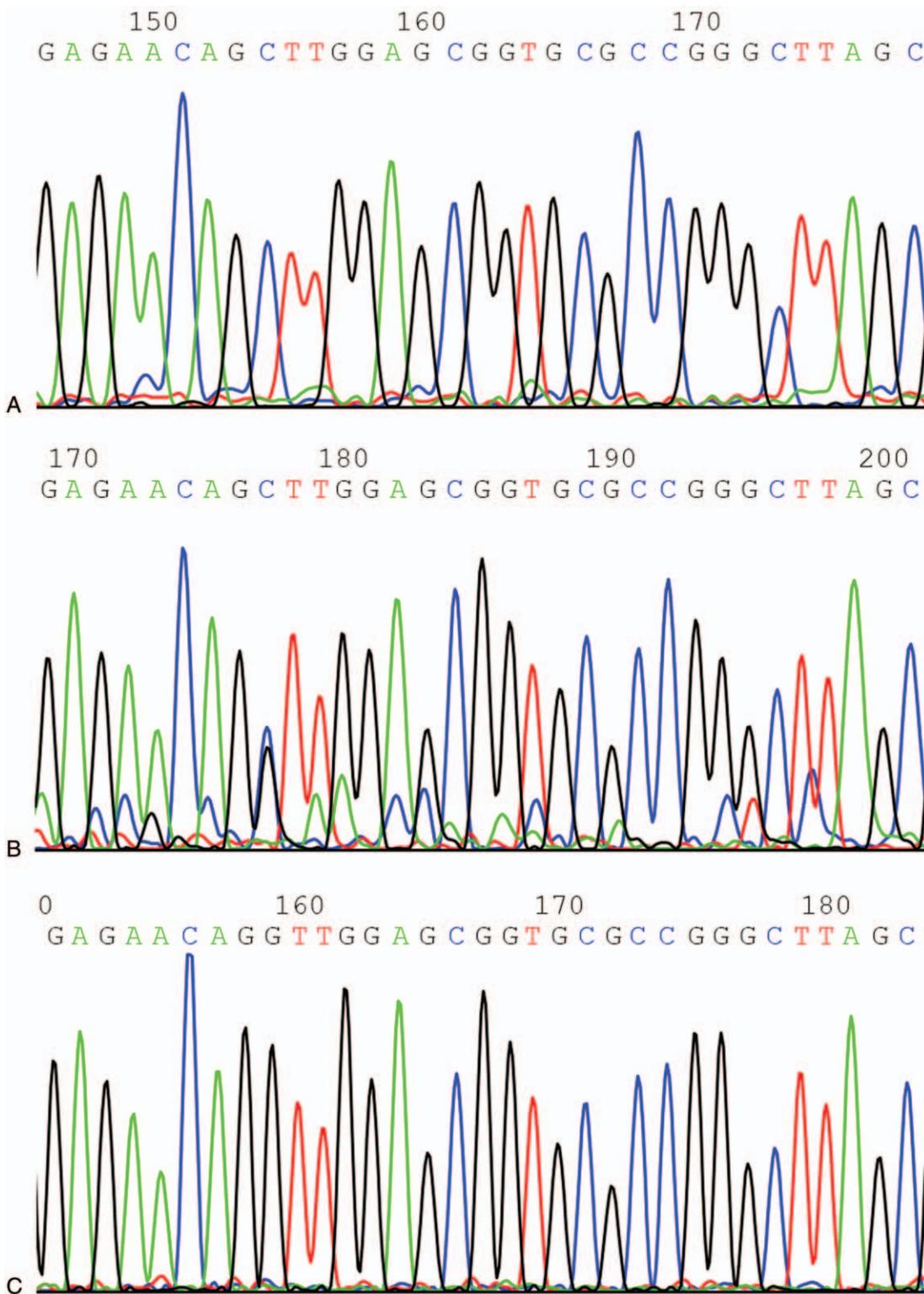


Figure 1. (A) The sequencing analysis results of the MIF-173GC polymorphism: CC. (B) The sequencing analysis results of the MIF-173GC polymorphism: CG. (C) The sequencing analysis results of the MIF-173GC polymorphism: GG.

obtain more detailed information and investigate the association between MIF and spinal TB, we studied the relationship between MIF and spinal TB with respect to gene variants, and transcript and serum levels of MIF.

Gene polymorphism plays an important role in the occurrence and development of TB. Several TB B susceptibility genes have been discovered, 1 of which is MIF. In recent years, it has been shown that MIF gene polymorphisms can affect host

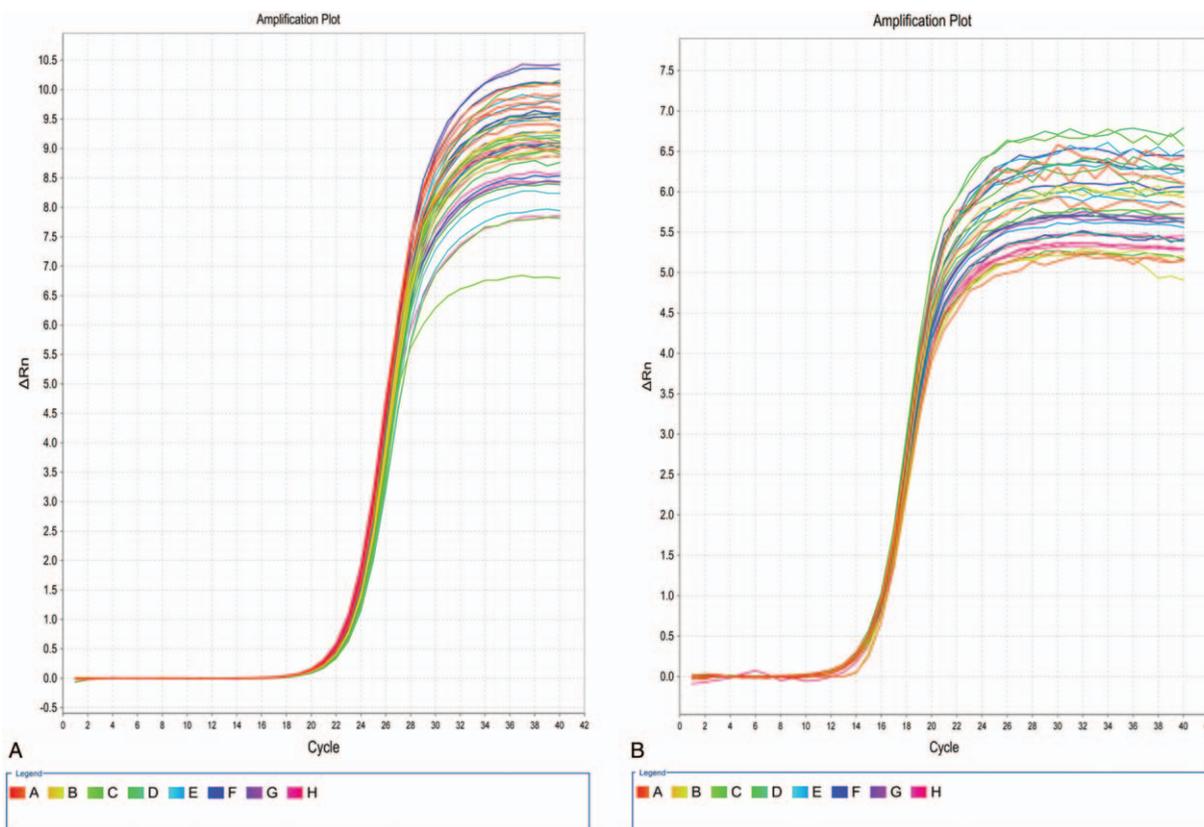


Figure 2. Amplification curves. (A) Migration inhibitory factor, the Ct value was within the range of 23–27. (B) beta-actin the Ct value was within the range of 13–15. MIF=migration inhibitory factor.

susceptibility to TB.^[13,14] In fact, some researchers have found that MIF-173G/C polymorphism may be involved in the development of pulmonary TB in Asians and Caucasians and the frequency of MIF-173CC homozygote and C allele in pulmonary TB group are significantly higher than that in healthy control group.^[15] Liu et al have found that the frequency of MIF-173CC homozygote and C allele in pulmonary TB group in Southwest China were significantly higher than that in healthy control group. Similarly, according to our data, the MIF C minor allele and MIF C carriers are more frequent in spinal TB patients than controls.

Interestingly, our study confirmed that the MIF transcript level in spinal TB patients is significantly lower than healthy controls;

Table 3
Comparison of migration inhibitory factor transcript levels and serum in patients and controls with different genotypes.

Group	Genotype	Case	mRNA ($2^{-\Delta\Delta CT}$)	MIF serum
Control		262	1.22±0.47	5.76±2.69
	GG	161	1.32±0.41	5.79±2.75
	GC+CC	101	1.17±0.65	5.82±2.61
spinal tuberculosis		254	0.36±0.14 ^a	11.38±3.79 ^a
	GG	123	0.35±0.13 ^a	11.89±4.31 ^{a,b}
	GC+CC	131	0.36±0.16 ^a	10.43±3.29 ^a

MIF=migration inhibitory factor.

^a Significant difference versus the control group $P<.001$.

^b Significant difference GG versus GC+CC in groups $P<.001$.

however, our findings showed that the MIF plasma levels were significantly higher in spinal TB patients compared with controls. Both Li and Liu et al have found that the level of serum MIF in TB group in Southwest China was also significantly higher than that in healthy control group,^[16,17] which was consistent with our study results. In addition, Xiang et al have found that the level of MIF plasma in Asian patients with pulmonary TB was significantly higher than that in the control group, but there was no significant difference among Africans.^[15] We also showed that the GG genotype may be associated with a higher serum concentration of MIF in spinal TB patients. Based on a review of the correlation between the expression of mRNA transcription and protein levels in many cases is not good. The mRNA and protein levels are a coupled process, but are not necessarily a consistent trend or miRNA may play a important role in this process. Therefore, a decrease in the level of mRNA transcription does not necessarily reduce the level of protein concentration expression.^[18] In addition, we also noticed a major difference in the MIF concentrations reported in different studies, owing to the following factors: ELISA kits; specimen preservation methods; populations investigated; and varying degrees of disease severity.

The ESR and CRP levels are reliable parameters when evaluating the treatment and prognosis of spinal TB.^[19,20] Our results indicated that the level of MIF serum protein are increased in spinal TB patients with high ESR and CRP levels. Therefore, when MIF enters the systemic circulation of TB patients, which causes an inflammatory reaction, the blood ESR and CRP levels

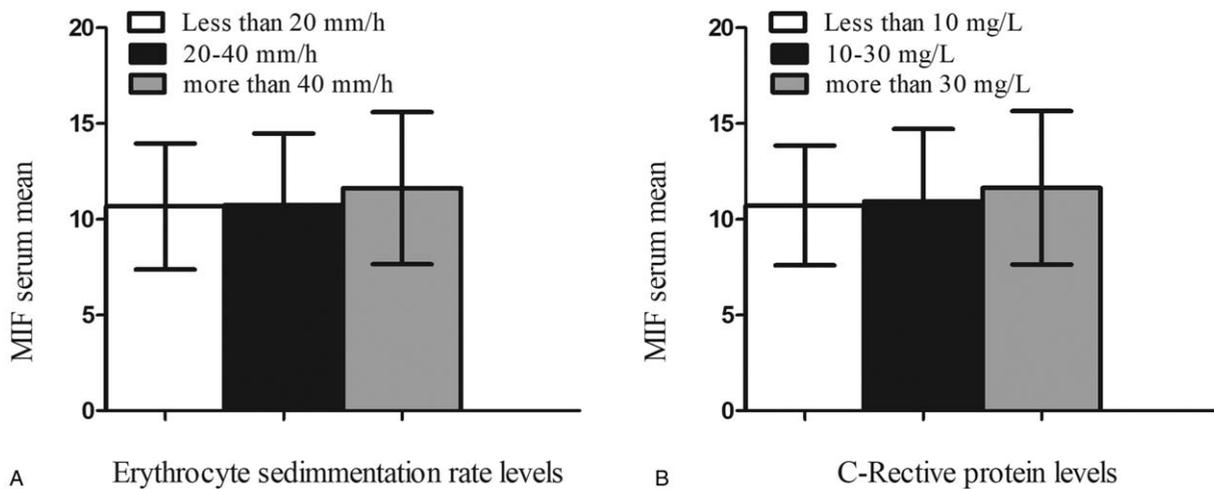


Figure 3. (A) Normalized serum levels of Migration inhibitory factor in the tuberculosis group with respect to erythrocyte sedimentation rate (ESR) levels. (B) Normalized serum levels of Migration inhibitory factor in the tuberculosis group with respect to C, -reactive protein (CRP) levels.

increase. Thus, we speculate that the blood ESR and CRP level are related to the expression of MIF, and may affect inflammation and the immune response in patients with spinal TB.

In conclusion, our study showed that the increased levels of MIF, as well as the MIF genetic variant, are associated with spinal TB. Furthermore, the C minor allele of the MIF-173GC genetic variant appears to be a risk factor for the development of spinal TB in the Shandong Province population of China. We believe that our report about the relationship between MIF polymorphisms and the transcription and plasma levels are the first reported in the Chinese spinal TB population. This study was relatively limited with 1 small cohort. The reason the levels of MIF mRNA transcription and protein expression do not exhibit a consistent trend warrants further study. The mechanism MIF plays in the development of spinal TB remains unclear. We will perform the study in a larger patient group. Further experimental studies are needed to confirm our findings.

Acknowledgments

We thank all of nurses and personnel of Weifang and Liaocheng People's Hospital for their cooperation in all the stages of the study.

Author contributions

The work presented here was carried out in collaboration between all authors. Thanks to all the authors for their significant contribution. XueJun Cao designed this study. Jun Wang, Bin Sheng, XiaoPeng Li, JianMin Sun and Lin Shi performed the experiments. GuoWei Wang and WenTao Wei completed the data analysis and the Specimen collection. Jun Wang wrote the paper. All authors have read and approved this manuscript.

References

- [1] Pandita A, Madhuripan N, Pandita S, et al. Challenges and controversies in the treatment of spinal tuberculosis. *Journal of clinical tuberculosis and other mycobacterial diseases* 2020;19:100151.
- [2] Bloom BR, Bennett B. Mechanism of a reaction in vitro associated with delayed-type hypersensitivity. *Science* 1966;153:80–2.
- [3] De la Cruz-Mosso U, Garcia-Iglesias T, Bucala R, et al. MIF promotes a differential Th1/Th2/Th17 inflammatory response in human primary cell cultures: Predominance of Th17 cytokine profile in PBMC from healthy subjects and increase of IL-6 and TNF-alpha in PBMC from active SLE patients. *Cell Immunol* 2018;324:42–9.
- [4] Benedek G, Meza-Romero R, Jordan K, et al. MIF and D-DT are potential disease severity modifiers in male MS subjects. *Proc Natl Acad Sci U S A* 2017;114:E8421–9.
- [5] Liang H, Yang X, Liu C, et al. Effect of NF- κ B signaling pathway on the expression of MIF, TNF-alpha, IL-6 in the regulation of intervertebral disc degeneration. *Journal of musculoskeletal & neuronal interactions* 2018;18:551–6.
- [6] Wang Q, Han W, Niu J, et al. Prognostic value of serum macrophage migration inhibitory factor levels in pulmonary tuberculosis. *Respiratory Res* 2019;20:50.
- [7] Parvizi J, Fassihi SC, Enayatollahi MA. Diagnosis of periprosthetic joint infection following hip and knee arthroplasty. *Orthopedic Clinics of North America* 2016;47:505–15.
- [8] Sanz-Reig J, Lizaur-Utrilla A, Miralles-Muñoz F. Risk factors for total hip arthroplasty dislocation and its functional outcomes. *Rev Esp Cir Ortop Traumatol* 2015;59:19–25.
- [9] Navab SAI, Hajzargarbashi SR, Mostafa S, et al. Infection after total hip arthroplasty. *J Bone Joint Surg* 2001;77:64–70.
- [10] Zhou Y, Tan CY, Mo ZJ, et al. P2X7 receptor in spinal tuberculosis: gene polymorphisms and protein levels in Chinese Han population. *Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases* 2018;57:138–44.
- [11] Zheng M, Shi S, Wei W, et al. Correlation between MBL2/CD14/TNF-alpha gene polymorphisms and susceptibility to spinal tuberculosis in Chinese population. *Bioscience Rep* 2018;38(1):.
- [12] Song H, Zhu Z, Zhou Y, et al. MIF/CD74 axis participates in inflammatory activation of Schwann cells following sciatic nerve injury. *J Mol Histol* 2019;50:355–67.
- [13] Areeshi MY, Mandal RK, Dar SA, et al. MIF-173 G > C (rs755622) gene polymorphism modulates tuberculosis risk: evidence from a meta-analysis and trial sequential analysis. *Sci Rep* 2017;7:17003.
- [14] Ma M, Tao L, Liu A, et al. Macrophage migration inhibitory factor-794 CATT microsatellite polymorphism and risk of tuberculosis: a meta-analysis. *Bioscience Rep* 2018;38: BSR20171626.
- [15] Tong X, Yan Z, Zhou Q, et al. Association between the MIF-173G/C polymorphism and serum mif levels with pulmonary tuberculosis: a meta-analysis. *Sci Rep* 2017;7:234.
- [16] Li Y, Zeng Z, Deng S. Study of the relationship between human MIF level, MIF-794CATT5-8 microsatellite polymorphism, and susceptibility of tuberculosis in Southwest China. *The Brazilian journal of infectious*

- diseases: an official publication of the Brazilian Society of Infectious Diseases 2012;16:383–6.
- [17] Liu A, Li J, Bao F, et al. Single nucleotide polymorphisms in cytokine MIF gene promoter region are closely associated with human susceptibility to tuberculosis in a southwestern province of China. *Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases* 2016;39:219–24.
- [18] MacKay VL, Li X, Flory MR, et al. Gene expression analyzed by high-resolution state array analysis and quantitative proteomics: response of yeast to mating pheromone. *Mol Cell Proteomics* 2004;3:478–89.
- [19] Kim JH, Ahn JY, Jeong SJ, et al. Prognostic factors for unfavourable outcomes of patients with spinal tuberculosis in a country with an intermediate tuberculosis burden: a multicentre cohort study. *The bone & joint journal* 2019;101-B:1542–9.
- [20] Ren HL, Jiang JM, Wang JX, et al. Is duration of preoperative anti-tuberculosis treatment a risk factor for postoperative relapse or non-healing of spinal tuberculosis? *European spine journal: official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society* 2016;25:3875–83.