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Association of tumor necrosis factor-α gene polymorphism with osteoarticular tuberculosis prognosis in a Hebei population

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ABSTRACT. This study investigated the association of tumor necrosis factor-α (*TNF-α*)-308, -238, and -863 polymorphisms with osteoarticular tuberculosis (OA-TB) prognosis in a Hebei population. Genomic DNA was extracted from venous blood samples of 120 OA-TB patients and 100 healthy volunteers. *TNF-α*-308, -238, and -863 were analyzed by PCR-restriction fragment length polymorphism; genotype and allele frequencies were calculated. Serum TNF-α level was significantly higher in OA-TB patients (283.16 ± 51.68 ng/L) than in control (122.54 ± 54.65 ng/L; $P < 0.05$). Higher frequency of *TNF-α*-308 GG genotype in healthy volunteers (91.0%) than in OA-TB patients (79.2%) indicated that it was a protective factor against OA-TB (OR = 0.405, 95%CI = 0.147-0.657, P = 0.007). Higher frequencies of *TNF-α*-308 GA genotype and *TNF-α*-308 allele (A) in OA-TB patients (20.8 and 10.4%, respectively) than in healthy volunteers (8.0 and 5.0%,

respectively) indicated an association with increased risk of OA-TB (OR = 3.112, 95%CI = 1.520-6.343, P = 0.003; OR = 3.109, 95%CI = 1.676-6.538, P = 0.006; respectively). Haplotype association analysis of *TNF-α* polymorphisms (-308/-238/-863) showed a higher frequency of *TNF-α* AGA in OA-TB patients (12.1%) than in healthy volunteers (3.5%), indicating that it was a risk factor for OA-TB (OR = 4.201, 95%CI = 1.80-9.91, P = 0.010). *TNF-α*-308 G/A and *TNF-α* AGA (-308/-238/-863) were associated with a predisposition to OA-TB, which could aid clinical detection, prevention, and prognosis of OA-TB.

Key words: Tumor necrosis factor-α; Gene polymorphism; Prognosis; Osteoarticular tuberculosis

INTRODUCTION

China is one of the 22 countries with a high-burden of tuberculosis (TB) and has the second highest number of TB patients in the world (World Health Organization, 2013). Osteoarticular TB (OA-TB) is one of the main types of extrapulmonary tuberculosis. There are about 30 million TB patients, of which 1-3% have skeletal involvement (Tuli, 2002), with causes that are directly related to poverty (Cheng, 2011).

Madan-Lala et al. (2014) reported that in the pathogenesis of OA-TB, *Mycobacterium tuberculosis* spreads regionally through lymphatic vessels or through primary lesions of lymphoid TB. In patients severely afflicted with OA-TB, without therapy, the disease can easily cause the malformation of spine and limbs, limit joint movement, and even lead to paraplegia. However, early diagnosis and identification of OA-TB are difficult to perform (Raja, 2004; Garg and Somvanshi, 2011).

Tumor necrosis factor-α (TNF-α) is a pleiotropic factor secreted by active macrophages. It not only participates in the resistance of macrophages to *M. tuberculosis* and tuberculous granuloma, but also induces the apoptosis of macrophages and the release of other cytokines (Li et al., 2013). The production of TNF-α is associated with the polymorphisms in its promoter region, which influences its transcription and expression (Garg and Somvanshi, 2011). The polymorphisms of *TNF-α* have been related to the pathogenesis of many diseases. *TNF-α*-308 G/A, located in the promoter region of *TNF-α*, was primarily described by Wilson et al. (1993) and was rediscovered and identified by Pantelidis et al. (1999). *TNF-α*-238 G/A is another common polymorphism (D'Alfonso and Richiardi, 1994), and *TNF-α*-863 C/A has been correlated with lower TNF-α levels (Heesen et al., 2004).

In our study, we focused on *TNF-α*-308, -238, and -863 in OA-TB patients from the Hebei region of China. We investigated the association between the generation of *TNF-α* polymorphisms and the development and treatment of OA-TB, providing possible clinical interventions with evidences.

MATERIAL AND METHODS

Patients

In total, 120 patients (72 males and 48 females of average age 40.1 ± 8.5 years) were

hospitalized in the Orthopaedic Department of the First Hospital of Hebei Medical University from January 1, 2011 to December 31, 2015. These patients were diagnosed with OA-TB, including cervical TB (Figure 1), lumbar TB (Figure 2), and spinal TB (Figure 3), based on their case histories, X-ray results, bacteriological examination, and histopathological examination. In total, 100 healthy volunteers, 70 males and 30 females, were included as controls. There was no significant difference in the gender and age of the cases and controls. Informed consent was obtained from every patient and volunteer.

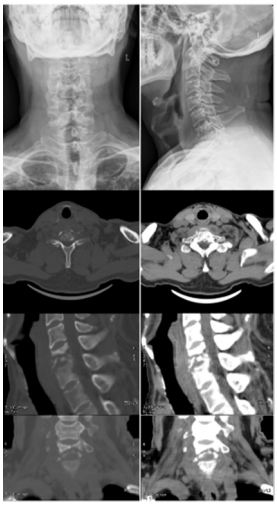

Figure 1. Male, 57 years old, with lesions at the 6th and 7th cervical and adjacent vertebrae. The cervical spinal cord was pressed. Diagnosed as cervical tuberculosis.

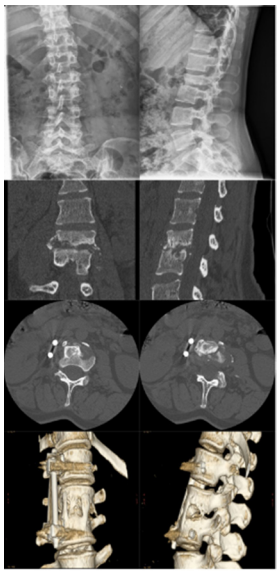

Figure 2. Female, 35 years old, with the 2nd and 3rd vertebral bodies damaged. Diagnosed as lumbar tuberculosis.

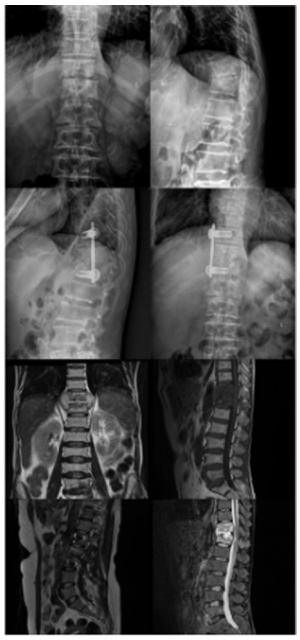

Figure 3. Male, 64 years old, with the 11th and 12th thoracic spine, the 5th lumbar vertebra, and the 1st sacral vertebra damaged. Diagnosed as spinal tuberculosis.

The following inclusive criteria were considered: 1) the patients had their ancestral home in Hebei; 2) there was no blood relationship among the patients; and 3) the patients had normal liver function, did not have HIV, autoimmune diseases, and/or diabetes mellitus, and had no history of the long-term use of hormones.

Genomic DNA extraction

Venous blood (5 mL) was collected from each patient and volunteer at 6:00 a.m. in an EDTA-K_2 anticoagulant tube (BD Bioscience, Beijing, China). QIAamp DNA Blood Mini Kit (QIAGEN International, Hong Kong, China) was used for genomic DNA extraction, according to the manufacturer protocol. The extracted DNA was stored at -20°C.

PCR-restriction fragment length polymorphism

The complete gene sequence of TNF-α was obtained from the NCBI database. The primers were designed using the Primer 5.0 software and were synthesized and provided by Invitrogen (Thermo Fisher Scientific Inc., Shanghai, China). The primer sequences are presented in Table 1. PCR was performed on a total volume of 25 μL, including 12.5 μL 2X PCR Mix (Thermo Fisher Scientific Inc.), 1 μL forward primer (10 μM), 1 μL reverse primer (10 μM), 2 μL DNA template, and 8.5 μL double distilled water, in a thermal cycler (Aeris; Esco Micro Pte. Ltd., Tianjin, China). PCR conditions are given in Table 2. PCR product was separated on a 1% agarose gel and was analyzed by a gel imaging system (Thermo Fisher Scientific Inc.). The residual product was stored at -20°C.

Table 1. Primers for PCR-restriction fragment length polymorphism of *TNF-α*-238, -308, and -863 SNPs.

SNP	Primer	Sequence
TNF-α-238	Forward	5'-CCGTGCTTGGTGCTTTGGACTA-3'
	Reverse	5'-AGAGCTGGTGGGGACATGTCTG-3'
TNF-α-308	Forward	5'-AATAGGTTTTGAGGGGCAAGG-3'
	Reverse	5'-AGGGAGCGTCTGCTGGCTG-3'
TNF-α-863	Forward	5'-CGAGTATGGGGACCCCCC-3'
	Reverse	5'-GAGTATGGGGACCCCCA-3'

SNP, single nucleotide polymorphisms; TNF-α, tumor necrosis factor-α.

Table 2. PCR steps and conditions.

No. of cycles	Step	Temperature (°C)	Time (s)
	Pre-heating	95	60
10	Denaturation	95	15
	Annealing	65	50
	Extension	72	40
20	Denaturation	95	20
	Annealing	59	50
	Extension	72	30
	Cooling	4	-
	Pre-heating	96	60
5	Denaturation	96	25
	Annealing	70	45
	Extension	72	25
21	Denaturation	96	25
	Annealing	65	50
	Extension	72	30
4	Denaturation	96	30
	Annealing	55	60
	Extension	72	90
	Cooling	4	-

Statistical analysis

All data were analyzed using SPSS 17.0 software, and the data are reported as means ± SD. Hardy-Weinberg equilibrium was used for analyzing the representative of the population, and the genotype and allele frequencies were calculated. The differences between the groups were analyzed by the chi-square and the Fisher exact tests. The associations among genotypes and alleles were calculated by reckoning the odds ratio (OR) and 95% credibility interval (CI) from logistic regression analyses. The Hardy-Weinberg equilibrium was calculated using the chi-square test. $P < 0.05$ was considered statistically significant.

RESULTS

Serum TNF-α level

As shown in Table 3, the mean serum TNF-α level in the OA-TB patients was 283.16 ± 51.68 ng/L, which was significantly higher than that in controls (122.54 ± 54.65 ng/L; $P < 0.05$). Additionally, the average TNF-α level in females was higher than in males, among both the OA-TB patients and healthy volunteers.

Table 3. Serum TNF-α level in OA-TB patients and healthy volunteers.

Group	TNF-α level (ng/L, means ± SD)		
	Males (cases)	Females (cases)	Total (cases)
OA-TB patients	279.60 ± 51.28 (72)	284.96 ± 56.40 (48)	283.16 ± 51.68 (120)
Healthy volunteers	122.06 ± 54.38 (70)	123.53 ± 59.30 (30)	122.54 ± 54.65 (100)
t	13.16	11.69	23.03
P value	<0.05	<0.05	<0.05

TNF-α, tumor necrosis factor-α; OA-TB, osteoarticular tuberculosis.

Genotype and allele frequencies of *TNF-α*-308, -238, and -863

All *TNF-α* SNPs were genotyped for the 120 OA-TB patients and 100 healthy volunteers. All of the SNPs in the patients and controls accorded with the Hardy-Weinberg equilibrium (P > 0.05).

As shown in Table 4, the *TNF-α*-308 G/A gene polymorphism was associated with predisposition to OA-TB. Higher frequency of the *TNF-α*-308 GG genotype was observed in healthy volunteers (91.0%) than in the OA-TB patients (79.2%), indicating that this genotype was a protective factor against OA-TB (OR = 0.405, 95%CI = 0.147-0.657, P = 0.007). In contrast, lower frequency of the *TNF-α*-308 GA genotype was observed in the healthy volunteers (8.0%) than in the OA-TB patients (20.8%), indicating that it was associated with an increased risk of OA-TB (OR = 3.112, 95%CI = 1.520-6.343, P = 0.003). Additionally, higher frequency of the *TNF-α*-308 allele (A) in the OA-TB patients (10.4%), than in the healthy volunteers (5.0%), indicated that this allele was a risk factor for OA-TB (OR = 3.109, 95%CI = 1.676-6.538, P = 0.006).

Table 4. Genotype and allele frequencies of TNF-α in OA-TB patients (N = 120) and healthy volunteers (N = 100).

SNP	Allele or genotype	OA-TB patients (%)	Healthy volunteers (%)	OR (95%CI)	P value
TNF-α-308					
Allele frequency	G	215 (89.6)	190 (95.0)	Ref.	-
	A	25 (10.4)	10 (5.0)	3.109 (1.676-6.538)	0.006*
Genotype frequency	GG	95 (79.2)	91 (91.0)	0.405 (0.147-0.657)	0.007*
	GA	25 (20.8)	8 (8.0)	3.112 (1.520-6.343)	0.003*
	AA	0 (0)	1 (1.0)	-	-
TNF-α-238					
Allele frequency	G	200 (83.3)	177 (88.5)	Ref.	-
	A	40 (16.7)	23 (11.5)	0.890 (0.528-1.542)	0.920
Genotype frequency	GG	87 (72.5)	82 (82.0)	Ref.	-
	GA	26 (21.7)	13 (13.0)	0.906 (0.544-1.673)	0.653
	AA	7 (5.8)	5 (5.0)	-	-
TNF-α-863					
Allele frequency	C	235 (97.9)	193 (96.5)	Ref.	-
	A	5 (2.1)	7 (3.5)	0.527 (0.134-2.326)	0.437
Genotype frequency	CC	116 (96.7)	94 (94.0)	Ref.	-
	CA	3 (2.5)	5 (5.0)	0.589 (0.149-1.886)	0.464
	AA	1 (0.8)	1 (1.0)	-	-

*P < 0.05, statistically significant; TNF-α, tumor necrosis factor-α; OA-TB, osteoarticular tuberculosis; SNP, single nucleotide polymorphism; Ref., reference = 1.

However, the allele and genotype frequencies of *TNF-α*-238 and -863 did not differ significantly between the OA-TB patients and healthy volunteers, indicating that -238 and -863 were not associated with the risk of OA-TB.

Haplotype association analysis of *TNF-α*-308, -238, and -863

The haplotype association analysis of *TNF-α* SNPs (-308/-238/-863) is shown in Table 5. Higher frequency of the *TNF-α* AGA haplotype was observed in the OA-TB patients (12.1%) than in the healthy volunteers (3.5%), indicating that *TNF-α* AGA was a risk factor for OA-TB (OR = 4.201, 95%CI = 1.80-9.91, P = 0.010). The other haplotypes were not associated with OA-TB (P > 0.05).

Table 5. Haplotype association analysis for *TNF-α*-308, -238, and -863 in OA-TB patients and healthy volunteers.

Haplotype	OA-TB patients (%)	Healthy volunteers (%)	OR (95%CI)	P value
TNF-α SNPs-308/-863/-238				
GGC	197 (82.1)	174 (87.0)	Ref.	-
GAC	1 (0.4)	3 (1.5)	0.430 (0.09-2.98)	0.602
GGA	3 (1.3)	2 (1.0)	1.862 (0.30-14.5)	0.684
GAA	4 (1.7)	3 (1.5)	0.695 (0.14-2.98)	0.710
AGC	4 (1.7)	6 (3.0)	0.576 (0.17-2.03)	0.428
AAC	0 (0)	1 (0.5)	-	-
AGA	29 (12.1)	7 (3.5)	4.201 (1.80-9.91)	0.010*
AAA	2 (0.8)	4 (2.0)	0.731 (0.18-4.36)	0.712

*P < 0.05, statistically significant; TNF-α, tumor necrosis factor-α; OA-TB, osteoarticular tuberculosis; SNPs, single nucleotide polymorphisms; Ref., reference = 1.

DISCUSSSION

OA-TB is caused by *M. tuberculosis*, which can diffuse to the bone and joints through the blood and lymphatic system, leading to abundant blood concentration of these pathogens and osteoarticular necrosis with heavy microbial burden (Onaitis et al., 2006). A previous study showed that TNF-α can bind to the receptor on the osteoclast precursors and can directly or indirectly regulate its differentiation, activation, and apoptosis (McKenna et al., 2006).

In our research, we studied the association of SNPs of *TNF-α*-308, -238, and -863 with OA-TB and found that the serum TNF-α level in the OA-TB patients was 283.16 ± 51.68 ng/L, which was significantly higher than that in controls (122.54 ± 54.65 ng/L; P < 0.05). The average TNF-α level in females was higher than that in males, among both the OA-TB patients and healthy volunteers. This indicated that the serum TNF-α level was related to the pathogenesis of OA-TB.

Of the *TNF-α* SNPs, we found that only the *TNF-α*-308 G/A gene polymorphism was associated with predisposition to OA-TB, whereas *TNF-α*-238 and -863 did not differ significantly between the OA-TB patients and healthy volunteers, indicating that *TNF-α*-308 was a risk factor for OA-TB. The *TNF-α*-308 GG genotype was present at a higher frequency in the healthy volunteers (91.0%) than in the OA-TB patients (79.2%), thereby behaving as a protective factor against OA-TB (OR = 0.405, 95%CI = 0.147-0.657, P = 0.007). The *TNF-α*-308 GA genotype was present at a lower frequency in the healthy volunteers (8.0%) than in the OA-TB patients (20.8%), indicating that it was associated with increased risk of OA-TB (OR = 3.112, 95%CI = 1.520-6.343, P = 0.003). Additionally, the *TNF-α*-308 allele (A) was more frequently observed in the OA-TB patients (10.4%) than in the healthy volunteers (5.0%), indicating that it was a risk factor for OA-TB (OR = 3.109, 95%CI = 1.676-6.538, P = 0.006).

The results of studies on the association of *TNF-α* SNPs with TB vary, probably due to the different types of TB and their geographic distribution. Vejbaesya et al. (2007) found

that *TNF-α*-308 and -238 are not associated with pulmonary TB in Thai patients, whereas Merza et al. (2009) observed that *TNF-α*-308 allele (A) is associated with susceptibility to pulmonary TB in Iranian patients. According to Li (2015), *TNF-α*-308 is associated with OA-TB in Tibetans in Qinghai area, whereas *TNF-α*-238 is not; this is similar to our results. This indicated that the two OA-TB afflicted populations, Tibetan and Chinese, have similar TNF-α SNPs, due to which the results were similar.

There have been many reports on the association of *TNF-α*-308, -238, and -863 with different diseases, such as hepatitis B virus infection (Du et al., 2006; Basturk et al., 2008) and breast cancer (Yang et al., 2011). In our study, we combined the three SNPs and the haplotype association analysis of *TNF-α* SNPs (-308/-238/-863) showed that the *TNF-α* AGA haplotype was present at a higher frequency in the OA-TB patients (12.1%) than in the healthy volunteers (3.5%), indicating that it was a risk factor for OA-TB (OR = 4.201, 95%CI = 1.80-9.91, P = 0.010). The other haplotypes were not associated with OA-TB (P > 0.05). This indicated that *TNF-α* AGA (-308/-238/-863) could be used in the detection, prevention, and prognosis of OA-TB.

However, owing to the use of a small sample size and specific population in our study, further investigations on the association of *TNF-α* SNPs with OA-TB are required.

In conclusion, OA-TB patients had higher serum TNF-α level than healthy individuals. The *TNF-α*-308 G/A gene polymorphism was associated with a predisposition to OA-TB, with lower *TNF-α*-308 GG but higher *TNF-α*-308 GA, *TNF-α*-308 allele (A), and *TNF-α* AGA (-308/-238/-863) frequencies in OA-TB patients than in healthy individuals. Therefore, *TNF-α* SNPs could aid clinical detection, prevention, and prognosis of OA-TB.

Conflict of interest

The authors declare no conflict of interest.

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