Original Article

MYCOBACTERIAL EXAMINATION OF INTRAOPERATIVE SPECIMENS AFTER A 1-MONTH STANDARD TREATMENT REGIMEN IN PATIENTS WITH SPINAL TUBERCULOSIS

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Abstract [Objective] The purpose of this study was to compare the negative conversion rate between intraoperative specimens of spinal tuberculosis and sputum specimens of pulmonary tuberculosis after 1 month of treatment with a standard anti-tuberculosis drug regimen. [Patients and Methods] We retrospectively reviewed the records of 111 patients who underwent anterior spinal fusion for spinal tuberculosis. All patients received anti-tuberculosis drug using a standard regimen with either INH, RFP, PZA, EB or SM (Method A) or a standard regimen with INH, RFP, EB or SM (Method B). Overall, 76 patients were treated with Method A and 35 patients were treated with Method B. Forty-five out of the 111 patients had intrathoracic lesions: 40 of these patients were smear-positive and 5 were smear-negative as well as PCR-positive. Nineteen patients were classified as having extra-pulmonary lesions such as miliary tuberculosis or tuberculous pleuritis. Forty-seven out of the 111 patients did not have intrathoracic lesions. All the patients underwent surgery 1 month after the initiation of the anti-tuberculous treatment. The negative conversion rate was compared between the intraoperative specimens of spinal tuberculosis and sputum specimens of the 40 smear-positive patients with pulmonary tuberculosis. [Results] The negative conversion rate of intraoperative specimens after 1-month treatment for spinal tuberculosis was 7.2% (Method A: 10.5%, Method B: 0%). The negative conversion rates of the sputum specimens were 82.5% (Method A: 78.2%, Method B: 88.2%) at 1 month, 92.5% (Method A: 87.0%, Method B: 100%) at 2 months, and 97.5% (Method A: 95.7%, Method B: 100%) at 3 months after treatment initiation. A comparison of the negative conversion rates of the sputum specimen at 1 month and the intraoperative specimen at the same period showed a significant difference ($p < 0.01$). [Conclusion] The negative conversion rate for intraoperative specimen in patients with spinal tuberculosis after 1 month of standard treatment was less than that of pulmonary tuberculosis at that time period.

Key words: Spinal tuberculosis, Standard treatment, Intraoperative specimen

INTRODUCTION

Following the introduction of rifampicin (RFP), short-course chemotherapy was extensively developed in the 1970s.[1] A four-drug regimen including RFP and pyrazinamide (PZA) was found to be especially effective with considerably low relapse rates. Throughout the 1980s, the regimen was used worldwide (including Japan) and became standard chemotherapy.[2,3] Currently, a standard four-drug regimen exists and consists of RFP, PZA, isoniazid (INH), and ethambutol (EB) or streptomycin (SM). Patients take RFP, PZA, INH, and EB or SM in the first 2 months followed by INH and RFP in the next 4 months (Method A). The standard three-drug regimen consists of INH, RFP and EB or SM, which are taken in the first 2 months followed by INH and RFP only in the next 7 months (Method B). Previous studies have shown satisfactory results in the use of these regimens in the treatment of spinal tuberculosis.[4,5] However, the duration of treatment administration is still controversial since there is no clear definition of healing spinal tuberculosis.[6] One of the difficulties in assessing spinal tuberculosis activity may be due to issues related to mycobacterial assessment. Unless a spinal biopsy is performed while a patient is undergoing treatment, mycobacterial activity cannot be assessed. However, unlike the sputum test for pulmonary tuberculosis, the spinal biopsy is not a practical procedure to perform periodically. On the other hand, during surgical treatment for spinal tuberculosis for a patient who has initiated treatment, intraoperative

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specimens can be collected and utilized for mycobacterial assessment. However, there may only be one opportunity for collection per patient. Knowing the mycobacterial status of intraoperative specimens after treatment initiation is valuable clinical information for determining the adequate duration of treatment. To the best of our knowledge, no previous study has reported on mycobacterial assessment of spinal tuberculosis after initiating standard treatment, including the four-drug regimen. Therefore, this study proposes to compare the negative conversion rate between intraoperative specimens of spinal tuberculosis and sputum specimens of pulmonary tuberculosis after 1 month of treatment with a standard anti-tuberculosis drug regimen.

PATIENTS AND METHODS

From January 2003 to January 2017, 111 patients who underwent anterior spinal fusion for spinal tuberculosis were retrospectively reviewed. There were 47 male and 64 female patients, and the average age of patients was 65 years (range ＝22 to 89 years). Spinal lesions were found in thoracic (n＝51), lumbar (n＝53), and other spinal (n＝7) regions. The average number of affected vertebrae was 2.2 (range＝2 to 4). Symptoms due to the spinal tuberculosis were pain (n＝108), paralysis (n＝24), and symptoms derived from large abscess or fistula formation (n＝3) (the symptoms overlapped between patients). Severity of the spinal tuberculosis lesions was classified using a staging system proposed by Kumar[7] as: Stage I (stage of implantation with no spinal deformity: n＝0), Stage II (stage of early destruction with kyphotic deformity less than 10 degrees: n＝0), Stage III (stage of advanced destruction with kyphotic deformity more than 10 degrees: n＝87), Stage IV (stage of neurological involvement: n＝24), Stage V (stage of residual deformity: n＝0), showing that all the patients in this study were at advanced stages of spinal tuberculosis. Patients received one of two anti-tuberculous drug regimens. First choice was Method A, consisting of INH, RFP, PZA, EB or SM. Method B consisted of INH, RFP, EB or SM and was administered to patients who could not tolerate the side effects of PZA. Before starting either treatment, *Mycobacterium tuberculosis* was confirmed in all patients by either needle biopsy of the spine or by sputum test. Overall, 76 patients were treated with Method A and 35 patients were treated with Method B. Treatment was administered for at least 9 months in both methods but could be extended to 12 months based on the severity of tuberculous lesions or presence of comorbidities. Forty-seven out of the 111 patients did not have intrathoracic lesions and 45 out of the 111 patients had intrathoracic lesions: 40 of these patients were smear-positive (23 treated with Method A and 17 treated with Method B) and 5 were smear-negative as well as polymerase chain reaction (PCR)-positive. Nineteen patients were classified as having extra-pulmonary lesions such as miliary tuberculosis (n＝15) or tuberculous pleuritis (n＝4). There was no patient with pulmonary tuberculosis whose sputum test showed smear-negative as well as culture-positive. Eight patients were immunocompromised with comorbidities including diabetes mellitus (n＝4), usage of oral steroid (n＝2), and malignancies (n＝2) (Table 1).

All of the patients underwent surgery 1 month from the start of the anti-tuberculous treatment, and intraoperative specimens were obtained for mycobacterial testing. Direct smears were examined by fluorescent microscopy and cultures of mycobacteria were identified by liquid broth cultures (Mycobacterial Growth Indicator Tube system). Excluded from the study were patients with any kind of drug resistance, patients who were treated for recurrent tuberculosis, patients who underwent a regimen converted to one other than the standard regimen, patients who underwent surgery at less than 4 weeks or at over 5 weeks after treatment initiation, and patients whose treatment period in our hospital was less than 3 months. The negative conversion rate (wherein specimens are smear-negative as well as culture-negative) was compared between intraoperative specimens of spinal tuberculosis and sputum specimens of smear-positive pulmonary tuberculosis. The chi-squared test was used to compare the negative conversion rate. Values are reported as means with standard deviations.

RESULTS

The negative conversion rate of intraoperative specimens after 1 month of treatment was 7.2% for all spinal tubercu-

Table 1 Patients' characteristics

Characteristics (n＝111)	n or Mean±SD
Age, years (mean±S.D.)	64.9±16.9
Male/Female	47/64
Location of spinal lesions	
Thoracic	51
Lumbar	53
Other spinal region	7
Symptoms due to spinal lesions	
Pain	108
Palsy	24
Abscess or fistula	3
Pulmonary lesions	45
Smear positive	40
Cavitary lesion	2
Non-cavity lesion	38
Smear negative	5
PCR positive	5
Culture positive	0
Miliary lesion	15
Pleural lesion	4
No intrathoracic lesion	47
Immunocompromised	8
Diabetes mellitus	4
Receiving systemic steroids	2
Malignancies	2
WBC count, cells/μl (mean±S.D.)	6489±2221
Lym count, cells/μl (mean±S.D.)	1152±494

losis patients; 10.5% for Method A; and 0% for Method B. The negative conversion rates of sputum specimen from 40 smear-positive patients with pulmonary tuberculosis was 82.5 % (Method A: 78.2%, Method B: 88.2%) at 1 month, 92.5% (Method A: 87.0%, Method B: 100%) at 2 months, and 97.5 % (Method A: 95.7%, Method B: 100%) at 3 months after treatment initiation (Table 2). A comparison of the negative conversion rates of the sputum specimen at 1 month and the intraoperative specimen at the same time period revealed a significant difference ($p<0.01$). It was notable that 72% of the intraoperative specimens showed positive culture. All the patients showed healing of spinal and pulmonary tuberculosis at the final follow-up. One patient, who died of general debility, showed smear and culture negative after treatment for 3 months. There were four patients whose intraoperative specimens showed negative culture outcomes of mycobacterial test. One of them had pulmonary tuberculosis with smear-negative as well as PCR-positive sputum specimen and minimal chest x-ray findings before treatment. The rest of them did not have pulmonary tuberculosis. Spinal needle biopsy before treatment showed that two patients were smear-positive as well as culture-positive and two patients were smear-negative as well as culture-positive. No extra-pulmonary tuberculosis other than spinal tuberculosis was noted. There was no patient with notable immunocompromising comorbidity. They were all treated with Method A (Table 3).

DISCUSSION

This study examined the negative conversion rates of spinal tuberculosis intraoperative specimen cultures after 1 month of treatment with a standard drug regimen. The negative conversion rates for pulmonary tuberculosis sputum test cultures for the same treatment period were reported by several authors such as Baba et al. (Method A: 50%, Method B: 16%),[3] British Thoracic Association (Method A: 38%, Method B: 29%)[2] and Wada et al. (Method A: 72%, Method B: 69%).[8] In this study, the sputum test outcomes (Method A: 78.2%, Method B: 88.2%) for patients with smear-positive pulmonary tuberculosis, who were treated for a month, were similar to previous reports. By contrast, the negative conversion rate (Method A: 10.5%, Method B: 0 %) regarding intraoperative specimens of spinal tuberculosis was significantly lower than previous reports. In our literature search, we identified only one report that studied the culture of intraoperative specimens for spinal tuberculosis. Allen et al. examined intraoperative specimens from 52 cases of spinal tuberculosis to investigate the efficiency of bacterial

Table 2 Bacteriological status of each specimen

	Sputum specimen of smear-positive pulmonary tuberculosis (n=40)	Intraoperative specimen of spinal tuberculosis (n=111)	p value
Before treatment			
Heavily smear-positive, culture-positive	4 (10.0)	—	
Moderately smear-positive, culture-positive	36 (90.0)	—	
Smear-positive, culture-negative	0 (0)	—	
After one-month treatment			
Heavily smear-positive, culture-positive	0 (0)	6 (5.4)	
Moderately smear-positive, culture-positive	4 (10.0)	74 (66.7)	
Moderately smear-positive, culture-negative	0 (0)	4 (3.6)	
Smear-negative, culture-positive	3 (7.5)	23 (20.7)	
Smear-negative, culture-negative	33 (82.5)	4 (3.6)	$p<0.01$
After two-month treatment			
Moderately smear-positive, culture-positive	3 (7.5)	—	
Smear-negative, culture-positive	0 (0)	—	
Smear-negative, culture-negative	37 (92.5)	—	
After three-month treatment			
Moderately smear-positive, culture-negative	1 (2.5)	—	
Smear-negative, culture-positive	0 (0)	—	
Smear-negative, culture-negative	39 (97.5)	—	

Figures in parentheses show percentage.

Table 3 Patients with negative intraoperative specimen

Age	Sex	Location of spinal lesion	Pulmonary lesion	Sputum test smear/culture	Spinal needle biopsy smear/culture	Regimen	Alb	WBC cells/μl	Lymp cells/μl	Comorbidity
70	F	T12/L1	—	Negative/negative	Positive/positive	A	3.1	4490	510	None
68	F	T9/10	—	Negative/negative	Positive/positive	A	2.2	7190	1270	None
68	F	L1/2	*r*Ⅲ1	Negative/PCR positive	Negative/positive	A	4.0	5350	800	None
64	M	L1/2	—	Negative/negative	Negative/positive	A	3.2	6290	1900	None

culture techniques for this type of specimen. They reported a 12% negative conversion rate after 2 weeks of treatment with a three-drug regimen (INH/RFP/SM), which is a poor negative conversion rate for 2 weeks of treatment.[9] The rate is similar to our outcome that was assessed after 4 weeks of treatment; therefore, negative conversion in spinal tuberculosis possibly occurs at a much slower pace when compared to pulmonary tuberculosis. Final outcomes of anti-tuberculous drug treatment for spinal tuberculosis were generally good in previous reports;[5,10] however, those outcomes were evaluated at final follow-up or at completion of the treatment regimen. Therefore, little is known about the effects in the early phase of treatment for spinal tuberculosis. Advanced spinal tuberculosis tends to show progressive bony destruction or development of kyphosis, even after initiation of treatment.[11,12] This worsening may occur because the spine is located deep in the body and can be affected by the accumulation of necrotic tissues that contain copious amounts of bacteria. *Mycobacterium tuberculosis* provokes immunological reactions[13,14] activated by cytokines such as tumor necrosis factor alpha (TNF-α) which also activates the RANK-RANKL-OPG pathway. This regulates osteoclast formation and activation, which leads to bone reabsorption in the infected area[15]. Therefore, unless surgical resection of the diseased tissue is performed, bone destruction may be continuously induced by the response to the residual bacterial components even after initiation of anti-tuberculosis treatment.

Inferior distribution of anti-tuberculosis drugs in pathologic bone tissue is supposed to be one of the factors that influences the refractory nature of osteoarticular tuberculosis. Concentrations of anti-tuberculosis drugs have been studied since the 1950s and the drugs have shown good distribution to abscess and synovial fluid,[16,17] and poor distribution to caseous necrotic tissue and sequestrum.[18,19] Treatment of spinal tuberculosis may be influenced by lesions that cause poor distribution of the drugs, as with a cavitary lesion of pulmonary tuberculosis. Our study showed a low negative conversion rate of spinal tuberculosis after 1 month of treatment utilizing anti-tuberculous drugs. This indicates that bony destruction may still be in progress at that time, which can be a motivator for extending the treatment for spinal tuberculosis. In this study, limitations such as retrospectively collected data and small sample size may have caused selection bias. All the patients had moderate to severe spinal tuberculosis that required surgery. If this study included mild cases of spinal tuberculosis, the negative conversion rate could be higher. The patients whose intraoperative specimens showed negative result in the mycobacterial test had relatively mild or no pulmonary tuberculosis and two of them showed smear negative spinal biopsy outcomes, indicating that the severity of the lesion might have affected the negative conversion rate of intraoperative specimens. Additionally, in the control group, there were a few cases with a large amount of bacilli discharge or cases with cavitary lesions, which may have influenced the negative conversion rate. Nevertheless, this study clearly showed that advanced spinal tuberculosis had low negative conversion rate even after 1 month of treatment with anti-tuberculous drugs.

CONCLUSION

The negative conversion rate of intraoperative specimens in patients with spinal tuberculosis is relatively low after a 1-month standard treatment.

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脊椎結核に対する抗結核薬標準治療開始 1 カ月後の術中検体抗酸菌検査

井澤　一隆

要旨：〔目的〕脊椎結核に対して標準治療 1 カ月間施行後に手術を行い，その術中検体の菌陰性化率と，同じ症例群の喀痰塗抹陽性例における菌陰性化率とを比較すること。〔対象および方法〕脊椎結核に対して前方固定術を施行した111例を検討した。治療法は標準治療 A 法76例，B 法35例であった。肺病変の合併は45例で，塗抹陽性40例（A 法23例，B 法17例），塗抹陰性（PCR 陽性）5 例，肺外結核（粟粒結核，結核性胸膜炎）の合併は19例で，合併なしは47例であった。全例抗結核薬開始後 1 カ月で手術が行われ，術中検体の菌陰性化率と，塗抹陽性肺結核40例の同時期の喀痰菌陰性化率とを比較した。〔結果〕術中検体の菌陰性化率は平均7.2%（A 法10.5%，B 法 0%），同時期の塗抹陽性例の喀痰菌陰性化率は平均82.5%（A 法78.2%，B 法88.2%）であり有意差が見られた。喀痰の菌陰性化率は治療開始後 2 カ月で平均92.5%（A 法87.0%，B 法100%），3 カ月で平均97.5%（A 法95.7%，B 法100%）であった。〔結論〕標準治療開始後 1 カ月での術中検体の菌陰性化率は同時期の肺結核と比べて有意に低かった。

キーワーズ：脊椎結核，標準治療，術中検体