Cross-Sectional Study

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Vertebral destruction in tuberculous spondylitis correlates with Toll-like Receptor 4 (TLR-4) levels: a cross-sectional study

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Background: Tuberculous (TB) spondylitis is a hazardous infectious disease causing significant spinal deformity. Increased toll-like receptor-4 (TLR-4) activity promotes more extensive infections in patients with TB spondylitis, so it has the potential to be used as a biomarker to predict the severity. This study aims to determine the relationship between TLR-4 levels and the degree of vertebral destruction in TB spondylitis patients.

Materials and methods: A cross-sectional study was conducted from May to October 2023. A total of 27 TB spondylitis samples were then measured for TLR-4 serum levels. Vertebral destruction is assessed based on the Spine At Risk Signs (SARS) criteria on X-ray and MRI examinations. Moreover, the degree of sensory and motor impairment was also assessed in this study. The Spearman correlation test assessed the correlation between TLR-4 levels and vertebral destruction.

Results: Most of the samples in this study were less than 30 years old (10 people, 37%), female (14 people, 51.9%), had spinal destruction at 1 level (11 people, 40.7%), had paraplegia (8 people, 29.6%), and had hypoesthesia (11 people, 40.7%). TLR-4 levels had a mean value of 8254.1 ± 1076.1 ng/ml. TLR-4 levels were positively correlated with the degree of vertebral destruction ($r = 0.599$, $P = 0.001$), motor disorders ($r = 0.632$.x, $P = 0.000$), and sensory disorders ($r = 0.574$, $P = 0.002$).

Conclusion: TLR-4 levels are associated with the severity of vertebral destruction in TB spondylitis, so it has the potential to be used as a prognostic biomarker.

Keywords: spondylitis, toll-like receptor, tuberculose, vertebral destruction

Introduction

Tuberculosis (TB) is a granulomatous infectious disease caused by *Mycobacterium tuberculosis*. This infection involves the participation of more than 90 antigens and various virulence factors, resulting from interactions between the pathogen and host mononuclear phagocytes and T lymphocytes[1]. Based on data from the WHO, in 2016, approximately one-third of the world's population was infected with *M. tuberculosis*, although only a tiny portion of those infected developed the disease[2]. About 10% of patients with extrapulmonary TB have skeletal involvement, with the spine being the area most commonly affected. The prevalence of TB spondylitis in each country has yet to be discovered with certainty[3,4]. The spine is involved in less than 1% of all TB cases. TB spondylitis is a hazardous type of bone TB as it can be associated with neurological deficits due to compression of adjacent neural structures and significant spinal deformity. Therefore, early diagnosis and management of TB spondylitis is of particular importance in preventing this severe complication[1,3,4].

HIGHLIGHTS

- Toll-like receptor-4 can promote tuberculosis spondylitis severity and maybe a potential prognostic biomarker for this condition.
- Toll-like receptor-4 were positively correlated with the degree of vertebral destruction, motor disorders, and sensory disorders.

TB spondylitis occurs due to the hematogenous spread of *M. tuberculosis* from the lungs as the primary organ. The progression of symptoms is related to failure to eliminate germs at the primary site. Increased germ activity at new sites is associated with a greater extent of infection and the resulting complications[5]. The failure of innate immunity to carry out elimination before disease occurs, along with the lack of presentation to adaptive immune cells, causes increasing bacterial growth[6,7]. This condition also triggers the virulence activity of germs, disrupting the stability of the host's immunity[8].

Vertebral damage is one of the severe manifestations of TB spondylitis. This condition correlates with vertebral body fracture and causes deformity, resulting in a shift in alignment to the

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posterior due to damage caused by *M. tuberculosis*[9]. When TB symptoms are more severe, the body's ability to eliminate them decreases. This happens because the immune system does not eliminate the infection properly from the beginning[10].

Toll-like receptors (TLR) are markers of immunity in response to infection or tissue damage, expressed to recognize pathogen-associated molecular patterns (PAMPs). This recognition protein plays a role in innate and adaptive immunity, determining the type of PAMPs. Protein in the form of PAMPs induces adaptive immunity better than lipids and carbohydrates[11]. TLR is a marker of adaptive immunity against *M. tuberculosis*, and this association is linked to the chronicity of TB symptoms. TB exhibits extrapulmonary symptoms, indicating a failure of adaptive immunity[12]. TLR-4 is one of the TLR family that reduces innate immunity to TB by inducing anti-inflammatory cytokines, thereby increasing resistance to *M. tuberculosis*[13]. Macrophages contribute to innate immunity against *M. tuberculosis* through TLR 7 and 9, further strengthened through Bacille Calmette-Guerin (BCG) vaccination[14]. The failure of the innate immune response is related to the avoidance of *M. tuberculosis*, triggered by the ability to block and acidify lysosomes in macrophages. In addition, *M. tuberculosis* inhibits apoptosis and autophagy, causing the host to remain alive[15].

TLR is associated with the severity of TB spondylitis[16]. Apart from playing a role in innate immunity, TLRs also contribute to changes related to the failure of adaptive immunity to the cytotoxic response of T helper 1 lymphocytes[17]. Increased TLR-4 activity promotes more widespread infection in patients with TB spondylitis due to a decreased inflammatory response in macrophages[18]. Additionally, it was found that increased osteoclast activity in patients with TB spondylitis was activated via TLR-4[19]. Recently, the mechanism of immune failure related to TLR in TB spondylitis has not been fully understood. Furthermore, research on the relationship between these two variables is still limited. This study aims to determine the relationship between TLR-4 and spinal destruction in TB spondylitis sufferers.

Methods

This research was conducted at our institution's hospital from May to October 2023 using a cross-sectional observational study design. The study protocol had been reviewed and approved by our institution's Health Research Ethics Committee under No. Letter 296/UN4.6.4.5.31/PP36 /2023 and No. Protocol UH23040231 on 11 May 2023. Each subject has received an explanation of the aims and benefits of the research and the risks that may occur and signed an informed consent form. In addition, the work had been reported in line with the Strengthening the reporting of cohort, cross-sectional and case-control studies in surgery (STROCSS) criteria[20].

All TB spondylitis patients who met the specified criteria were recruited in the study. TB spondylitis was diagnosed at the discretion of the doctor in charge based on the results of the history, physical examination, laboratory, and radiology. Patients who were aged 19–50 years and had a diagnosis of TB spondylitis were the inclusion criteria for this study. On the other hand, if the patient is confirmed to have extrapulmonary TB other than the spine and suffers from osteoporosis or malignancy, they will be excluded from this study.

The sample was initially interviewed to obtain initial characteristic data in the form of demographic and clinical data. The clinical parameters assessed include the motor and sensory disorders experienced. The assessment results are normal, paraparesis, and paraplegia for motor disorders. Meanwhile, the assessment results for sensory disorders are normal, hypoesthesia, and anesthesia. After that, TLR-4 levels were measured from the patient's blood specimens using the PCR method at the Clinical Pathology Laboratory at the research location. The results of measuring TLR-4 levels are expressed in ng/ml units. In addition, the level of vertebral destruction is assessed based on the results of an X-ray radiological examination of the spine using the Spine At Risk Signs (SARS) criteria and MRI, which is characterized by a lytic appearance on the bones, changes in the normal shape of the vertebrae, and vertebral collapse. The assessment results group the level of vertebral destruction based on the number of vertebrae affected, namely one vertebra, 2–3 vertebrae, and 4–6 vertebrae.

The collected data was processed using the Statistical Package for Social Sciences (SPSS) software version 26.0 (IBM). The TLR-4 measurement data was initially checked for normality using the Shapiro–Wilk method. It was found that the data was not normally distributed. The Mann–Whitney *U* test examined the relationship between TLR-4 levels and gender. Furthermore, to assess the relationship between TLR-4 levels and age group variables, spinal damage, motor disorders and sensory disorders, the Kruskal–Wallis test was used. The correlation between TLR-4 levels and spinal damage, motor disorders, and sensory disorders was analyzed using the Spearman correlation test. The test is considered significant with a *P*-value <0.05 with a 95% CI.

Results

Baseline characteristics of the study participants

The sample recruited for this study amounted to 27 people and remained until the end of the study. The initial characteristics of the research subjects are shown in Table 1. Most of the participants in this study were less than 30 years old (10 people, 37%). Regarding sex, there were 14 female patients (51.9%) and 13 male patients (48.1%). Based on spinal destruction in these patients, there were 11 people (40.7%) with a total level of spinal destruction of 1 level. In assessing neurological function, motor disorders were found in five people (18.5%) with paraparesis and eight people (29.6%) with paraplegia. Moreover, the sensory function assessment found that 11 people (40.7%) experienced hypoesthesia, and four (14.8%) experienced anesthesia. TLR-4 levels in these patients had a mean value of 8254.1 ± 1076.1 pg/ml.

Association of TLR-4 with vertebral destruction, motoric disorders, and sensory disorders

Table 2 shows differences in TLR-4 levels based on the degree of vertebral damage, motor disorders, and sensory disorders. The highest median TLR-4 level was found in grade 3 vertebral destruction, followed by grade 2 and grade 1 ($P = 0.006$). In addition, the highest median TLR-4 levels were found in paraplegic conditions, followed by paraparesis and normal conditions ($P = 0.006$). However, concerning sensory disturbance parameters, the highest levels of TLR-4 were

Table 1

Characteristics of study participant

Variables	*N* (%)	Mean ± SD
Age[a]		
< 30 years	10 (37)	24.5 ± 3.06
30–39 years	8 (29.6)	32.87 ± 3.13
≥ 40 years	9 (33.3)	49.77 ± 7.06
Sex[b]		
Male	13 (48.1)	
Female	14 (51.9)	
Vertebral destruction		
1 vertebrae	11 (40.7)	1 ± 0
2–3 vertebrae	7 (25.9)	1.57 ± 0.53
4–6 vertebrae	9 (33.3)	4.44 ± 0.72
Motoric disorders		
Normal	14 (51.9)	
Paraparesis	5 (18.5)	
Paraplegia	8 (29.6)	
Sensoric disorders		
Normal	12 (44.4)	
Hypoesthesia	11 (40.7)	
Anesthesia	4 (14.8)	
TLR-4 level		8.254.1 ± 1.076.1

[a]Kruskal–Wallis test, $P = 0.751$.
[b]Mann–Whitney *U* test, $P = 0.488$.

found in hypoesthesia conditions, followed by anesthesia and normal conditions ($P = 0.008$).

Correlation of TLR-4 with vertebral destruction, motoric disorders, and sensory disorders

Table 3 shows the correlation between TLR-4 levels with vertebral destruction, motor disorders, and sensory disorders. There was a positive and significant correlation between TLR-4 levels and the degree of vertebral damage ($r = 0.599$, $P = 0.001$). Apart from that, there was a positive and significant correlation between TLR-4 levels and the degree of motor impairment ($r = 0.632$, $P = 0.000$) and the degree of sensory impairment ($r = 0.574$, $P = 0.002$).

Table 2

Comparison of TLR-4 level according to vertebral destruction, motoric disorders, and sensory disorders severity

Variables		Median	Interquartile range	*P*
Vertebral destruction				
1 vertebrae	11	3405.850	4177.760	0.006
2–3 vertebrae	7	9617.156	10748.430	
4–6 vertebrae	9	10939.499	7876.360	
Motoric disorders				
Normal	14	3510.603	4907.240	0.006
Paraparesis	5	7456.348	7468.520	
Paraplegia	8	13227.083	7190.060	
Sensory disorders				
Normal	12	3510.603	3799.130	0.008
Hypoesthesia	11	10939.499	8590.530	
Anesthesia	4	9846.149	8406.870	

Kruskal–Wallis test.

Table 3

Correlation between TLR-4 level and vertebral destruction, motoric disorders, and sensory disorders severity

Variables	r	*P*
Vertebral destruction	0.599	0.001
Motoric disorders	0.632	0.000
Sensory disorders	0.574	0.002

Spearman Correlation test.

Discussion

This study examines the relationship of TLR-4 to vertebral destruction in patients with TB spondylitis. The total samples collected were 27 people with TB Spondylitis. The majority of the sample in this study was under 30 years old, namely 10 people or around 37% of the total sample. This study aligns with a retrospective review conducted by Garg *et al*.[21] in 2022 on 1652 patients. It was found that the average age of the study population was 38.2 years, with almost a third of patients infected in the fourth decade of life (31–40 years). Another study by Ismiarto *et al*.[22] reported that young adults are the most commonly affected by TB spondylitis in endemic areas. However, in developed countries, it is more common in older people. Apart from that, in line with Mijaya *et al*.[23] research, the 15–24-year age group is a group of TB spondylitis sufferers because this age group is an active age group, has minimal knowledge about health and has a riskier lifestyle.

Based on sex, in this study, the ratio between men and women was similar, namely 13 people (48.1%) and 14 people (51.9%), respectively. These results are in line with the study of Zeng *et al*.[24] Yang found that the male-to-female ratio was 1:1 in TB spondylitis cases, with no statistically significant change over the 9-year study period. Research conducted by Wang *et al*.[25] also reported that the male-to-female ratio was 1.10.

The majority of bone destruction in this study occurred in only one bone. In line with the study of Wang *et al*., of the 597 total patients, 452 people (75.8%) were diagnosed with TB spondylitis with less than three vertebral destructions. Early diagnosis allows rapid therapeutic intervention and prevention of possible complications, thereby reducing the involvement of vertebral destruction. Disc space narrowing and vertebral body destruction are the most common changes seen on plain radiographs but may be normal in the early stages of the disease[25].

The most common motor disorder in this study was found to be paraplegia. The most common sensory function disorder is hypoesthesia. Previous support reported that 94.9% of TB spondylitis patients were found to have neurological deficits (sensory and motor). Neurological examination may show motor or sensory function changes in the extremities[22].

In this study, TLR-4 levels were positively correlated with the degree of sensory and motor disorders. This further strengthens the relationship between these two variables in previous studies[16]. Although research on the relationship between the two is still limited, several mechanisms that are thought to play a role are explained as follows. TLR has a significant role in innate immunity. In addition, TLRs can also cause changes related to the failure of adaptive immunity to the cytotoxic response of T helper 1 lymphocytes[17]. Increased TLR-4 activity promotes more widespread infection in patients with TB spondylitis due to

decreased inflammatory response in macrophages[18]. In addition, there was an increase in osteoclast activity in patients with TB spondylitis, which was activated via TLR-4[19]. In contrast, Sepehri *et al*. and Branger *et al*. highlight the positive role of TLR-4 in inducing immune responses and protecting against *M. tuberculosis* infection. Despite Branger's study being limited to animal models, it reveals that the part of TLR-4 in TB pathogenesis is complex[12,26]. This diversity in TLR-4's role in TB underlines the need for further research to understand its impact fully.

There are several limitations to our study. Firstly, there is a lack of evaluation for variables like comorbidities and BCG vaccination status, which may be linked to TLR-4 expression and immune activation. Additionally, no further analysis was carried out regarding the diagnostic value of TLR-4 as the gold standard for assessing the severity of TB spondylitis. This analysis can strengthen the function of TLR-4 in predicting the severity of TB spondylitis.

Conclusion

TLR-4 levels have the potential to be used as a biomarker in predicting the severity of TB spondylitis. This is primarily found based on the level of damage, sensory disturbances, and motor disturbances suffered.

Ethical approval

The study protocol has been reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University under No. Letter 296/UN4.6.4.5.31/PP36 /2023 and No. Protocol UH23040231 on 11 May 2023.

Consent

Written informed consent was obtained from the patient for publication and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

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The study was self-funded.

Author contribution

J.A.: conceived the study, administered the project, conducted formal analysis, and wrote the first draft of the manuscript; P.A.N.W., M.S., A.B., and F.H.: contributed in the methodology, formal analysis, writing original draft, reviewing, and editing; P.A.N.W.: wrote the first draft of the manuscript; J.A.: supervised the whole study. All authors reviewed and approved the final version of the manuscript.

Conflicts of interest disclosures

Nothing to declare.

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Data availability statement

Any datasets generated during and/or analyzed during the current study are available upon reasonable request.

Provenance and peer review

This not an invited paper.

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