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Study on the correlation of the TLR4/MyD88 axis with the degree of inflammatory response in patients with synovitis of the knee joint

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ABSTRACT

This work aims to provide a novel reference for future diagnosis and treatment of synovitis of the knee joint (SKJ) by analyzing the correlation of the TLR4/MyD88 axis with the degree of inflammatory response in SKJ patients. First, this study retrospectively analyzed the clinical data of 46 SKJ patients (research group, RG) treated in our hospital from January 2021 to December 2022 and 52 concurrent healthy controls (control group, CG). Concentrations of TLR4, MyD88 and inflammatory factors (IFs) in peripheral blood were measured, and differences in TLR4 and MyD88 between groups were observed to explore the diagnostic performance of the two for SKJ. Additionally, the correlation of TLR4 and MyD88 with IFs and Western Ontario Mac Master (WOMAC) scores in SKJ patients was discussed. Through the above experiment, we found that TLR4 and MyD88 presented higher mRNA levels in RG than in CG ($P<0.05$), both of which had excellent diagnostic efficiency for SKJ. Pearson correlation coefficients identified a positive correlation of TLR4 and MyD88 mRNA with IFs and WOMAC scores ($P<0.05$). Therefore, The TLR4/MyD88 axis is activated in SKJ patients and is strongly related to the intensification of inflammatory responses.

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Introduction

Synovitis of the knee joint (SKJ) is a kind of aseptic inflammation that may occur at all ages (1). In China, its incidence rate is about 9.56%, but it can reach 78.5% or even higher among people aged over 60 (2, 3). Although the pathogenesis has not been completely clarified, KJ is clinically considered to be related to obesity, long-term physical labor, trauma, etc. (4). In addition, due to the fact that SKJ usually has no special early clinical symptoms other than paroxysmal swelling and pain, most patients do not pay attention to the treatment, resulting in disease progression into a severe stage at the time of diagnosis (5). At this time, SKJ will not only be accompanied by severe pain and symptoms such as severe limitation of joint motion and quadriceps femoris atrophy, but also the invasion of articular cartilage due to changes in synovial membrane morphology, increasing the possibility of disability (6). Therefore, the early evaluation of SKJ is of great significance to ensure patient outcomes.

Clinically, the diagnosis of SKJ is based on multiple inspections such as blood routines, inflammatory factors (IFs), rheumatoid factors, tuberculosis sensitivity test, X-ray, computed tomography (CT), arthroscopy, and joint fluid collected through arthrocentesis, which involves a complicated collection process of test samples and causes great pain to patients (7, 8). Finding an accurate, rapid and convenient SKJ evaluation scheme is therefore the focus of current clinical research. In recent years, researchers have begun to focus on cellular signaling pathways (9). Of them, the TLR4/MyD88 axis initially confirmed to mainly play a role in malignant neoplastic diseases, has become one of the hotspots in clinical research (10, 11). As the research deepens, the TLR4/MyD88 axis has also been proven to be closely related to the inflammatory reaction of cells and tissues and is involved in the development and development of osteoarthritis (12, 13). Therefore, we speculate that the TLR4/MyD88 axis may also play an important role in SKJ, but its exact clinical significance is not yet clear.

Based on this, this study aims to provide clinical evidence and guidance for future clinical diagnosis and treatment of SKJ by analyzing the correlation of the TLR4/MyD88 axis with the degree of inflammatory response in SKJ patients.

Materials and Methods

Patient data

This study retrospectively analyzed the clinical data of 46 SKJ patients (research group, RG) treated in our hospital from January 2021 to December 2022 and 52 concur-

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rent healthy controls (control group, CG). Approval from the hospital's Ethics Committee of our hospital, as well as informed consent from all subjects, have been obtained.

Criteria for patient enrollment and exclusion

RG: The patients included fulfilled all the following criteria: in accordance with the diagnostic criteria for SKJ (14) and confirmed diagnosis of SKJ by X-ray examination; positive for floating patella test or presence of excessive effusion in the knee joint shown by magnetic resonance imaging (MRI); yellow or light yellow joint puncture fluid with no fat droplets on the surface. In contrast, patients with malignant tumors, heart, liver, kidney and other organ dysfunction, severe immune diseases, hematological system diseases, or cognitive impairment were excluded.

Sample collection and detection

Fasting cubital vein blood was collected from both groups and divided into two parts: one was used for polymerase chain reaction (PCR) quantification of TLR4 and MyD88 and the other for enzyme-linked immunosorbent assay (ELISA) measurement of IFs interleukin-1β/6 (IL-1β/6) and tumor necrosis factor-α (TNF-α).

PCR

We isolated total RNA from blood with Trizol, verified its purity, and reverse-transcribed it into cDNA for the configuration of the PCR reaction system. The PCR reaction was 40 cycles of 95°C for 30s, 95°C for 30s, and 60°C for min. TLR4 and MyD88 mRNA expression, normalized against GAPDH, was calculated by $2^{-\Delta\Delta CT}$.

ELISA

Plasma obtained via centrifugation of blood samples was used to determine serum IL-1β/6 and TNF-α levels following the instructions of the kits all supplied by Beijing TransGen Biotech, China.

Endpoints

TLR4 and MyD88 expression in SKJ were analyzed to determine the diagnostic value of the two in SKJ. Additionally, the correlations of the two with IFs and Western Ontario Mac Master (WOMAC) scores (15) (higher scores suggest more severe joint symptoms) were discussed.

Statistical methods

This study used SPSS25.0 for statistical analysis and P<0.05 as the level of statistical significance. The comparison of measurement data (χ±s) and count data [n(%)] employed the independent samples t-test and χ^2 test, respectively. Diagnostic value was determined by receiver operating characteristic (ROC) analysis and the correlation was identified using Pearson correlation coefficient analysis.

Results

Comparison of baseline data

Age, sex, smoking history and other baseline data were collected for comparative analysis. The results showed no statistical difference between RG and CG (P>0.05, Table 1), indicating comparability.

Comparison of TLR4 and MyD88 levels

According to PCR results, peripheral blood TLR4 and MyD88 mRNA levels in RG were (2.63±0.35) and (2.13±0.36), respectively, compared with (2.02±0.53) and (1.73±0.36) in CG, suggesting markedly up-regulated TLR4 and MyD88 mRNA in SKJ cases than in healthy controls (*P<0.01*, Figure 1).

Diagnostic performance of TLR4 and MyD88 for SKJ

As indicated by the ROC analysis, SKJ can be effectively diagnosed based on elevated peripheral blood TLR4 and MyD88 mRNA expression, with the diagnostic sensitivity and specificity of TLR4 being 94.23% and 58.70%, respectively, and those of MyD88 being 59.62% and 89.13%, respectively (*P<0.01*, Figure 2 & Table 2).

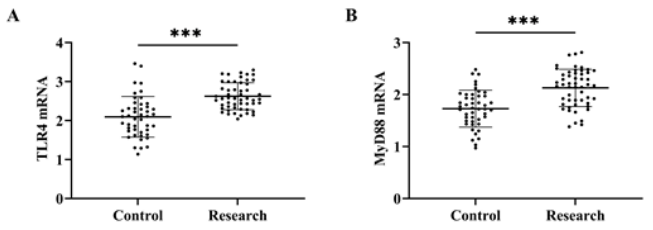

Figure 1. A: Comparison of TLR4 mRNA in the research and control groups. B: Comparison of MyD88 mRNA in the research and control groups. ****P<0.001.*

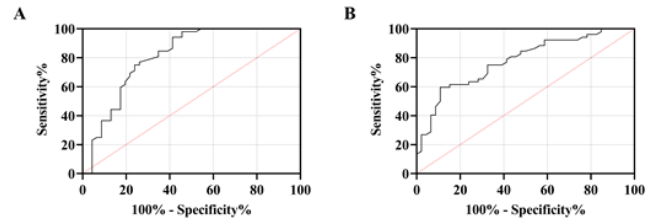

Figure 2. Diagnostic performance of TLR4 and MyD88 for SKJ. A: TLR4 mRNA diagnosis of ROC occurring in SKJ. B: MyD88 mRNA diagnosis of ROC occurring in SKJ.

Table 1. Comparison of baseline data.

Group	n	Male / Female	Age	Course of SKJ	Smoking Yes/No	Affected side Left/right/bilateral
Control	46	28 (60.87)/18 (39.13)	64.74±10.43	38.24±0.10	22 (47.83)/24 (52.17)	25 (54.35)/15 (32.61)/6 (13.04)
Research	52	34 (65.38)/18 (34.62)	66.52±8.19	36.69±11.13	22 (42.31)/30 (57.69)	27 (51.92)/20 (38.46)/5 (9.62)
χ^2 (t)		0.214	0.945	0.717	0.300	0.517
P		0.644	0.347	0.475	0.584	0.772

Table 2. Diagnostic performance of TLR4 and MyD88 for SKJ.

	Cut-off	Sensitivity	Specificity	Yoden Index	Area under curve (AUC)	95%CI	P
TLR4 mRNA	>2.17	94.23%	58.70%	52.93	0.812	0.722-0.901	<0.001
MyD88 mRNA	>2.13	59.62%	89.13%	47.75	0.778	0.688-0.869	<0.001

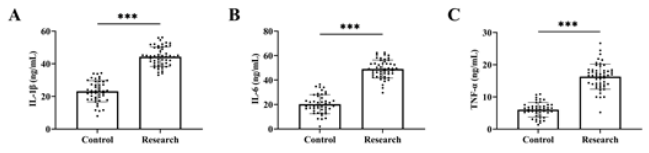

Figure 3. A: Comparison of IL-1β in the research and control groups. B: Comparison of IL-6 in the research and control groups. C: Comparison of TNF-α in the research and control groups. ****P<0.001*.

Comparison of IF levels

The detection results of IFs showed that the serum concentrations of IL-1β, IL-6, and TNF-α in RG were (44.33±5.96 ng/mL), (49.02±7.35 ng/mL) and (16.31±3.88 ng/mL), respectively, all of which were increased compared with CG (*P<0.05*, Figure 3).

Correlation of TLR4 and MyD88 with IFs in SKJ patients

Pearson correlation coefficient analysis identified a positive correlation of TLR4 and MyD88 mRNA with IL-1β, IL-6, and TNF-α in SKJ patients (P<0.05, Figure 4), indicating that the TLR4/MyD88 axis activation predicts the intensification of inflammation.

Association of TLR4 and MyD88 with WOMAC scores in SKJ patients

The WOMAC score of RG was (79.90±7.09) points. Pearson correlation coefficient analysis showed that TLR4 and MyD88 mRNA levels in SKJ patients were also positively correlated with WOMAC scores (*P<0.05*, Figure 5), confirming a close relationship between TLR4/MyD88 axis activation and pathological progression of SKJ.

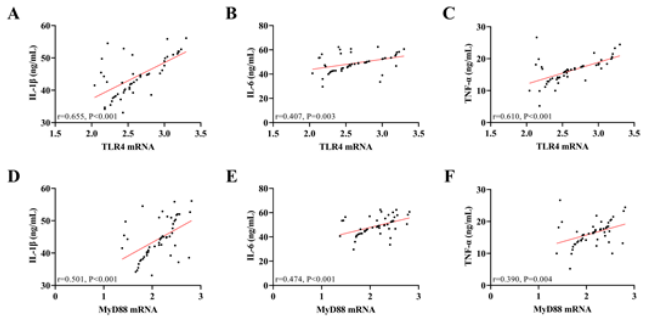

Figure 4. Correlation of TLR4 and MyD88 with IFs in SKJ patients. A: Correlation of TLR4 mRNA and IL-1β. B: Correlation of TLR4 mRNA and IL-6. C: Correlation of TLR4 mRNA and TNF-α. D: Correlation of MyD88 mRNA and IL-1β. E: Correlation of MyD88 mRNA and IL-6. F: Correlation of MyD88 mRNA and TNF-α.

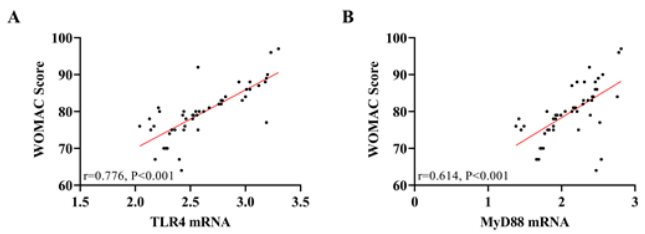

Figure 5. Association of TLR4 and MyD88 with WOMAC score in SKJ patients. A: Correlation of TLR4 mRNA and WOMAC score. B: Correlation of MyD88 mRNA and WOMAC score.

Discussion

SKJ, an inflammatory disease mediated by factors such as synovial macrophages, is mainly due to the stimulation of synovial membrane by knee sprains and surrounding soft tissue injuries, leading to cell fluid exudation and massive accumulation in joints and causing knee inflammation (16). Patients with knee weakness, swelling and pain should seek medical advice in time to avoid further deterioration of the disease into muscle atrophy; otherwise, there will be joint deformity and rigidity and the final loss of mobility as the disease progresses (17). Therefore, the diagnosis and treatment of SKJ have long been a hot spot in clinical research. By analyzing the role of the TLR4/MyD88 axis in SKJ, this study can offer new suggestions for future diagnosis and treatment of SKJ.

Significantly higher peripheral blood TLR4 and MyD88 mRNA levels were found in SKJ patients versus healthy controls in this study, indicating obvious activation of the TLR4/MyD88 axis in SKJ, which was consistent with previous studies (18, 19). TLR4 is a transmembrane signal-transducing protein widely distributed in the surface layer of cell membranes, which can recognize and perceive some certain injury- and pathogen-related molecules, activate signal transduction, promote the synthesis and secretion of various IFs, and induce inflammatory reactions (20). While MyD88 is the first discovered adapter protein matching Toll-like receptor signal transduction pathway, which is a key protein transmitting information downstream; one end of Myd88 can recognize the kinase structure of TLR4 and form a polymer, and the other end recruits IL-1 receptor-associated kinases to self-detach after phosphorylation and bind to TRAF-6 to form a complex (21). Therefore, the TLR4/MyD88 axis has been widely concerned in multiple inflammatory diseases such as gastroenteritis and nephritis (22, 23). In SKJ, although few studies have shown the specific role of the TLR4/MyD88 axis, inhibition of its activation state has been reported to effectively promote the growth of osteoblasts in osteoarthritis (24), which undoubtedly supports the important relationship between the TLR4/MyD88 axis and skeletal inflammatory diseases. Moreover, through ROC analysis, we found that TLR4 and MyD88 mRNA had excellent diagnostic value for SKJ, which is of great significance for SKJ whose early diagnosis is relatively complex and lacks reliable clinical evaluation indicators at present. We believe that the early diagnosis rate of SKJ can be improved by detecting TLR4 and MyD88 mRNA in the future so that patients can be managed symptomatically as soon as possible and patient safety can be improved.

Further, to confirm the specific role of the TLR4/MyD88 axis in SKJ, we analyzed its correlation with the levels of IFs in SKJ patients. Similarly and unsurprisingly, SKJ patients showed elevated IL-1β, IL-6, and TNF-α than healthy controls. As we all know, inflammatory cells infiltrating synovial tissue secrete IFs such as TNF-α, IL-1β and IL-6, the main pathological factors that persist and aggravate SKJ (25). According to Pearson correlation coefficient analysis, TLR4/MyD88 signal in SKJ patients was positively correlated with IL-1β, IL-6, and TNF-α, which verifies the above viewpoint and confirms the close relationship between the TLR4/MyD88 axis and the intensification of inflammatory reaction in SKJ. Inhibition of TRAF-6 mRNA expression has been shown to reduce inflammation, which may be related to the induction of phosphorylation of the IκB kinase complex by TRAF-6 and the subsequent activation of NF-κB, a gene that participates in the transduction of various inflammatory pathways (26). Therefore, we speculate that the mechanism underlying the involvement of TLR4/MyD88 axis in SKJ may be that NF-κB will be transferred from cytoplasm to nucleus after IκB kinase is activated, initiating the expression of relevant genes in the nucleus, activating pro-IFs, and triggering an inflammatory cascade. Of course, this needs further confirmation through more basic experiments.

Finally, we found that TLR4/MyD88 was also positively correlated with WOMAC scores of patients, further confirming the relationship between the TLR4/MyD88 axis and the pathological development of SKJ. This also suggests that the status of the TLR4/MyD88 axis is expected to be an objective indicator for the development of SKJ in the future, and immunotherapy targeting TLR4/MyD88 may become a new direction of SKJ therapy after further understanding of its specific mechanism of action in SKJ.

However, more experiments are needed to test the above viewpoint. In addition, due to the limited experimental conditions, there are still many shortcomings to be addressed in this study. For example, we should further detect TLR4/MyD88 expression in SKJ by Western blot and immunohistochemistry. Furthermore, it is necessary to extend the study period to evaluate the relationship between the TLR4/MyD88 axis and the prognosis of SKJ patients.

The TLR4/MyD88 axis is significantly activated in SKJ patients, and the increase of its level is closely related to the aggravation of inflammatory reaction and pathological development of SKJ. Detection of peripheral blood TLR4 and MyD88 mRNA expression can help effectively diagnose the occurrence of SKJ. An in-depth understanding and mastering of the mechanism of the TLR4/MyD88 axis in SKJ may lay a reliable foundation for future molecular immunotherapy of SKJ.

Ethical approval
Not applicable.

Consent to publish
All authors gave final approval of the version to be published.

Competing interests
The authors report no conflict of interest.

Author contributions
Min Li and Ayinigeer Mierzhati conceived and designed the project, Yanyan Yang wrote and revised the paper. Xia Lu generated the data. Xiaoli Sun analyzed the data. All authors gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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