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Original Article

Detection of anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis patients undergoing total knee arthroplasty

Chong-Jun Guo[1], Jin-Han Lv[1], Dong-Sheng Niu[1], Tao Ma[2], Shou-Xuan Sun[3], Li-Xin Li[1], Xin Zhao[2], Long Wu[2], Qun-Hua Jin[3]

[1]Department of Orthopedics, Ningxia People's Hospital, 148 West Huaiyuan Road, Yinchuan 750021, People's Republic of China; [2]School of Clinical Medicine, Ningxia Medical University, Yinchuan 750004, People's Republic of China; [3]Department of Orthopedics, General Hospital of Ningxia Medical University, Yinchuan 750004, People's Republic of China

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Abstract: Rheumatoid arthritis (RA) is the most common chronic inflammatory joint disorder and anti-cyclic citrullinated peptide antibody (anti-CCP Ab) is regarded as a serological marker for diagnosing early and late RA. In the present study, we aimed to determine the levels of anti-CCP Ab in serum, synovial tissue (ST) and synovial fluid (SF) in RA patients undergoing total knee arthroplasty (TKA). 23 patients were included. Rheumatoid factor (RF) and anti-CCP Ab in serum were detected prior to surgery and then at 1, 3, 6 and 12 months after TKA. Synovial samples were obtained by knee arthroscopy and used for anti-CCP detection. One month after TKA, anti-CCP levels were significantly reduced ($P < 0.01$) in RA patients. However, their levels were not significantly different between pre-surgery and 1 year post-surgery ($P > 0.05$). Furthermore, anti-CCP levels in ST were much higher than in serum. These findings suggest that RA patients should continue antirheumatic therapy after TKA. ST is the preferred place for the synthesis of anti-CCP Ab.

Keywords: Rheumatoid arthritis, anti-CCP antibody, total knee arthroplasty, synovial tissue

Introduction

Rheumatoid arthritis (RA) is the most common inflammatory autoimmune disease, causing progressive joint destruction as a result of chronic synovitis. Joint contractures, fixed flexion and valgus deformities are especially evident in large joints, complicating treatments [1]. For those late RA patients, total knee arthroplasty (TKA) has proven to be a highly successful treatment for advanced RA [2].

Anti-cyclic citrullinated peptide (CCP) antibody (one of anti-citrullinated peptide antibodies, ACPA) is potentially important surrogate marker for diagnosis and prognosis in RA [3-5]. Like rheumatoid factor (RF), it has a well-documented high sensitivity and specificity for RA [6]. Moreover, it is an independent predictor of joint lesion, radiological damage and progression in patients suffering from RA [7-10]. Therefore, the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for RA give official confirmation for the use of ACPA testing in RA diagnosis [11].

Few studies reported anti-CCP Ab levels after surgery. In the present study, we tested the levels of anti-CCP Ab in serum, synovial tissue (ST) and synovial fluid (SF) in RA patients before and after TKA.

Materials and methods

Patients

This study was carried out on 23 RA patients scheduled to undergo TKA. All subjects underwent unilateral TKA and they were recruited and enrolled from November 2012 to May 2014. They were 20 females and 3 males. Their ages

Table 1. Clinical characteristics of RA and OA patients with arthroscopy

	RA (N = 23)	OA (N = 10)	P
Age (mean ± SD) (years)	55 ± 7	55 ± 5	> 0.05
Range (years)	50-58	52-70	
Sex (Female/Male)	20/3	8/2	> 0.05
Disease duration (years)	16 ± 5	15 ± 3	> 0.05
DAS28	2.8 ± 3	2 ± 0.5	< 0.05
Anti-CCP (U/ml) (pre-surgery)	200 ± 15	4 ± 2	< 0.01
RF (IU/ml) (pre-surgery)	135 ± 10	15 ± 10	< 0.01
Anti-CCP (U/ml) (1 month post-surgery)	123 ± 12	4 ± 2	< 0.01
RF (IU/ml) (1 month post-surgery)	125 ± 15	3 ± 1	< 0.01

DAS28, 28-joint count Disease Activity Score Data from pre-surgery and post-surgery were compared.

ranged from 50 to 68 years with a mean value of 55 ± 7 years (**Table 1**). Their duration of the disease ranged from 8 to 22 years with a mean value of 16 ± 5 years. The patients fulfilled the 1987 revised American College of ACR criteria for RA [12]. Twenty osteoarthritis (OA) patients without joint complaints or any rheumatological disease were selected as controls. They were 8 females and 2 males and their ages ranged from 52 to 70 years with a mean value of 55 ± 5 years (**Table 1**). Patients with any history of previous surgery or trauma to the affected knee were excluded, as were patients who refused to participate in the study. This study was approved by the institutional review board and ethical committee of Ningxia Medical University. All patients gave the informed consent.

Laboratory tests

Determination of rheumatoid factor (RF): Blood samples were collected from patients. RF was measured by immunoturbidimetry using Cobas integra RFII (Roche Diagnostics, Mannheim, Germany) according to the instructions of the manufacturer. Positivity is ≥ 20 IU/ml.

Determination of serum anti-CCP antibodies: Anti-CCP antibodies were detected by ELISA (DIASTAT Axis-Shield, Dundee, United Kingdom) according to the instructions of the manufacturer. Positivity is ≥ 5 U/ml.

Preparation of synovial tissue

During TKA surgery, synovium was sequestered, formalin-fixed, and embedded in paraffin for slide preparation. Synovial tissue sections were stained with hematoxylin and eosin (H&E) and analyzed by a light microscope. SF were collected in heparinized tubes at the time of joint capsulotomy, centrifuged at 450 g for 10 min to remove debris, and the supernatants were collected.

Assessment of knee pain and joint function

Visual analogue scale (VAS) for pain, the Hospital for Special Surgery (HSS) score, and range of motion (ROM) were evaluated before surgery and after TKA.

Statistical analysis

All the data were analyzed using SPSS 19.0 statistical package (SPSS Inc, Chicago. IL, USA). Two-tailed t-tests for paired data were used to compare results between pre-surgical and postsurgical visits. *P* values less than 0.05 were considered statistically significant.

Results

Histological analysis

Histological examination revealed signs of synovitis. Synovial hyperemia and edema were seen (**Figure 1**) in RA patients. There were a large number of inflammatory cells especially lymphocytes in the knee of RA group. While mild synovial thickening and only few inflammatory cells were found in OA group.

Anti-CCP levels

Patients were followed up for 1 year after TKA. Baseline anti-CCP levels in sera ranged from 180 to 235 U/ml (mean ± SD: 200 ± 15 U/ml; n = 23) for the RA group and from 1 to 7 U/ml (mean ± SD: 4 ± 2 U/ml; n = 10) for the OA group. One week after surgery, anti-CCP levels began to reduce. As seen from **Table 1**, their postsurgical levels at one month were sharply decreased to nearly 125 U/ml. However, their levels were not significantly different between baseline and one-year post surgery for RA group (P > 0.05). For OA group, anti-CCP levels did not differ before or after TKA.

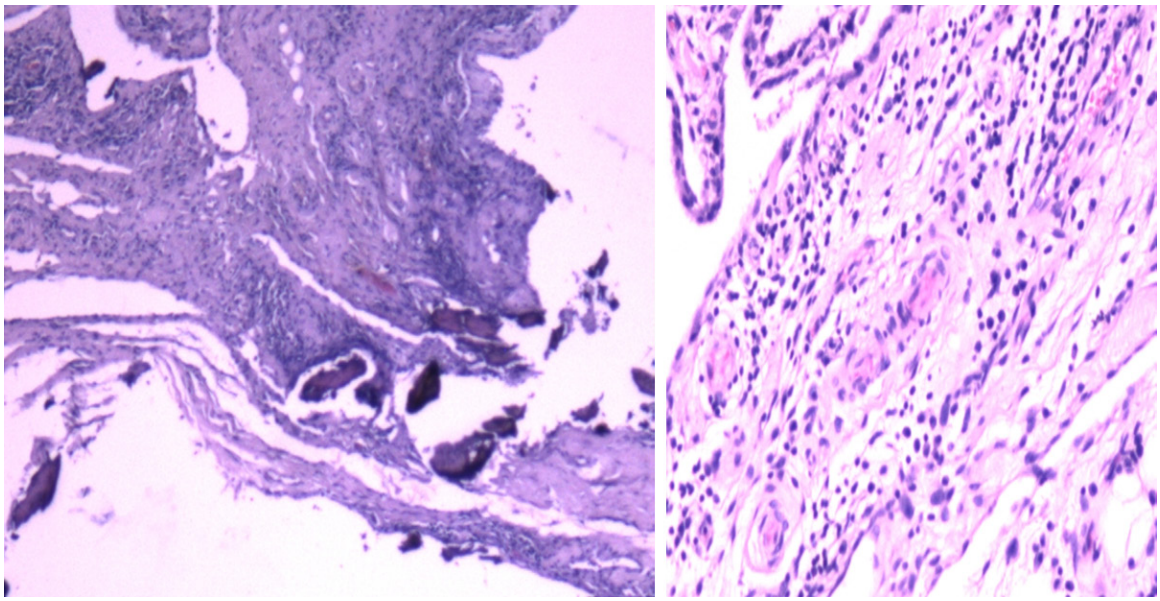

Figure 1. HE staining of arthritic synovial tissue. Sections of RA (left) and OA (right) synovial tissue were probed with anti-CCP antibodies. Original magnification ×100.

Table 2. Anti-CCP levels in ST and SF of RA patients

	RA		OA	P
(U/ml)	Active disease	Inactive disease		
ST	239 ± 15	235 ± 9	4 ± 1	< 0.05
SF	230 ± 15	232 ± 11	4 ± 1	< 0.05
Serum (pre-surgery)	225 ± 16	215 ± 13	4 ± 1	< 0.05

ST: synovial tissue; SF: synovial fluid. Data from RA and OA group were compared.

Considering these change, we observed anti-CCP distribution in ST and SA of RA patients. We wonder whether their levels would change with disease activity. However, from **Table 2**, we did not observe a decrease of anti-CCP in RA patients with inactive disease.

VAS pain reduction and knee function

The average VAS value for RA group prior to surgery was comparable with that of OA group (4.5 ± 0.9 and 4.5 ± 0.3, respectively; not significant) (**Table 3**). Pain reduction (VAS values) was statistically significant for both RA and OA groups with respect to preoperative level one month after TKA ($P < 0.05$), although it seemed more evident in OA patients.

Knee activity was significantly increased after TKA, with approximately 30 % increase from baseline in RA group, especially in active RA patients ($P < 0.05$). Throughout the study, patients in OA group showed higher ROM values in comparison with those in RA group, especially at 12 months after TKA (data not shown).

Discussion

The pathological changes in RA initially occur in synovium and mainly is chronic synovitis which is characterized by synovial hyperplasia, increased SF and pannus formation. The study on the distribution of anti-CCP antibodies in RA serum, SF and synovial tissue and the origin of these antibodies helps to understand the pathogenesis of RA [13]. It has been confirmed specific anti-CCP antibody secreting B-lymphocytes were identified from the peripheral blood, SF and bone marrow of patients [13].

All the 23 patients did not take antirheumatic drugs after TKA. The levels of anti-CCP Abs were significantly decreased after 1 week and 1 month post-surgery, but at 3 and 6 months post-surgery, they did not reduce anymore. Twelve months after TKA, their levels restored and were not significant different compared with preoperative examination. Although substantial synovial tissues were resected during surgery, this surgery did not change the system-

Table 3. VAS score and ROM before and after TKA

	RA		OA	P
	Active disease (N = 13)	Inactive disease (N = 10)		
VAS				
pre-surgery	4.5 ± 0.9	3.8 ± 0.8	4.5 ± 0.3	> 0.05
1 month post-surgery	2.8 ± 0.8	2.5 ± 0.6	2.1 ± 0.3	< 0.05
ROM				
pre-surgery	65° ± 7°	55° ± 5°	62° ± 5°	> 0.05
1 month post-surgery	85° ± 9°	88° ± 5°	91° ± 7°	< 0.01
HSS				
pre-surgery	51.3 ± 10.6	52.1 ± 9.8	54.7 ± 12.5	> 0.05
1 month post-surgery	69.8 ± 5.3	72.1 ± 4.8	73.1 ± 6.4	< 0.05

VAS: visual analogue scale; ROM: range of motion Data from pre-surgery and post-surgery were compared.

ic disease status except alleviating the knee pain. These findings indicate that the usefulness of measuring anti-CCP to monitor the clinical response to therapy is controversial [14]. After surgery, some patients still complained the pain in elbow and other small joints. We also found growth of synovial tissue in RA patients converted to surgery. In the study, pain reduction and functional recovery of joint were observed in both groups and improved until 12 months after surgery. Nevertheless, within 1 month after TKA, results seemed more evident among OA patients, but without significant differences compared to RA patients. One of OA patients even complained of pain at 1 year follow-up after surgery.

The high sensitivity and specificity of anti-CCP Ab for the diagnosis of RA is beneficial to identify with other rheumatic diseases [15]. In spite of the short follow-up of our study, the 23 patients were found anti-CCP positive. However, these 10 OA patients were negative for anti-CCP antibodies, regardless of in serum, SF or ST, indicating plasma cells secreting anti-CCP Ab were not present in OA patients [16]. Given the limited number of patients and short follow-up time, an expanded sample size and extended observation time are needed in order to confirm the above results. Future studies should focus on the role and mechanism of anti-CCP Abs in synovium damage.

Conclusions

TKA can significantly improve knee pain of patients with late RA, Anti-CCP Abs are mainly present in the joints of RA patients. Although synovial tissues were resected during TKA, anti-rheumatic therapy is necessarily needed after surgery. Rheumatoid synovium contributes to synthesis of anti-CCP antibodies.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Qun-Hua Jin, Department of Orthopedics, General Hospital of Ningxia Medical University, 804 South Shengli Road, Yinchuan, Ningxia 750004, China. Tel: +86-951-6744457; Fax: +86-951-6745560; E-mail: jinqunhua@sina.com

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