# Osteoarthritis Patients' Synovium IL-7R Expression and Value Research of Its Clinical Diagnosis

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# ABSTRACT

**Objective:** To discuss the expression of IL-7R genes in the synovium of osteoarthritis (OA) patients and the value of its clinical significance.

**Methods:** Forty-one patients who had total knee arthroplasty were selected for the OA group, from the Department of Orthopaedics in our hospital, and 36 patients who had arthroscopic surgery because of meniscus injury and cruciate ligament injury were selected for the control group. Fresh synovial tissues were collected for gene-chip analysis, real-time quantitative polymerase chain reaction (PCR) detection, haematoxylin-eosin (HE) staining experiment, immunohistochemistry experiment and detection of IL-7R gene expression. Comparison was made between the results of these two groups.

**Results:** The results of gene-chips and real-time PCR detection showed that the expression of IL-7R in OA synovial tissues in the knees was lower than that in the normal synovial tissues in the knees. The statistical analysis of IL-7R mRNA showed that the expression of IL-7R mRNA in OA synovial tissues in the knees  $(0.13 \pm 0.55)$  was lower than that in the normal synovial tissues in the knees  $(0.93 \pm 0.12, p = 0.001)$ . The result of HE staining showed that synovial tissues in the OA group had something similar to papillary hyperplasia and thickening. Immunohistochemistry research showed that IL-7R expression mainly existed in the cytoplasm of synovial tissues in the knees and the expression of IL-7R in OA synovial tissues was lower than that in the normal synovial tissues.

*Conclusion: IL-7R* expression existed both in the normal synovial tissues in the knees and OA synovial tissues, but the latter was lower.

Keywords: IL-7R expression, osteoarthritis, real-time quantitative polymerase chain reaction

# Expresión del receptor IL-7R en la membrana sinovial de pacientes con osteoartrosis e investigación del valor de su diagnóstico clínico

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# RESUMEN

**Objetivo:** Analizar la expressión de genes de IL-7R de pacientes de osteoartritis (OA) en la membrana sinovial y el valor de su significación clínica. **Métodos:** Cuarenta y un pacientes con artroplastia total de rodilla (ATR) fueron seleccionados para el grupo OA del Departamento de Ortopedia en nuestro hospital, mientras que 36

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Correspondence: Dr DF Zhang, Department of Orthopaedics, Huaihe Hospital, Henan University, Kaifeng, Henan Province, 475000, China. Email: doudongmeikf@163.com pacientes que tuvieron cirugía artroscópica debido a lesión del menisco y lesiones de ligamento cruzado, fueron seleccionados para el grupo de control. Se recogieron muestras de tejido sinovial fresco para llevar a cabo el análisis del chip genético, la detección de la reacción en cadena de la polimerasa (RCP) cuantitativa en tiempo real, el experimento con tinción hematoxilina-eosina (HE), el experimento de inmunohistoquímica (IHQ), y la detección de la expresión del gen IL-7R. Se hizo una comparación entre los resultados de estos dos grupos. **Resultados:** Los resultados del análisis de los chips genéticos y la detección de RCP en tiempo real mostraron que la expresión de IL-7R en el tejido sinovial de OA en las rodillas, fue menor que la de los tejidos sinoviales normales en la rodilla. El análisis estadístico del ARNm de IL-7R mostró que la expresión IL-7R ARNm en los tejidos sinoviales en las rodillas (0.13  $\pm$ 0.55) fue menor que en los tejidos sinoviales normales en las rodillas  $(0.93 \pm 0.12, p = 0.001)$ . El resultado de la tinción HE mostró que los tejidos sinoviales en el grupo OA tenían algo similar a un engrosamiento e hiperplasia papilar. La investigación inmunohistoquímica mostró que la expresión de IL-7R se hallaba presente principalmente en el citoplasma de los tejidos sinoviales en las rodillas, y que la expresión de IL-7R en los tejidos sinoviales de OA era menor que en los tejidos sinoviales normales.

**Conclusión:** La expresión de IL-7R se hallaba presente tanto en los tejidos sinoviales normales en las rodillas como en los tejidos sinoviales de OA, pero era menor en estos últimos.

Palabras claves: Expresión de IL-7R, osteoartritis, reacción en cadena de la polimerasa cuantitativa en tiempo real

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# **INTRODUCTION**

Osteoarthritis (OA) is a chronic progressive osteoarthropathy whose morbidity increases with ageing. The statistics of the World Health Organization show that more than half of persons over 65 years of age have clinical manifestations of OA (1). At present, the therapeutic methods are mainly non-surgical methods, such as analgesia, protection and delaying cartilage degeneration in the early stage. If cartilage is damaged badly in the later period, joint-cleaning and total knee arthroplasty (TKA) will be needed (2, 3). Longterm conservative treatment and surgery is expensive. Currently, gene therapies for OA are the hotspots in the world (4, 5). IL-7R is a kind of Type 1 cytokine receptor. It is a necessary factor in the proliferation and atomization process of lymphopoiesis and if lacking will cause a barrier to the growth of B and T lymphopoiesis (6).

Recently, research has found that IL-7R expression increases a lot on the surface of immune cells in patients with rheumatoid arthritis; interdicting IL-7R can effectively alleviate the severity of arthritis (7, 8). The current study on the relationship between IL-7R and OA shows that interdicting IL-7R can reduce the protein expression of RANKL, inhibit the signal pathway of RANK/ RANKL, lower the formation and maturity of osteoblast and influence chondrocytes (9, 10). This study mainly discusses the expression of IL-7R genes in the synovial tissues of OA patients and the value of its clinical significance.

#### SUBJECTS AND METHODS

#### **Subjects**

For the OA group, 41 patients with TKA were selected from the Department of Orthopaedics in our hospital. They were aged between 40 and 79 years, and the average age was  $66.4 \pm 9.2$  years. The control group had 36 patients who had arthroscopic surgery because of meniscus injury and cruciate ligament injury. They were aged between 26 and 60 years, and the average age was  $38.1 \pm 11.3$  years. The synovial tissues from the suprapatellar bursa in the knees of the two groups of patients were excised, placed in sterile tubes which were put in liquid nitrogen 30 minutes later and kept at  $-80^{\circ}$ C. Osteoarthritis patients met the criteria of class III and class IV of Kellgren-Lawrence.

## **Diagnosis standards of osteoarthritis**

Gonyalgia, bone rings when joints exercise, age  $\ge 40$  years, morning stiffness  $\le 30$  minutes and abnormal X-ray of knee joints were considered diagnostic.

# **Exclusion standard**

Other diseases of the blood system, arthritic disease, disease in the immune system and systematic disease in the whole body.

# **Experimental methods**

# Gene chips

Three fresh and normal synovial tissue samples were excised, and 10 fresh synovial tissues were taken from OA patients for gene-chip detection. The result of genechips was verified with real-time PCR (entrust Shanghai Sangon Biological Engineering Technology and Service Co, Ltd).

# Haematoxylin-eosin staining and immunohistochemistry experiment

Synovial tissue samples were placed in 4% paraformaldehyde solution, fixed for 24 hours at 4°C for dehydration and paraffin embedding. All samples were sliced into 4  $\mu$ m and fixed on the glass slide. The samples were stained with HE and immunohistochemistry carried out.

Immunohistochemistry experiment: seal EGPO with 3% H<sub>2</sub>O<sub>2</sub>, dispose it with heat-induced epitope retrieval, incubate it with 10% normal sheep serum, add primary antibodies, then it was placed into the fridge overnight at 4°C; it was vibrated and cleaned with PBS buffer solution (30 minutes each) and add second antibodies and tri-anti-effects, with DAB colouration, haematoxylineosin (HE) staining, and conventional sealing.

#### Real-time polymerase chain reaction

Primer design: Primer sequences of IL-7R 5'-CTGTTGGACATCTCGGCCTGT-3', are: 3 - TATCGCACCTTCTGTAAGTTCG-5 ; primer sequences of GAPDH are: 5 '-TGTTGCCATCAATGACCCCTT-3 ', 3'-GCGACTCATCGAGCACCTC-5'. Ribonucleic acid (RNA) in the synovial tissues was extracted with Trizol, the first chain cDNA was synthesized by RT kit bought from Applied Biosystems, after PCR amplified IL-7R, the real-time PCR started. IL-7R expression in the patellar synovial tissues was detected in 77 patients and likewise the expression of OA IL-7R genes.

#### Statistical treatment

The data were statistically analysed with SPSS17.0,  $(\bar{x} \pm s)$  represents measurement data, *t*-test was adopted,

and p < 0.05 meant that the difference was of statistical significance.

# RESULTS

#### Gene chips

Figure 1 shows the result of gene chips. It tested 22 882 genes, among which increasing expressed genes were 450, declining expressed genes were 809; the expression of IL-7R in OA synovial tissues was lower than that in normal synovial tissues. We used real-time PCR to verify the result of gene chips. The result showed that the expression of IL-7R in OA synovial tissues was lower than that in the normal synovial tissues.



Fig. 1: Screening results of gene chips. H2, H3, H4, H5, H7, H8, H9, H10, H11 and H12 are OA synovial tissues. GH1, GH2 and GH3 are normal synovial tissues.

# Real-time polymerase chain reaction analysis

## Electrophoresis results of total RNA

Electrophoresis results showed that real-time PCR had high specificity (Fig. 2).



Fig. 2: Electrophoresis results of total RNA. Rows 26–38 take 1st-cDNA chains of H2, H10, H3, H11, H4, H12, H5, GH1, H7, GH2, H8, GH3 and H9's samples as models.

#### IL-7R mRNA statistical analysis

The relative quantitative expression analysis of IL-7R mRNA in OA synovial tissues and normal synovial tissues in knees showed that the expression  $(0.13 \pm 0.55)$  of IL-7R mRNA in OA synovial tissues was lower than that  $(0.93 \pm 0.12)$  in the normal synovial tissues (p = 0.001).

# Real-time polymerase chain reaction detection results

Real-time polymerase chain reaction results verified that IL-7R in OA synovial tissues was lower than that in the normal synovial tissues (Fig. 3).



Fig. 3: Real-time polymerase chain reaction detection results. N1 and N2 are RNA extracted from normal synovial tissues in the knees. Syn1 and Syn2 are RNA extracted from OA synovial tissues in the knees. GAPDH is an internal control.

#### Haematoxylin-eosin staining results

Figure 3 shows HE staining results of the normal group and OA group. Haematoxylin-eosin staining in the normal synovial tissues showed fiber cells arranged regularly. We can see the thin synovial lining, and the layers were fewer (Fig. 4A). Haematoxylin-eosin staining in the OA synovial tissues showed that the synovial tissues had something similar to papillary hyperplasia. We can see the thick synovial lining, and the layers were more. Moreover, the bigger macrophages and fibroblasts can be seen, and the fluff of the synovial tissues became thicker and more fibrotic (Fig. 4B).



Fig. 4: Haematoxylin-eosin staining results. A: the control group; B: the OA group.

# Immunohistochemistry study on IL-7R

Immunohistochemistry research showed that IL-7R expression mainly existed in the cytoplasm of synovial tissues in the knees, and the expression of IL-7R in OA synovial tissues was lower than that in the normal synovial tissues (Fig. 5).



Fig. 5: Immunohistochemistry results. A: normal synovial tissues; B, C and D: OA synovial tissues in the knees.

#### DISCUSSION

Reports on IL-7R and OA in the knees are rare in China. This study examined the normal synovial tissues and OA synovial tissues in the knees and tested normal synovial tissues and OA synovial tissues with gene chips. Screened by gene chips, the expression of IL-7R in OA synovial tissues was lower than that in the normal synovial tissues. Compared with traditional PCR quantitative analysis, real-time PCR can greatly improve the accuracy of quantification. The traditional quantification tests through mid-points, optical density scanning the agarose electrophoresis banding, which is the PCR product. Its deficiency is that the accuracy of results is greatly influenced by the PCR plateau. Real-time PCR can effectively avoid this problem. Taking advantage of the Ct value and starter template, real-time PCR can quantify the linear relationship of numbers, and it has good reproducibility.

This study applied HE staining to normal synovial tissues and OA synovial tissues. The result indicated that HE staining in normal synovial tissues showed fiber cells arranged regularly. There was thin synovial lining, and the layers were fewer. Haematoxylin-eosin staining in OA synovial tissues showed that the synovial tissues had something similar to papillary hyperplasia, with thick synovial lining, and the layers were more. Moreover, bigger macrophages and fibroblasts can be seen; the fluff of the synovial tissues became thicker and more fibrotic. Immunohistochemistry result indicated that IL-7R expression showed up in the cytoplasm in synovial tissues, its expression in OA synovial tissues declined compared with that in the normal synovial tissues. Realtime PCR proves this result further. The experimental results indicated that IL-7R expression showed up in both normal synovial tissues and OA synovial tissues, but its expression in OA synovial tissues was less.

Some studies have demonstrated good treatment effects through treating collagen induced joint model with IL-7R (11, 12). Some reports show that IL7 can strongly activate Th1 and Th17 cells, but IL7 activation effect on Th2 cells is very weak. Therefore, blocking IL-7R can weaken the activation of Th1 and Th17 cells so as to weaken the expression of inflammatory factor IL-7R and interferon- $\beta$  (13, 14). Some other studies verify that interdicting IL-7R can reduce the protein expression of RANKL, inhibit the signal pathway of RANK/RANKL, lower the formation and maturity of osteoblast and influence chondrocytes (15).

In conclusion, IL-7R expression existed both in the normal synovial tissues in the knees and OA synovial tissues, but the latter was lower. The effect of IL-7R in treating OA needs further study.

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