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Cross Sectional Evaluation of Interleukin-4 and Collagen Type-1 in Knee Osteoarthritis.

Marlina[1]*, Miftahul Jannah[1], Andini Khairunnisa[1], Maya Anggrelana Zalmi[1], Hirowati Ali[2], Rizki Rahmadian[3], Rustini[1], and Fithriani Armin[1].

[1]Faculty of Pharmacy, Andalas University
[2]Department of Biochemistry, Faculty of Medicine, Andalas University
[3]Department of Orthopedic Surgery, Faculty of Medicine, Andalas University/M. Djamil Hospital, West Sumatra

ABSTRACT

Our present study is to evaluate the association of IL-4 and COL-1 in synovial tissue from knee osteoarthritis patients in Padang, West Sumatra. in this study carried the gene detection using real-time PCR. There are twelve samples of synovial tissue in patients with knee osteoarthritis obtained from 12 patients after total knee replacement surgery in several hospitals in Padang, from April to June 2016. From the results of statistical calculations using paired T test, it can be stated that there is no significant difference from the gene expression data of type I collagen and IL-4 gene seen from the p value = 0.7443. High content of collagen -1 (COL-1) has been reported for its subsequent poor mechanical properties in fibrotic articular cartilage tissue which forms the surfaces of synovial joints. Previous investigation in the process of healing, showed COL-I contained only modest amounts of glycosoaminoglycans (GAG) in poor mechanical properties of fibrocartilage and insufficient integration to native cartilage, often leading to degeneration of the repaired. Meanwhile, IL-4 exhibits as one of potent anti-inflammatory activities in OA. The increased concentration of IL-4 exhibited inhibiting effect of proteoglycans in the articular cartilage. Ratio of expression type I collagen gene in sinovyal tissue of knee osteoarthritis up regulation compared to IL-4 gene.

Keywords: Knee Osteoarthritis, Collagen tipe-1, Interleukin-4

Corresponding author

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INTRODUCTION

Osteoarthritis (OA) is one of the most arthritis diseases and the incidence is increasing appropriately elderly population. OA cause of degenerative joint disease characterized by age-related regressive change in articular cartilage (1). OA is a common cause of pain and reduced physical function in the elderly. Synovial inflammation contributes to dysregulation of chondrocyte function, favouring an imbalance between the catabolic and anabolic activities of the chondrocyte in remodelling the cartilage extracellular matrix (2). Osteoarthritis (OA) is a common joint disease involving a cascade of catabolic events that ultimately lead to abnormal and degraded articular tissues. Although OA has been considered as a non inflammatory disease, mild to moderate inflammatory changes are also seen in OA synovial tissue at certain stages of the disease. Arthroscopic evaluations of knee joint show that about 25% of subjects with painful knee OA have inflamed synovial tissue and these patients are at increased risk for radiological progression (3).

There is a multifactorial etiology of OA that might include both systemic and local biomechanical factors. Systemic factors include age, sex, estrogen levels, racial and genetic susceptibility, bone density, and many nutritional factors. The occurrence of synovitis after trauma to the knee joint may result in progressive patellofemoralchondropathy (4; 5). The synovium is an intra-articular mesenchymal tissue and essential for the normal joint function. It is involved in many pathological characteristic processes and sometimes specific for this distinctive tissue. Although osteoarthritis (OA) is commonly described as a non-inflammatory joint disease, synovial inflammation is increasingly recognized as contributing to the symptoms and progression of OA (6). Cartilage destruction contribute by released of collagenolytic enzymes (7). The major elements of the extracellular matrix are collagen fibers which represent about 65 to 80 % dry weight of tendon. These collagen fibers, which are composed of type I collagen (95 % of collagens) (8), and of some minor collagens (collagen III, V and X), provide the tendons with strength to withstand high loads. Proteoglycans, such as decorin, glycoproteins and elastin also composed tendon matrix (8,9). Progressive of OA today is generally based on the level of inflammatory. The secretion of inflammatory factors such as proinflammatory cytokines and mediators can inhibit the metabolism of joint tissues that accelerate OA. For example, Interleukin and TNF, expenditures can trigger produces MMP-1, which can degrade collagen type I, which will cause the weakness of the extracellular matrix and further aggravate the injury to the joints (10).

Proinflammatory cytokines play a role in exacerbating the inflammation but anti-inflammatory cytokines play a role in modulating the inflammatory response and protect joint tissue (11). Interleukin 4 is one of the anti-inflammatory cytokine that is primary active molecule in mechanical transduction and inhibit the inflammatory mediators (12). IL-4 is known as one of the anti-inflammatory cytokine that has the potential to keep the damage to the cartilage in arthritis to not getting worse (13). In previous studies have noted that IL-4 potentially limiting the cartilage damage caused by pro-inflammatory mediators such as interleukin 1 (14). Based on the theory we will observe gene expression of type I collagen and IL-4 gene, we assume that if expression of type I collagen upregulation and than expression of IL-4 gene will be upregulation too.

MATERIAL AND METHODS

Present study included 12 outpatients with knee OA (10 women, 2 men; mean (SD) age 59,6 (5,6) who were attending the department of orthopedic surgery in several hospitals in Padang, West Sumatra. All patients fulfilled the American College of Rheumatology criteria for Primary Knee OA.

Synovial Tissues Samples

There are twelve samples of synovial tissue in patients with knee osteoarthritis obtained after total replacement knee surgery in several hospitals at Padang, from April to June 2016.

RNA Extraction and Realtime PCR

Total RNA was extracted from synovial tissue using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to manufacturer's protocol. The concentration and purity of the RNA were determined by calculating the ratio of the absorbance at 260 and 280 nm. All samples were stored at -80^{0}C for further analyses. One microgram of total RNA was synthesized into complementary DNA using iScript reverse

transcriptase (Bio-Rad Laboratories, Hercules, CA, USA). Quantitative real-time PCR were performed using Thermocycler. The sequences of primers as follows : Type I Collagen, forward: 5`- CCCCCTCCCCAGCCACAAAG -3`, reverse: 5`-TCTTGGTCGGTGGGTGACTCT-3`, and IL-4, forward: 5`- GAAACGGCTCGACAGGAAC -3`, reverse: 5`-CTCTGGTTGGCTTCCTTCAC-3`. Amplification was carried out as follows: 36 cycles consisting of 30 sec of denaturation at 95°C, 1 min of annealing at 60°C, 1 min of extension at 65°C with an initial denaturation step of 10 min at 95°C, and final extension of 10 min in at 65°C. Relative mRNA levels were normalized to those of β-actin and are calculated as $2^{\Delta\Delta Ct}$.

RESULTS

The characteristics and clinical condition of the patients in this study were patients with knee osteoarthritis in the fourth grade and the age range from 51 until 67 years old. There are twelve samples of knee osteoarthritis consist of two are men and ten are womens. All tissue taken after total knee replacement. From this table seems that osteoarthritis is often more affected to women than men.

Table 1 : Characteristic of the Patients

Range Age	**50-67 y.o**
Sex	Female : 10 Male : 2
Cause Of OA	Trauma

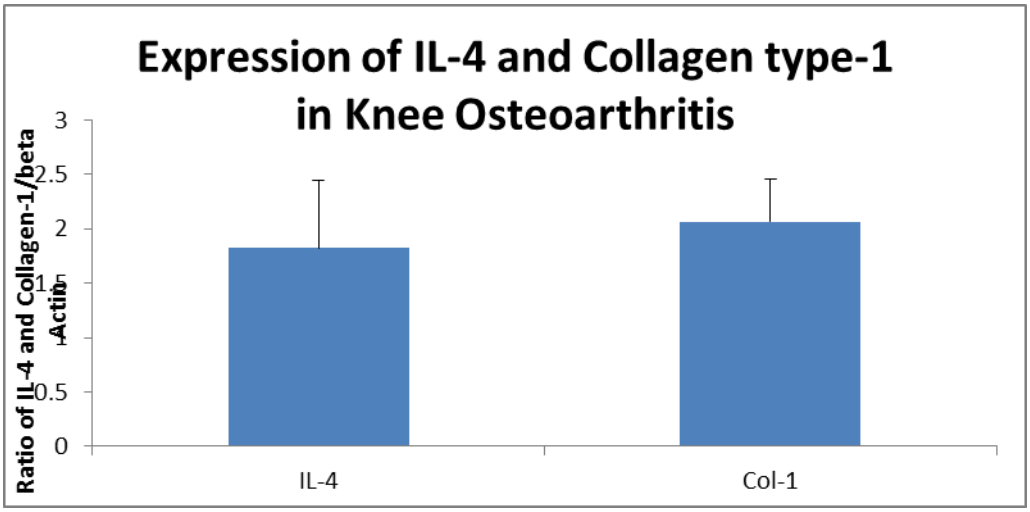

Figure 1 : chart expression of IL-4 and Collagen type-1 in Knee Osteoarthritis

From the results of statistical calculations using paired T test, it can be stated that there is no significant difference from the gene expression data of type I collagen and IL-4 gene seen from the p value = 0.7443.

DISCUSSION

Synovial tissue of osteoarthritis patient in inflammatory. When osteoarthritis of the knee has reached the fourth grade, there have been many osteophytes, there is no gap joints, there is a subchondral cysts and sclerosis this is can cause dismobilities and patient should be knee replacement surgery. In addition proinflammatory cytokines and will increasingly be produced in osteoarthritis. When the production of proinflammatory cytokines and increased the Matrix Metalloproteinase (MMP) will increasingly be produced. While MMP will degrade proteoglycans and collagen type I. As feedback from this condition, chondrocytes will

proliferate and synthesis molecules of collagen and proteoglycans. Type I collagen gene expression is stimulated due to the increased expression of TGF-β_1 gene expression, resulting in increased binding of TGF-β_1 on cell membrane receptors and fibroblasts which would result in a fibrosis.

From the results of real-time PCR, seen the average CT value for type I collagen of 30.1. The results from calculation of the ratio type I collagen gene expression with reference genes obtained average value ratio of 2.06, this value is large enough so that it can be assumed that the type I collagen gene expression in these tissues is high. These results are relevant to several previous studies including Miosge et. al (2004) which shows the results of type I collagen gene expression in osteoarthritis of the knee were higher in satge III (CT value of 27.7 and ratio of 0.99) compared with stage I (CT value of 31.2 and ratio of 0.0015). On the other hand the relevant results were also seen in the results of research Martin et. al (2001) which shows the average gene expression of collagen type I in patients with osteoarthritis is higher than normal patients 3050.6-fold. Recent research Zhong et. al (2016) also states that the expression of type I collagen gene will upregulation with the development of osteoarthritis disease, so it can be said type I collagen gene expression is upregulation in synovial tissue of knee osteoarthritis patients in Padang .

Interleukin 4 as antiinflammatory cytokines has been found in synovial knee osteoarthritis at our research refers to downregulation. While, actually IL 4 involved in articular cartilago normal and mechanisme anabolic and protective effect in osteoarthritis. Interleukin 4 in osteoarthritis case refers that IL 4 fail to protect the cartilago.

In this research we try to analysed expression of interleukin 4 and type 1 collagen gene in Indonesian especially minangnese. Some studies have found and shown that they are research about polymorphisms Interleukin 4 gene like Vargiolu et al reported that there is no association between osteoarthritis and IL 4 gene polymorphism in Caucasian population but they studies shown that association between interleukin 4 receptor and hand osteoarthritis ini Caucasian population (12). Then Forster et al in their study reported that IL 4 gene influence to hip osteoarthritis in Caucasian females (15).

In this research we got that interleukin 4 expression is low. Which no significant difference from the gene expression data of type I collagen and IL-4 gene. Then we can conclude too that women aged more than 50 years old are high risk to afffected of osteoarthritis and osteoarthritis is often more affected to women than men.

CONCLUSION

The result of research in synovial tissue with osteoarthritis can be concluded that no significant difference from the gene expression data of type I collagen and Interleukin 4 gene from knee osteoarthritis patients in Padang. Ratio of expression type I collagen gene compared with Beta Actin gene in synovial tissue with knee osteoarthritis more than IL-4 gene in Padang.

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