Clinical Significance of Inflammatory Factors Within the Synovial Fluids in Patients With Knee Osteoarthritis for Predicting Meniscus Degeneration Progression

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Research article

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Abstract

Background: This research aims to investigate the expressions and clinical significance of inflammatory factors in the synovial fluid of the meniscus degeneration with knee osteoarthritis (OA).

Methods: A total of 100 patients with meniscus degeneration in knee OA were divided into stage II and III groups based on MRI imaging. The expressions of inflammatory factors including interleukin-1 β (IL-1 β), IL-17, tumor necrosis factor- α (TNF- α) and tumor growth factor- β 1 (TGF- β 1) in the knee joint fluid were determined by means of an ELISA and chemiluminescence assay. International general Lysholm score and correlation between the levels of four cytokines were further analyzed.

Results: Compared with stage II group, patients in stage III group had significantly increased levels of IL-1 β in the synovial fluid along with increases in TNF- α ($P < 0.05$) and higher levels of IL-17 ($P < 0.05$). In addition, stage II group also showed a decrease in TGF- β 1 ($P < 0.05$). The levels of IL-1 β , TNF- α and IL-17 were negatively correlated with Lysholm score whilst TGF- β 1 was positively correlated with Lysholm score ($P < 0.05$). The values of the area under the receiver operating characteristic (ROC) curves for the four cytokines in the diagnosis of meniscus degeneration in knee OA were 0.652, 0.604, 0.714 and 0.391 respectively.

Conclusions: The high expression levels of IL-1 β , TNF- α and IL-17, and the low expression levels of TGF- β 1 in knee joint fluid are related to the severity of meniscus injury in patients with knee OA. These cytokines are associated with the pathological development of knee injury and ultimately lead to the decline of knee joint function.

Introduction

With an increasingly aging population, the frequency of people with knee osteoarthritis (OA) is increasingly prevalent. Studies in developing countries such as China and India show that the incidence of knee OA has reached up to 28.7%, and is associated with a number of independent risk factors such as gender, age and obesity [1, 2]. The knee OA is characterized by a reduction in bone mass and bone micro-structure deterioration, representing osteo-articular degeneration and articular cartilage degradation, which was influenced by genetics, biomechanics, trauma, dysplastic tissues in addition to body weight [3]. As one of the most important weight-bearing joints in the human body, knee OA is very common and can have major consequences on quality of life such as loss of mobility. For knee OA, a large number of neutrophils and oxidative stress metabolites are locally induced, which can enhance damage and degeneration of the meniscus [4]. The meniscus plays an irreplaceable role in lubricating joints and maintaining the bio-mechanical properties of the knee joints, and so chronic degeneration is also an important cause of knee OA [5, 6, 7].

Numerous factors influence knee OA progression. It has been suggested that the elevated levels of inflammatory cytokines within the joint environment were associated with OA in addition to knee trauma [8, 9].

Currently, confirmation of linkage with OA has been recorded for interleukin-1 (IL-1), IL-6, tumor necrosis factor- α (TNF- α), IL-10 and Macrophage Migration Inhibitory Factor (MIF) [10, 11]. However, a panel of IL-1 β , TNF- α , tumor growth factor- β 1 (TGF- β 1) and IL-17 in the synovial fluid and meniscus injury remains unclear. The main aim of this study was to determine the clinical significance of inflammatory factors in the articular fluid of meniscus degeneration in knee OA.

Materials And Methods

Study participants

The study was conducted at the Jilin Clinical Research Center for Knee Disease, Associated Hospital of Beihua University from January 2017 to January 2018. A total of 100 cases of patients with knee OA and meniscus degeneration treated were enrolled from the hospital. Human experimental protocols to collect the clinical samples were approved by ethical committees of Associated Hospital of Beihua University (formal ethical approval number: Protocol Number 2016-10-1), and written informed consent was gained from all participants in the study.

Inclusion, exclusion criteria and groups

The diagnosis of knee OA was based on the Kellgren and Lawrence system in combination with magnetic resonance imaging (MRI) grading modified Outerbridge system [12, 13]; if (1) knee OA was diagnosed by X ray showing single knee OA (asymmetry joint space narrowing, subchondral bone sclerosis and cystic degeneration, hyperosteoecy of the joint edges and osteophyte formation, articular mobile corpus or joint deformity), while displaying clinical symptoms such as pain. (2) Meniscal degeneration was graded by MRI: Grade 0 included $\frac{1}{4}$ normal articular cartilage, Grade I $\frac{1}{4}$ superficial softening; Grade II included $\frac{1}{4}$ superficial fracturing or defibrillation involving $<1.25\text{cm}$ area, the appearance of mild high signal lines in the meniscus, the sac margin of the meniscus was direct to the free margin, but it did not affect the joint margin, and the capsule edge of the meniscus reached the free edge. Grade III included $\frac{1}{4}$ fibrillation or fissuring involving $>1.25\text{ cm}$ area. The appearance of slightly high signal lines within the meniscus showed a significant effect on the joint margin of the meniscus. Grade IV included $\frac{1}{4}$ full-thickness cartilage wear by exposing the subchondral bone. The meniscus form was incomplete, partial defect, irregular thinning and morphological disappearance or meniscus was separated from the joint sac.

Inclusion criteria were defined as knee OA, meniscus degeneration MRI grade II or III, 18-75 years old, and agreement to participate in this study. Exclusion criteria were defined as other serious diseases such as tuberculosis of the knee, history of previous knee surgery, treatment with sodium hyaluronate injection for special treatment in the past month, rheumatism / rheumatoid arthritis, infection, connective tissue disease, and inability to complete the study.

All patients included in the study were divided into stage II group (patients with OA of Grade II, $n = 57$) and stage III group (patients with OA of Grade III, $n = 43$) based on the MRI grading modified Outerbridge classification. The age of patients ranged from 42 - 73 years (average 61.61 ± 5.53 years), with 22 males

and 35 females in the group I. The disease course ranged from 2 -11 years (average disease course of 5.88 ± 2.02 years). The age of patients ranged from 40 - 74 years (average 61.65 ± 4.92 years), 16 cases were males and 27 cases females in the group II. The disease course ranged from 1 - 12 years (average disease course of 6.22 ± 1.98 years).

Data collections

Cytological measurement of knee joint fluid samples was performed. Primary outcomes included levels of IL-1 β , TNF- α , TGF- β 1 and IL-17 in the knee joint fluid. For each operative case, a minimum of 1 ml of synovial fluid was aspirated from the knee joint prior to orthopedic surgery via direct needle aspiration. Samples were placed in a BD Vacutainer test tube and centrifuged at 3,000 rpm for 10 minutes. The supernatant was stored at 20 °C until the assay. The level of IL-1 β , TNF- α and TGF- β 1 was measured using an automated chemiluminescence analyzer (Siemens immune 1000, Berlin, Germany) according to the files (EP15-A2, EP6-A2, C28-A2) of the Clinical Laboratory Standardization Institute (CLSI) [14]. IL-17 levels were measured by means of an enzyme-linked immunosorbant assay (ELISA) with the Quantikine® HS Human IL-17 Immunoassay kit (R&D Systems, MN, USA). The limit of detection was lower than 2 pg/mL for IL-1 β , IL-17 and 5 pg/mL for TNF- α , TGF- β 1, and the intra-batch CV and the inter-batch CV were < 9 % and <15 %. Additionally, Lysholm score was determined according to eight scoring items including lameness, support, interlocking, instability, pain, swelling, stair climbing, and squat according to the previously described [15]. The total score that can be obtained is 100, with a lower score indicating more severe symptoms.

Statistical analysis

Data are expressed as values with standard deviation (mean $\pm$ SD). Statistical analysis was performed using SPSS (version 22.0) and with MATLAB (version R2010b; Math Works). The data of each group were tested for normality and homogeneity of variance (Levene's test). Normality of data distribution was assessed by the Jarque-Bera test. The measured data from the two groups were analyzed by independent sample *t*-test. When the cytokine concentrations were non-Gaussian distribution, Mann–Whitney–Wilcoxon (MWW) tests was utilized. The count data from the two groups were analyzed using a chi-square test. Analysis of variance (one-way ANOVA) and post hoc Bonferroni multiple comparisons test was utilized to compare differences between groups meeting normality and homogeneity of variance. Receiver operating characteristic (ROC) curves and areas under the ROC curve (AUC) were used to evaluate the clinical usefulness of each of four markers, which represented the diagnostic probability and accuracy of a true result in ascertaining the knee OA. Correlations between cytokines and clinical score were assessed for significance using the non-parametric Spearman's rank correlation coefficient test. *P* values of < 0.05 indicated statistically significant differences.

Results

Expressions of inflammatory factors in knee joint fluid

Demographic data analysis of 100 cases of patients was given in Table 1. There were no statistical differences in the age, gender and disease course between the two groups ($P>0.05$). Compared with stage II group, IL-1 β in the synovial fluid of

stage III group was significantly higher (25.53 ± 6.60 vs. 21.84 ± 4.96 ng/ml, $P = 0.003$). Also, TNF- α showed a demonstrable increase (37.86 ± 5.54 vs. 34.87 ± 7.72 ng/ml, $P = 0.026$), along with IL-17 (39.31 ± 8.55 vs. 32.45 ± 8.30 ng/ml, $P = 0.000$). In contrast, TGF- β 1 was significantly reduced (57.94 ± 13.78 vs. 64.54 ± 16.24 ng/ml, $P = 0.031$), as showed in Table 2.

Analysis of the correlation between Lysholm score and inflammatory factors

Lysholm score was significantly lower in stage III group compared with the stage II group (52.28 ± 7.13 vs. 60.79 ± 6.44 , $P = 0.000$) (Figure 1). Lysholm score and inflammatory factors in study patients demonstrated that IL-1 β , TNF- α and IL-17 were negatively correlated with Lysholm score ($P < 0.05$), whilst TGF- β 1 was positively correlated with Lysholm score ($P < 0.05$) (Table 3).

Diagnostic values of inflammatory factors on the knee OA

The values of the area under the ROC curve for IL-1 β , TNF- α , IL -17 and TGF- β 1 were 0.652, 0.604, 0.714 and 0.391 respectively. The optimum diagnostic cut-off values were 22.75 ng/ml (specificity 0.674, sensitivity 0.614) for IL-1 β , 34.05 ng/ml (specificity 0.767, sensitivity 0.491) for TNF- α , 36.45 ng/ml (specificity 0.605, sensitivity was 0.316) for IL -17 and 62.55 ng/ml (specificity 0.372, sensitivity 0.491) for TGF- β 1, as shown in Figure 2. The findings indicated that the diagnostic tests in cytokines patterns had high sensitivities to predict knee OA heterogeneity and measure progression.

Discussion

In recent years, traffic and sports injuries, and the aging of the population have caused an increasing occurrence of knee OA, which is one of the major diseases in the middle-aged and elderly [16]. For patients with knee OA, the vast majority are associated with meniscus injuries. The main physiological function of the meniscus is to lubricate the joints and deepen the joints. Meniscus also has multiple functions such as absorbing shock, cushioning pressure, transmitting loads, protecting articular cartilage and maintaining the biomechanics of the knee joints. It plays an important role in maintaining the normal function of the knee joints [17, 18].

Knee injury including trauma, meniscus tears or the chronic cartilage degeneration, which resulted in a large number of infiltrating neutrophils and release of inflammatory cytokines into synovial fluid in addition to oxidative stress products, leads to knee OA [19]. The level of inflammation in the knee is low in normal physiological conditions. For knee OA, many cells such as articular cartilage become apoptotic, causing a range of inflammatory reactions in knee joints. In spite of multiple factors concerned in the injury process, the ability to predict knee OA heterogeneity and measurable progression is more likely to

exhibit the development of disease and administration of modifying drugs in advance during a defined time period [20].

In the present study, we compared two groups of patients with knee OA concerning the role of inflammatory mediators in the process of chronic degeneration in knee OA from MRI of knee Grade II to Grade III. Our findings indicated that the elevated levels of TNF- α , IL-1 β and IL-17, and decreased TGF- β 1 suggested that inflammatory factors were involved in the degeneration of the meniscus. In previous studies, several studies have confirmed that TNF- α and IL-1 β within synovial inflammation were associated with meniscus or cartilage degeneration as primary inflammatory mediators in humans or animals. Our study was in agreement with these reporters [21, 22]. High levels of TNF- α , IL-1 β and IL-17 in knee traumatic areas demonstrated the level of meniscus or cartilage damage and the presence of a local inflammatory response that triggers early knee OA [23].

To illustrate, both TNF- α and IL-1 β are pro-inflammatory factors, which have been detected in synovial fluid associated with knee trauma and OA [24]. TNF- α can act on articular cartilage through both autocrine and paracrine pathways to degrade the cartilage matrix of the knee joints. In addition, TNF- α can also produce matrix metalloproteinases by stimulating synovial fibroblast nuclear chondrocytes and promote degradation of proteoglycans and collagen in the meniscus cartilage of knee joints. IL-1 β can inhibit the proliferation of chondrocytes by interfering with the metabolism of meniscus chondrocytes, and activate the matrix metalloproteinase signaling pathway. Consequently, both TNF- α and IL-1 β lead to the degradation of articular cartilage and accelerate chronic degeneration of the meniscus [25].

IL-17 is also a pro-inflammatory cytokine, which has been declared to increase synovitis and joint destruction following intra articular injection [26]. IL-17 is mainly secreted by Th17 and can promote the proliferation and activation of T cells. Activated T cells produce a large number of cytokines such as TNF- α and IL-1 β , to accelerate meniscus injury. Additionally, TGF- β 1 can regulate the proliferation, differentiation and synthesis of matrix of articular chondrocytes, and antagonize the action of other inflammatory factors such as IL-1 β and TNF- α [27]. In addition, TGF- β 1 can accelerate the metabolism of proteoglycan, stimulate the synthesis of collagen and glucosamine in articular cartilage, and inhibit the production of matrix metalloproteinases. TGF- β 1 can therefore promote cartilage repair and inhibit the degeneration of the meniscus [28]. Our study indicated that TGF- β 1 was an inhibitor of inflammation, which was administrator of modifying TGF- β 1 drugs to interfere with the progression of knee OA in advance. To the best of our knowledge, we are the first report to highlight the high level of IL-17 as pro-inflammatory and low level of TGF- β 1 as anti-inflammatory cytokines.

In the present study, we applied ROC curves as a means of evaluating diagnostic tests to quantify the overall ability of two methods to discriminate among four outcomes. The values of the area under ROC curves of L-1 β , TNF- α , IL-17 and TGF- β 1 in the diagnosis of knee meniscus degeneration were 0.652, 0.604, 0.714 and 0.391 respectively. IL-1 β , TNF- α and IL-17 were negatively correlated with Lysholm score whilst TGF- β 1 was positively correlated with Lysholm score. Our findings demonstrated that the more severe the meniscus degeneration, the higher the levels of IL-1 β , TNF- α , IL-17, and the lower the level of

TGF- β 1, which can consequently impact knee joint function. These findings further confirmed the role of various inflammatory factors in the pathogenesis of meniscus degeneration of the knee OA. Levels of inflammatory mediators are significantly related to severity and can be widely used to predict the clinical outcome of patients [29].

In summary, the high expression of IL-1 β , TNF- α , and IL-17, and low expression of TGF- β 1 in knee joint fluid were related to the severity of meniscus injury and mediated the progressive decline of knee joint function. At the same time, these inflammatory mediators involved in the development of the injury, eventually leading to decreased knee function.

Abbreviations

IL-1 β : interleukin-1 β ; IL-17: interleukin-17; OA: osteoarthritis; ELISA: enzyme-linked immunosorbant assay; TNF- α : tumor necrosis factor- α ; TGF- β 1: tumor growth factor- β 1

Declarations

Acknowledgments

Not applicable

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Authors' contributions

Dalin Wang conceived, designed the experiments and wrote a draft manuscript. Mingcheng Li analyzed, interpreted the results of the experiments and revised the manuscript. Zhe Wang and Weihai Jiang performed the experiments. All authors read and approved the final manuscript.

Availability of data and materials

All data generated or analyzed during this study are available in this published article.

Ethics approval and consent to participate

Human experimental protocols to collect the clinical samples were approved by ethical committees of Associated Hospital of Beihua University (formal ethical approval number: Protocol Number 2016-10-1), and written informed consent was gained from all participants in the study.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests. The authors alone are responsible for the content and writing of the paper.

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Tables

Table 1 Comparison of basic clinical data recorded across the two groups of patients

Groups	n	Age	Gender (males)	Course (years)
StageⅡ	57	61.61 ± 5.53	22 (38.60%)	5.88 ± 2.02
Stage III	43	61.65 ± 4.92 [#]	16 (37.21%) [#]	6.22 ± 1.98 [#]
<i>t</i> value		0.038	0.020	0.840
<i>P</i> value		0.970	0.887	0.403

Note: Data are presented as mean±standard deviation of two groups.

[#]*P*>0.05 compared with the StageⅡgroup determined by ANOVA and post hoc Bonferroni multiple comparisons test.

Table 2 Expressions of inflammatory factors in the knee joint fluid of the two groups

Groups	n	IL-1 β (ng/ml)	TNF- α (ng/ml)	TGF- β 1 (ng/ml)	IL-17 (ng/ml)
Stage II	57	21.84 $\pm$ 4.96	34.87 $\pm$ 7.72	64.54 $\pm$ 16.24	32.45 $\pm$ 8.30
Stage III	43	25.53 $\pm$ 6.60*	37.86 $\pm$ 5.54*	57.94 $\pm$ 13.78*	39.31 $\pm$ 8.55*
<i>t</i> value		3.067	2.255	2.193	4.026
<i>P</i> value		0.003	0.026	0.031	0.000

Note: IL=interleukin; TNF=tumor necrosis factor; TGF=tumor growth factor

Data are presented as mean $\pm$ standard deviation.

**P*<0.05 compared with the Stage II group determined by ANOVA and post hoc Bonferroni multiple comparisons test.

Table 3 Correlation between Lysholm score and inflammatory factors

Category	IL-1 β	TNF- α	TGF- β 1	IL-17
<i>r</i> value	-0.250	-0.238	0.201	-0.348
<i>P</i> value	0.012	0.017	0.044	0.000

Note: IL=interleukin; TNF=tumor necrosis factor; TGF=tumor growth factor

Correlation between IL-1 β , TNF- α , IL-17 and TGF- β 1 in knee OA patients. Logarithm-transformed IL-1 β , TNF- α and IL-17 levels negatively correlate with Logarithm-transformed Lysholm score of knee OA (*P*< 0.05). whilst Logarithm-transformed TGF- β 1 levels positively correlate with Logarithm-transformed Lysholm scores of knee OA (*P*< 0.05).

Figures

Figure 1

Comparison of Lysholm score in two patient groups Note: IL=interleukin; TNF=tumor necrosis factor; TGF=tumor growth factor * $P<0.05$ compared with the stage III group determined by ANOVA and post hoc Bonferroni multiple comparisons test.

Figure 2

Diagnostic values of inflammatory factors in the joint fluid of knee meniscus degeneration Note: ROC=Receiver operating characteristic; AUC=areas under the ROC curve; IL=interleukin; TNF=tumor necrosis factor; TGF=tumor growth factor; The values of the area under the ROC curve for IL-1β, TNF-α, IL-17 and TGF-β1 were 0.652, 0.604, 0.714 and 0.391 respectively.