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Clinical Therapy of Shujin Huoxue Tablet Combined with Sodium Hyaluronate on Inflammatory Factors of Synovial Fluid (IL-6, TNF-α) in the Treatment of Knee Osteoarthritis: Clinical Trial Study

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Authors' Contribution
GW, XT and JD conceived the research idea and designed the study. LL, JL and BZ visited the dairy farms and collected the data. GW and XT performed data analysis. GW, LL and JD wrote the paper.

Key words
Shujin Huoxue tablets, Sodium hyaluronate, Combination therapy, Knee osteoarthritis, Synovial fluid inflammatory.

ABSTRACT

Sodium hyaluronate are used to reduce pain and improve function in patients with knee osteoarthritis (KOA) and in traditional Chinese medicine, Shujin Huoxue Tablets (SJHXT) is known to be a useful medicine for KOA. The aim of this study was to investigate the effects of SJHXT combined with sodium hyaluronate on synovial inflammation in a patients with KOA. A total of 180 patients with knee osteoarthritis who were treated in our hospital were enrolled as the study subjects who were divided into study group and reference group, each with 90 cases. The reference group received sodium hyaluronate injection treatment, while the study group was given SJHXT combined with sodium hyaluronate. The therapeutic effects were compared between the two groups. Comparison of Lysholm knee score scale, WOMAC score, and joint VAS score between the two groups showed that the study group had a significant advantage over the reference group after treatment, $p<0.05$. Comparison of synovial fluid inflammatory factors before and after treatment between the two groups indicated more obvious improvement in the study group than in the reference group, $p<0.05$. Comparison of overall treatment efficiency of the two groups showed significantly higher efficiency in the study group than in the reference group, $p<0.05$. To conclude SJHXT combined with sodium hyaluronate can produce good therapeutic effects for patients with knee osteoarthritis, which can positively affect the synovial fluid inflammatory factors so that effectiveness of adjuvant treatment can be improved.

INTRODUCTION

Knee osteoarthritis (KOA), the most frequent degenerative articular disorder in middle-aged and the elderly, is considered one of the primary causes of pain and functional disability (Long *et al*., 2020; Vos *et al*., 2010). KOA may be triggered by excessive joint forces, defects to the articular cartilage or subchondral bone. Chondrocytes become metabolically active and initiate inflammatory processes that secrete several inflammatory cytokines that work synergistically to stimulate synthesis of enzymes that break down cartilage. Key cytokines include interleukin-1 (IL-1β), tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) (Ricci *et al*., 2017). IL-1β and TNF-α are commonly increased in inflamed joints, and these cytokines activate other inflammatory chemokines such as monocyte chemotactic proteins and others (Ding *et al*., 2017). These changes are progressive and frequently identified by radiographic and symptomatological signs.

Intriguing, but very limited evidence also suggests a role for oxidative stress-related degradation processes in human OA (Regan *et al*., 2008). Oxidative stress represents the imbalance between endogenous antioxidant defenses and the free radical induced pro-oxidant. Relevant to osteoarthritis, byproducts of lipid oxidation such as 4-hydroxynonenal (4-HNE) induce cell damage and death of chondrocytes (Abusarah *et al*., 2017; Benabdoune *et al*., 2016). An imbalance of antioxidant defenses relative to oxidative processes has been shown to exist in human OA. The levels of oxidatively damaged byproducts such as lipid peroxides, are high in synovial fluid in patients with OA (Regan *et al*., 2008; Qin *et al*., 2019).

synovial fluid contains high levels of hyaluronic acid (HA) that help to maintain high fluid viscosity of the joint by attenuating inflammation and preserving the normal cartilaginous matrix (Lee *et al*., 2019; Fu *et al*., 2019). HA is a polysaccharide produced by the chondrocytes and synoviocytes. While HA may help to lubricate and cushion the joint (Altman, 2003), it can help maintain cartilage matrix and minimize inflammation. In OA, the molecular weight and concentration of HA are reduced (Bagga *et*

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al., 2006), thereby lowering fluid viscosity and elasticity. Protection against articular injury is compromised and OA damage ensues. In vitro data suggest supplemental HA can suppress IL-1β production (Takahashi *et al*., 1999), and may increase synovial fluid viscosity.

Sodium hyaluronate are used to reduce pain and improve function in patients with KOA, or for patients with severe knee OA who wish to delay joint replacement surgery or for whom surgery is contraindicated (Bruyere *et al*., 2016). On the other handin traditional Chinese medicine, SJHXT is known to be a useful medicine for KOA patients (Dong, 2007). SJHXT have the main ingredients as follows: safflower, rhizoma cibotii (prepared), mistletoe, herba lycopi, caulis spatholobi, chinese starjasmine stem, lycopodium clavatum, rhizoma cyperi (prepared), cortex periplocae, native copper (forged) (Shaoqi *et al*., 2016). We hypothesize that SJHXT and sodium hyaluronate can reduce the overall inflammatory response in human KOA. The function of SJHXT is to relax tendons and activate collaterals, quicken blood and dissipate stasis. It is applicable to arthralgia and myalgia, limb contracture, low back pain, traumatic injuries (Shaoqi *et al*., 2016). Clinical practice and anecdotal evidence suggest that HA may be more beneficial in mild to moderate OA (Dagenais, 2006). However, most of evidence on disease severity and age has been derived from animal and human models of OA (Kyostio-Moore *et al*., 2018; Plaas *et al*., 2011; Piuzzi *et al*., 2018).

This study analyzed the clinical efficacy and influence of Shujin Huoxue tablet combined with sodium hyaluronate on inflammatory factors of synovial fluid in the treatment of knee osteoarthritis, and made the following report.

MATERIALS AND METHODS

Patients

Patients (N=180) with chronic knee OA were recruited from the UF Orthopaedics Clinics. The inclusion criteria were a diagnosis of knee OA, degenerative joint disease or joint. The patients were treated in our hospital from January 2016 to June 2019. Inclusion and exclusion criteria are: unilateral knee disease and degenerative disease; repeated pain in the affected joint within one month; no analgesiaor drug therapy three months before the diagnosis; no pregnant and lying-in woman, no allergy, no liver and kidney dysfunction, no cardiovascular and cerebrovascular diseases, no cancer, no trauma and viral infection. Patients and their families have the right to know and signed an informed consent form. The patients were randomly divided into study group and reference group, each with 90 cases. The study group had 50 males and 40 females, with an average age of 60.9±3.2 years. The reference group had 88 males and 82 females, with an average age of 59.8±3.5 years. Comparison of relevant data of the two groups reveals comparability, $p>0.05$.

Reagents and instruments

The enzyme-linked immunosorbent assay (EL-ISA) kits for IL-6, hs-CRP, and TNF-α were pur-chased from Duo-Set kits; R&D Systems (Minneapolis, MN, USA). Bio-Rad 680 Microplate Reader was purchased from Bio-Rad Laboratories, Inc. (Her-cules, CA, USA). The arthroscopy system Dyonics was purchased from Smith and Nephew (Memphis, TN, USA).

Methods

The reference group was given routine sodium hyaluronate injection treatment, which was to intra-articularly inject sodium hyaluronate injection (manufactured by Shandong Bausch & Lomb Freda Pharmaceutical Co., Ltd., batch number 140603, specification 2 ml: 20 mg). Patients took a sitting position and bent the knee at 90° angle. After routine sterilization, the joint effusion was removed. With the lateral knee eye as the puncture point, sodium hyaluronate injection was injected into the affected joint cavity using a syringe, 2ml each time and once a week. Five weeks constitute a course of treatment. There were two courses of continuous treatment. The study group was treated with Shujin Huoxue tablets combined with sodium hyaluronate. Sodium hyaluronate was given with the same measure as the reference group. SJHXTare for oral administration (Pharmaceutical Factory of Beijing Tongrentang Science and Technology Development Co., Ltd., lot number 140702, specification 0.3g* 60 tablets), three times a day and 5 tablets each time. Five weeks constitute a course of treatment. There were two courses of continuous treatment. At the end the knee joint synovial fluid was extracted before the surgeryfollow-up by the same orthopedic physician. A total of 2 mL synovial fluid was extracted as specimens. The supernatant was separated after centrifugation, which was kept in the -80°C freezer for future ELISA testing.

VAS , Lysholm and WOMAC scores before and after surgery

The VAS and Lysholm scores of 180 KOA patients were evaluated before the surgery and at the six-month follow-up by the same orthopedic physician. VAS scores range from 0 to 10 points (Norris *et al*., 2018). 0: painless; 1-3: a slight pain which can be tolerated; 4-6: pain affects sleep but still can be endured; 7-10: intense pain affecting and sleep, which cannot be toler-ated any longer. Lysholm score is an interna-tionally applied rating scale for knee function general evaluation, which includes limp, sup-

port, locking, instability, pain, swelling, stair-climbing, and squatting. >90 is defined as ex-cellent; 84-90 is defined as good; 65-83 is defined as fair; <65 is defined as poor.

At baseline and at each follow-up visit the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (Lee *et al*., 2017). For each of the 24 questions of the WOMAC index patients had to give a mark using a 5 point Likert scale (0=none, 1=mild, 2=moderate, 3=severe, 4=extreme) giving a total score ranging from 0 to 20 for WOMAC pain sub-score, 0 to 68 for WOMAC function and 0 to 96 for WOMAC total.

Observation indicators

The overall treatment efficiency was compared between the two groups. Evaluation was made according to the "Guidelines for the Clinical Research of New Drugs in Traditional Chinese Medicine". If Lysholm knee score scale (Lysholm score) is improved by more than 70% after treatment, the treatment is significantly effective; if the Lysholm score is improved by 30%-69% after treatment, the treatment is effective (Hao *et al*., 2017) if the Lysholm score is improved by less than 30%, the treatment is ineffective. At the same time, the two groups were compared in terms of Lysholm score, osteoarthritis index rating score (WOMAC score), visual analog scale of knee joint (joint VAS score), synovial fluid inflammatory factor interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), high-sensitivity C-reactive protein (hs-CRP) levels.

Inflammatory factors measurement

The concentration of IL-6, hs-CRP, and TNF-α in joint synovial fluid was determined by ELISA using the commercially available enzyme-linked immunoabsorbent assays according to the manufacturer's instructions (Quantikine, R&D Systems, Minne-apolis, MN, USA). Optical densities were determined using a microtiter plate reader (Multiscan RC Type 351; Labsystems, Helsinki, Finland) at 405 nm, with a correction wavelength set at 540 or 570 nm. The blank was subtracted from the duplicate readings for each standard and sample without any knowledge of survival or other clinical data. Concentrations are expr-essed as ng/mL for IL-6and TNF-α and mg/L for hs-CRP. All of the analyses and calibrations were carried out at least in duplicate. The mean values were used for statistical analyses.

Statistical analysis

For statistical analysis, IBM SPSS 21.0 statistical software was used to analyze the data. Continuous data were expressed as mean ± average ($\bar{x} \pm s$). Categorical data were expressed as percentages. Intergroup comparisons were performed using Chi-square test (or Fisher's exact test) or analysis of variance or rank sum test. All the tests were performed using a two-sided test of difference, where the inspection level α of 0.05 and a difference with $P<0.05$ were considered statistically significant.

RESULTS

Comparison of Lysholm score, WOMAC score and VAS score before and after treatment in the two groups

According to Lysholm, WOMAC and VAS scores, all patients had varying degrees of pain before surgery patients were painless after surgery. The difference was statistically significant (P=0.023). As shown in Table I, after different treatment modes are implemented, the study group (experimental group) had more satisfactory improvement in various indices than the reference group (control group), p=0.021; however, there is no significant difference between the two groups before treatment, p>0.05.

Table I.- Comparison of Lysholm, WOMAC and VAS score ($\bar{x} \pm s$).

	Control (reference) group		Experimental (study) group	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**
Lysholm score	48.79±4.58	72.18±5.47	48.66±4.29	86.58±6.59
WOMAC score	69.74±5.32	40.96±5.34	69.70±5.12	28.47±5.37
VAS score	4.71±0.24	3.49±0.15	4.60±0.11	2.01±0.26

Table II.- Comparison of synovial fluid inflammatory factors levels between the two groups ($\bar{x} \pm s$).

	Control (reference) group (n=90)		Experimental (study) group (n=90)	
	Before treatment	**After treatment**	**Before treatment**	**After treatment**
hs-CRP (mg/L)	11.40±3.21	8.05±1.79	11.24±3.10	6.02±1.20
IL-6 (ng/ml)	207.50±20.36	145.40±19.05	206.88±32.18	113.33±30.82

TNF-α (ng/L)	0.85±0.39	0.79±0.22	0.87±0.19	0.23±0.31

Comparison of synovial fluid inflammatory factors levels between the two groups

According to the results of the ELISA test, IL-6, hs-CRP, and TNF-α expression in the Study group before treatment was 206.88±32.18 ng/mL, 11.24±3.10 mg/L, and 0.87±0.19 ng/L, respectively; it was 113.33±30.82 ng/mL, 6.02±1.20 mg/L, and 0.23±0.31 ng/L in the group with after treatment. The expression was significant increased as before treatment scores were increased (p=0.023). As shown in Table II, the study group has better improvement in synovial fluid inflammatory factors than the reference group after treatment, (p=0.021).

Comparison of overall treatment efficiency in the two groups

As shown in Table III, the study group has higher overall treatment efficiency than the reference group, p=0.023.

Table III.- Comparison of overall treatment efficiency in the two groups [n (%)].

	Control (reference) group	Experimental (study) group
Significantly effective	50	65
Effective	22	19
Ineffective	18	6
Total effective rate	72 (80.00)	84 (93.33)

DISCUSSION

At present, there is no radical cure for knee osteoarthritis. The treatment is mainly to implement drug control in the early and middle stages to alleviate the disease progress, and surgical treatment is needed later. According to the epidemiological characteristics of this disease, anti-inflammatory and analgesic drugs are often used, but the effect is unsatisfactory.

The results of this study showed that the study group treated with SJHXT combined with sodium hyaluronate had significantly higher total effective rate than the reference group. There was a positive correlation between the severity of knee osteoarthritis and the levels of synovial fluid inflammatory factors IL-6, TNF-α, and hs-CRP (*P* values =0.023). In addition, the after treatment WOMAC and VAS scores were decreased significantly compared with before treatment ones; and the after treatment Lysholm scores were significantly increased compared with before treatment ones. The differences were statistically significant (*P* values =0.023).

It is known that pro-inflammatory cytokines, such as IL6 and TNF-α, exert catabolic action and contribute in a number of ways to joint degeneration in KOA, including proteinase activation and suppression of type II collagen, one of the main components of the extracellular matrix (Mobasheri and Batt, 2016; Mabey and Honsawek, 2015). According to our results, treatment was effective in reducing pro-inflammatory cytokine levels in the synovial fluid.

We found Comparison of overall treatment efficiency in study group has higher overall treatment efficiency than the reference group, p=0.021. The sodium hyaluronate can cover and protect joint tissues, improve lubrication and penetrate into denatured cartilage that In our study and many studies, its effect on KOA was also observed (Watson, 2018; Hirose *et al*., 2012; Curran, 2010; Altman *et al*., 2018). Previous Chinese medicine scholars have dialectically recognized knee osteoarthritis from multiple perspectives, and implemented traditional Chinese medicine to treat the disease, generally achieving good results. Related studies have pointed out that the occurrence of knee osteoarthritis is closely related to vein occlusion of the joint. The promoting factors are liver and spleen deficiency, liver hematogenesis, liver deficiency, lack of blood, limb malnutrition, exogenous and endogenous pathogenic factors. The main components of SJHXT are safflower, rhizoma cibotii, mistletoe, herba lycopi, caulis spatholobi, and Chinese starjasmine stem, lycopodium clavatum, rhizoma cyperi. Where, safflower can effectively promote blood circulation and remove blood stasis, which can also reduce swelling and ease pain, remove toxic material and stasis. Mistletoe and herba lycopi can relieve exterior syndrome and stop pain,remove meridian obstruction and eliminate dampness. Caulis spatholobi can enrich and activate the blood, remove meridian obstruction. Lycopodium clavatum andrhizoma cyperi can relieve pain, reduce swelling and regulate menstruation. The mixture of the medicines can relax tendons and activate collaterals, quicken blood and dissipate stasis, ease pain and reduce swelling (Dong, 2007; Shaoqi *et al*., 2016; Han and Gellhorn, 2018; Yaowu *et al*., 2017).

Overall, our results showed for the first time that the Patients treated with Shujin Huoxue tablets combined with sodium hyaluronate had higher decrease in IL-6, TNF-α, and hs-CRP levels, indicating that down-regulation of joint fluid inflammatory factors brings therapeutic effect.

CONCLUSION

In summary, Shujin Huoxue tablets can improve

the therapeutic effect on knee osteoarthritis, reduce inflammation and pain, which is safer and worthy of further clinical research and application. However, this study had short follow-up time and a small sample size was studied, so the long-term effect requires further study. These findings demonstrate the benefits of SJHXT combined with sodium hyaluronate, confirming its potential and as a pharmacological treatment for joint inflammation in the KOA.

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Statement of conflict of interest

The authors declare no conflict of interest.

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