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Study the relevance between inflammatory factors and estradiol and their association with knee osteoarthritis in postmenopausal women

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Abstract. – OBJECTIVE: To investigate whether serum levels of inflammatory factors and estradiol (E2) are involved in the pathogenesis of postmenopausal women with knee osteoarthritis (OA).

PATIENTS AND METHODS: 58 randomly patients diagnosed with postmenopausal knee OA that underwent orthopedic surgery from October 2013 to October 2016 in our hospital were selected. These patients, considered as the experimental group, according to the degree of cartilage damage, were divided into light, medium and heavy groups. 58 patients with menstrual disorders without knee OA were in the control group. 35 cases without osteoarthritis were included in the normal control group. Serum levels of interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), erythrocyte sedimentation rate (ESR), high sensitive C- reactive protein (CRP), estradiol (E2) and IL-1, IL-6 and TNF-α levels in the synovial fluid of the experimental group were measured.

RESULTS: The serum levels of IL-1, IL-6, TNF-α, and CRP in the normal control group, the control group and the experimental group were gradually increasing, the difference was statistically significant ($p<0.05$). The level of serum E2 was gradually decreasing ($p<0.05$); the difference of ESR between normal control group and control group had no significant difference ($p>0.05$), but the level of ESR in experimental group was higher than the normal control group and the control group ($p<0.05$). The serum levels of IL-1, TNF-α in experimental group of mild, moderate and severe sub-group were gradually increasing, the difference was statistically significant ($p<0.05$); while the level of IL-6 in the early, middle stage of OA increased significantly, and the late was reduced ($p<0.05$). The level of E2 was gradually decreased in the mild, moderate and severe sub-group of the experimental group, which had statistically significant difference ($p<0.05$). The level of serum E2 in the experimental group was positively correlated with the levels of IL-1, IL-6 and TNF-α in synovial fluid ($p<0.05$).

CONCLUSIONS: The lack of estradiol is associated with the pathogenesis of OA in postmenopausal women, the inflammatory factors of IL-1, IL-6, TNF-α in postmenopausal increased in serum and synovial fluid may promote and aggravate the OA.

Key Words:
Osteoarthritis, Interleukin, Tumor necrosis factor -alpha, C-reactive protein, Estradiol.

Introduction

Postmenopausal osteoarthritis (OA) is the most common joint disease in people at the age of over 45 years old, especially in the knee, and its disability rate is high, seriously affecting the patient's health and quality of life[1]. In the premenopausal and early perimenopausal periods, circulating estradiol is the predominant estrogen, and it is decreased with menopause[2]. Whether the level of estrogen in the blood mediates OA pathophysiology has been controversial[3-5]. In recent years, the study on the etiology of inflammatory factors in postmenopausal OA has become a hot topic in academia. A study has shown that levels of interleukin-1 (IL-1), IL-6, tumor necrosis factor-alpha (TNF-α) and other inflammatory factors significantly change with the

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changes in the female hormone level[6]. This study aimed to study the relationship of various inflammatory factors and estradiol (E_2) with the pathogenesis of knee OA in postmenopausal women, and to explore their clinical values.

Patients and Methods

Patients

The patients treated in the Department of Bone and Joint Surgery of our hospital from October 2013 to October 2016 were selected. In the experimental group, 68 patients at an average age of (61.35±4.82) years old were diagnosed with postmenopausal (cessation of menstruation ≥ 12 months)[2] knee OA, whose mean course of disease was (9.85±2.96) years, and the mean body mass index (BMI) was (25.58±2.64) kg/m^2. Patients in the experimental group were divided into the mild group, the moderate group and the severe group according to the degree of cartilage injury. In the control group, 58 patients at an average age of (50.39±5.42) years old had menstrual disorder, but were not definitely diagnosed with knee OA, whose mean course of disease was (3.47±1.89) years, and the mean BMI was (24.13±2.97) kg/m^2. In the normal control group, 35 patients at an average age of (44.86±5.17) years old had normal menstruation with no special discomfort in the knee, whose mean BMI was (24.08±3.21) kg/m^2. According to the results of arthroscopy, the experimental group was divided into the mild group (n=15, few fibrosis in the articular cartilage surface), the moderate group (n=25, fiber bundle changes in a large amount of articular cartilage with crab-like appearance) and the severe group (n=18, cartilage necrosis and detachment as well as subchondral bone exposure). All OA patients were in line with the Guidelines for the Treatment of OA of the Knee (2007 Edition). This study was approved by the Ethics Committee of Shandong Jining No.1 People's Hospital. Signed written informed consents were obtained from all participants before the study. Exclusion criteria: 1) patients with knee joint congenital malformations or OA caused by recent traumas; 2) patients with signs of infection; 3) patients suffering from rheumatoid autoimmune arthritis; 4) patients receiving ovariectomy or suffering from gynecological diseases affecting estrogen secretion; 5) patients with serious liver or kidney function impairments; 6) patients experiencing acute cardiovascular accident recently.

Collection of General data of Patients

Data of patients were recorded, including age, height, weight, history of menstruation and course of disease. The fasting body weight of patients wearing single-layer garment was measured, and their height was measured after they took off shoes and hats. Each index was measured for 2 times to take the average value. BMI = weight (kg)/height (m)^2.

Detection of Serological Indexes

5 mL antecubital venous blood of patients was extracted the next morning after the hospitalization under the resting and fasting state. 2 mL of it were sent to the Clinical Lab of the hospital for the detection of high-sensitivity C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and E_2 by specialists, and the average E_2 level in the follicular phase and luteal phase was taken in the normal control group and the control group. Besides, the remaining 3 mL were centrifuged in the laboratory, and the supernatant was taken for subpackage and placed at -80°C for standby application. Double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to detect levels of serum IL-1, IL-6, and TNF-α.

Collection of Synovial Fluid Specimens

2 mL synovial fluid of patients in the experimental group was extracted and centrifuged to take supernatant for subpackage, followed by storage at -80°C for standby application. Concentrations of IL-1, IL-6, and TNF-α in synovial fluid were measured by ELISA.

Statistical Analysis

Data were analyzed using Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS Inc., Chicago, IL, USA) and recorded using mean ± standard deviation ($\bar{x}$±s). *t*-test and χ^2-test were used for statistical analysis. Pearson correlation analysis was conducted for two factors. $p<0.05$ represented that differences in all results were statistically significant.

Results

Detection Results of Serum IL-1, IL-6, and TNF-α in Each Group

Levels of serum IL-1 in the experimental group, the control group and the normal control group were (345.6±57.5) pg/mL, (297.2±37.1) pg/mL and (158.4±29.3) pg/mL, respectively;

levels of serum IL-6 were (36.5±4.6) pg/mL, (29.4±3.3) pg/mL and (17.2±2.4) pg/mL, respectively; levels of serum TNF-α were (304.9±51.8) pg/mL, (246.8±43.5) pg/mL and (108.4±7.7) pg/mL, respectively. Levels of serum IL-1, IL-6, and TNF-α in the experimental group had statistically significant differences compared with those in the control group and the normal control group (p<0.05), and there were significant differences between the control group and normal control group (p<0.05) (Figure 1).

Detection Results of Serum CRP and ESR in Each Group

Levels of serum CRP in the experimental group, the control group and the normal control group were (42.1±10.4) pg/mL, (7.2±5.9) pg/mL and (1.8±5.1) pg/mL, respectively; CRP levels showed a step-wise increase in the normal control group, the control group and the experimental group, and intergroup differences were statistically significant (p<0.05). ESR levels were (31.9±25.4) pg/mL, (15.8±7.6) pg/mL, (13.8±5.1) pg/mL, respectively; there was no significant difference in ESR level between the normal control group and the control group (p>0.05), but that in the experimental group was higher than those in the normal control group and the control group (p<0.05) (Figure 2).

Detection Result of Serum E2 in Each Group

Levels of serum E_2 in the experimental group, the control group and the normal control group were (15.4±4.6) pg/mL, (49.56±10.3) pg/mL and (72.5±10.2) pg/mL, respectively; E_2 levels showed a step-wise decrease in the normal control group, the control group and the experimental group, and intergroup differences were statistically significant (p<0.05) (Figure 3).

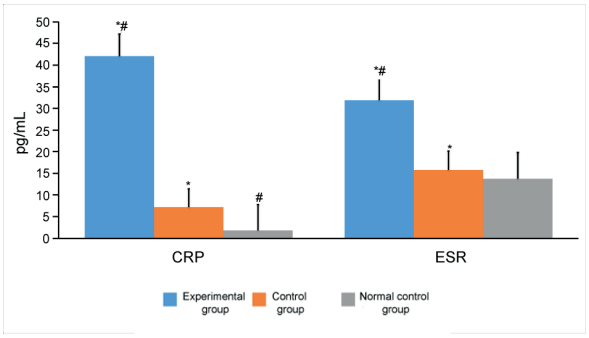

Figure 2. Detection results of serum CRP and ESR in each group. Note: *Compared with the normal control group, p<0.05; #compared with the control group, p<0.05.

Comparisons of the Detection Results of IL-1, IL-6, and TNF-α in synovial Fluid in the Experimental Group

Levels of IL-1 and TNF-α in synovial fluid of patients in the mild group, the moderate group and the severe group were increased progressively, which were statistically different in patients with OA at different severities (p<0.05), indicating that the severer the OA is, the higher the levels of IL-1 and TNF-α will be. IL-6 levels were significantly increased in the mild and moderate OA groups, but decreased in the severe OA group (p<0.05) (Figure 4).

Detection Results of Serum E2 in Different Subgroups of the Experimental Group

Levels of serum E_2 in the mild group, the moderate group and the severe group were (21.2±4.2) pg/mL, (16.3±4.5) pg/mL and (11.5±4.3) pg/mL,

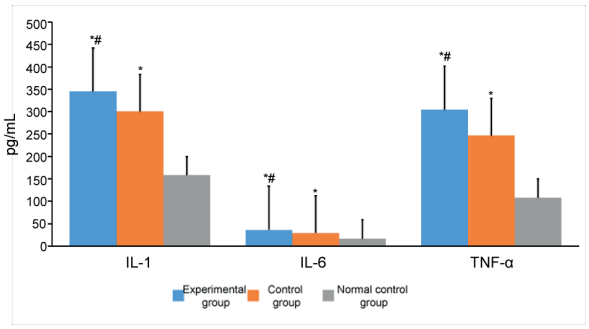

Figure 1. Detection results of serum IL-1, IL-6 and TNF-α in each group. Note: *Compared with the normal control group, p<0.05; #compared with the control group, p<0.05.

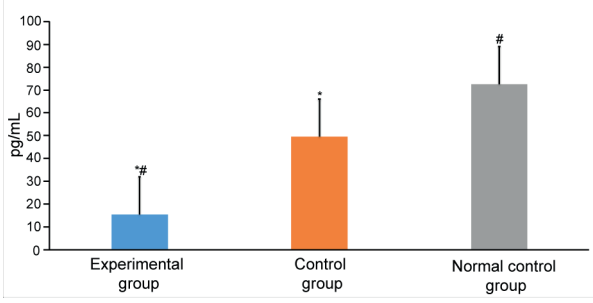

Figure 3. Detection result of serum E2 in each group. Note: *Compared with the normal control group, p<0.05; #compared with the control group, p<0.05.

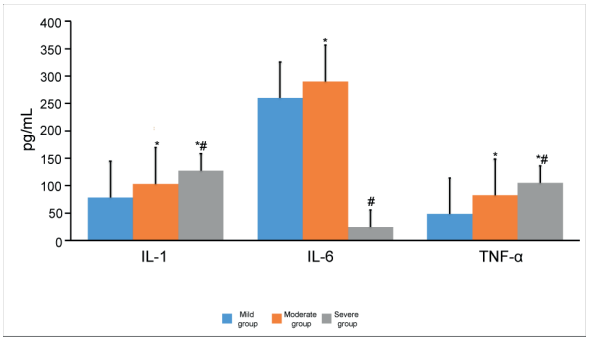

Figure 4. Comparisons of the detection results of IL-1, IL-6 and TNF-α in synovial fluid in the experimental group. Note: *Compared with the normal control group, $p<0.05$; #compared with the mild group, $p<0.05$.

respectively; E_2 levels in the mild group, the moderate group and the severe group declined progressively, and intergroup differences were statistically significant ($p<0.05$) (Figure 5).

Correlation Analyses of Serum E_2 with IL-1, IL-6, and TNF-α in Synovial Fluid

Pearson correlation analysis results showed that the level of serum E_2 was negatively correlated with levels of IL-1, IL-6 and TNF-α ($r=-0.375$, $p<0.05$; $r=-0.269$, $p<0.05$; $r=-0.281$, $p<0.05$).

Discussion

Epidemiological surveys and analyses show that up to 45% of the Chinese population at the age of over 55 years old suffers from OA[7], which is more commonly seen in women, seriously affecting the quality of life and health. From the beginning of menopause, the hypofunction of ovarian secreting estrogens and the dysregulation of the autonomic nervous center lead to the decline and gradual disappearance of ovarian functions[8]. In recent years, IL-1, IL-6, TNF-α and other cytokines have become the research hotspot in the pathogenesis of OA[9]. The inflammation level in the body changes with changes in female estrogen level[10]. The study results showed that levels of serum E_2 showed a step-wise decrease in the normal control group, the control group and the experimental group, and intergroup differences were statistically significant ($p<0.05$). In each subgroup of the experimental group, E_2 levels showed a step-wise decrease in the mild group, the moderate group and the severe group, and intergroup differences were statistically significant ($p<0.05$).

Lee et al[11] pointed out that estrogen is involved in the occurrence and development of OA. A study has shown that OA is associated with a decrease in the level of serum E_2, and the decrease in estrogen may increase the risk of OA[12]. Another report has shown that there is a larger possibility of hip and knee OA symptoms appearing in postmenopausal women with faster development of lesions than men at the same age[13]. Therefore, female knee OA was selected as the study object in this work. A study has shown that estrogen has anti-inflammatory effects, which will become weakened with the decrease in the estrogen level after menopause[14]. At present, estrogen has been found to have anti-inflammatory effects in the following four ways. Firstly, on the one hand, estrogen inhibits the expression of cyclooxygenase-2 (COX-2) and its activity, thereby reducing the production of prostacyclin. At the same time, estrogen reduces the stimulation of lipopolysaccharide (LPS) to COX-2[15]. On the other hand, the combination of estrogen receptors with nuclear factor inhibitors blocks the expression of IL-6 genes and inhibits the activation of IL-1-induced COX-2[16]. Secondly, estrogen can antagonize the expression of nitric oxide synthase and NO release in cells, and at the same time, estrogen inhibits endothelial nitric oxide synthase by reducing the expression of angiotensin II receptor[17]. Thirdly, estrogen can promote the apoptosis of relevant T lymphocytes and play a role in inhibiting inflammatory reactions[18]. Fourthly, it regulates the activation of specific signal transduction pathways, such as mitogen-activated protein kinase (MAPK) signaling transduction pathway that increases the level of inflammation, and effectively reduces levels of IL-1, IL-6, and TNF-α. In the pre-menopausal and the early menopausal periods, circulating

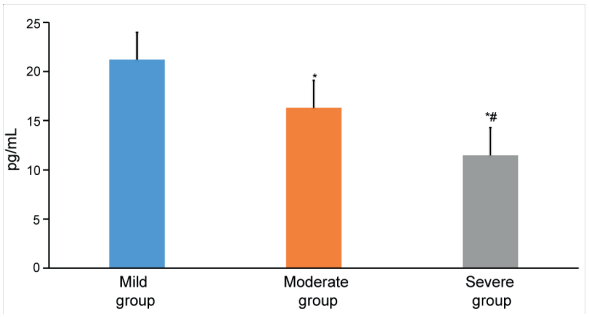

Figure 5. Detection results of serum E_2 in different subgroups of the experimental group. Note: *Compared with the mild group, $p<0.05$; #compared with the moderate group, $p<0.05$.

Table I. Correlation analyses of serum E2 with IL-1, IL-6 and TNF-α in synovial fluid.

Index	E_2	
	r	*p*
IL-1	-0.375	<0.05
IL-6	-0.269	<0.05
TNF-α	-0.281	<0.05

estradiol is the major estrogen and is decreased with menopause. The rapid decline in estrogen level in postmenopausal women is an important cause of OA[19]. The results of this research showed that levels of serum IL-1, IL-6, TNF-α, and CRP were increased progressively in the normal control group, the control group and the experimental group. Levels of IL-1, IL-6, TNF-α, and CRP in the control group were higher than those in the normal control group, and those in the experimental group were higher than those in the control group; intergroup differences were statistically significant ($p<0.05$). There was no significant difference in ESR level between the normal control group and the control group ($p>0.05$), but ESR level in the experimental group was higher than those in the normal control group and the control group ($p<0.05$). Levels of IL-1 and TNF-α in synovial fluid of the experimental group showed a step-wise increase in the mild group, the moderate group and the severe group; levels of IL-1 and TNF-α in the control group were higher than those in the normal control group, and those in the experimental group were higher than those in the control group; intergroup differences were statistically significant ($p<0.05$). However, IL-6 levels were significantly increased in the mild and moderate OA groups but decreased in the severe OA group ($p<0.05$). IL-1 was first discovered in monocyte culture supernatants by Gray et al[20] in 1972 with a wide range of biological effects. IL-1 is considered as a core factor for the inflammatory response and mediates many destructive reactions[21]. Normal chondrocytes produce almost no IL-1, whereas IL-1 levels in the synovium and cartilage are significantly increased in patients with OA, and are positively correlated with the severity of the disease[22]. Studies have shown that IL-1 may affect the occurrence and development of OA by influencing the living microenvironment of articular cartilage, promoting the development of the synovial membrane inflammation, inhibiting the synthesis of cartilage matrix, promoting the degradation of cartilage matrix and reducing the repair ability of cartilage[23-25]. IL-6 is a glycopeptide with a molecular weight of 26 kDa, and its gene is located on chromosome 7[26]. IL-6, a central mediator regulating immune and inflammatory responses *in vivo*, is produced by macrophages and is an important pro-inflammatory cytokine[27] that has been considered as an important mediator-giving rise to joint destruction. Studies have shown that in the first place, through degrading collagens, promoting cartilage absorption and digesting the matrix proteoglycans, IL-6 induces the loss of matrix moisture[28,29], disrupts the matrix composition, deteriorates the microenvironment in which chondrocytes live, thus forming a vicious cycle between matrixes and chondrocytes. In the second place, IL-6 and its receptors promote the formation, activity, differentiation and growth of osteoclasts, and its concentration is related to the degree of joint destruction[30]. TNF-α is an early mediator of local inflammation. Through activating neutrophils, stimulating and inducing the production of prostaglandin E_2 by synovial cells, the synthesis and secretion of matrix metalloproteinase in cartilage are promoted, and the degeneration of cartilage is accelerated, thus accelerating the process of osteoarthritis[15]. Clinically, CRP test is an indicator with important values in the treatment process of inflammatory diseases[31]. In addition, it can assess whether infection exists and whether the disease is in the active stage[32]. Although plasma CRP is commonly used in the auxiliary diagnosis of systemic inflammatory responses, it begins to increase slowly in the body 8-12 h later, and its concentration is regulated by the immunity of the body. The half-life period of CRP is long, and CRP returns to normal after the body's inflammation is controlled for a while, so it is not suitable to use it in the evaluation of efficacy and prognosis. Therefore, the clinical ESR and CRP are often used as auxiliary diagnostic indicators for OA.

Conclusions

We showed that the lack of estradiol is associated with the pathogenesis of OA in postmenopausal women. The increased levels of IL-1, IL-6, TNF-α and other inflammatory factors in serum and synovial fluid after menopause may promote and aggravate the occurrence of OA. Expanding the sample size and conducting multi-center prospective investigations may yield more meaningful results.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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