PERIPHERAL BLOOD B-CORRELATION BETWEEN CROSSLAPS, EMMPRIN LEVELS AND DISEASE SEVERITY IN POSTMENOPAUSAL PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: *To explore peripheral blood β-Correlation between CrossLaps, EMMPRIN levels and disease severity in postmenopausal patients with rheumatoid arthritis.*

Methods: *89 patients with postmenopausal rheumatoid arthritis treated in the rheumatology and Immunology Department of our hospital from October 2019 to November 2020 were selected as the case group of this experiment; 56 healthy postmenopausal women in the physical examination center of our hospital were selected as the control group. According to the disease activity score (DAS28), the disease severity was divided into 35 cases in the low activity group, 28 cases in the moderate activity group and 26 cases in the high activity group. The general data and knee motion of different groups were compared, and the expression differences of β-Crosslaps, IL-6 and EMMPRIN in peripheral blood of different groups were studied, and the correlation between the above indicators and the severity of disease was analyzed.*

Results: *Compared with moderate and low activity groups, the levels of β-Crosslaps and IL-6 in peripheral blood of high activity group were significantly increased (P<0.05); compared with the medium and low activity groups, the knee mobility of the high activity group decreased significantly (P<0.05); compared with the medium and low activity groups, the EMMPRIN level of the high activity group increased significantly (P<0.05); according to the Pearson correlation test, the knee mobility and peripheral blood β-CrossLaps, serum IL-6 and EMMPRIN showed a positive correlation (P<0.05).*

Conclusion: *Peripheral blood β-CrossLaps, IL-6 and EMMPRIN are highly expressed in postmenopausal RA patients. The more serious their condition is, the smaller their knee joint activity is, and the more serious their condition is.*

Keywords: *Peripheral blood β-Crosslaps, EMMPRIN, postmenopausal rheumatoid arthritis, disease severity, relevance.*

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease, often characterized by chronic joint inflammation, which is a synovial microenvironment disorder caused by the joint action of multiple factors on the body, and can develop into synovitis and damage of joints, articular cartilage and other surrounding bone tissues[1]. The incidence of RA in women is significantly higher than that in men, often presenting with symmetrical arthritis, joint swelling and pain, joint space narrowing and cartilage damage, etc. When the disease progresses to the later stage, it can lead to bone loss and aggravate the bone damage of the body, resulting in joint deformity and dysfunction[2]. According to the data survey, the incidence and disability rate of RA are on the rise, which has a serious impact on the life and work of patients and affects the prognosis of patients[3]. The cause of RA is still unclear, and it may be closely related to genetic factors, hormones, environment and mental factors. In the early stage of RA, muscle atrophy and sedimentation may occur, and even lifelong disability may occur[4]. The Extracellular

matrix metalloproteinase inducer (EMMPRIN) is a highly glycosylated transmembrane protein in the immunoglobulin superfamily, which plays an important role in cell to matrix and cell to cell. It can affect the secretion of MMPs by fibroblasts and accelerate the differentiation of macrophages into osteoclasts[5]. Serum β collagen special sequences (β-Crosslaps) belong to the degradation products of c-terminal peptides of typeyI collagen degradation, which can be used as markers of bone resorption and have important value in bone metabolic diseases[6].

There are few relevant studies on EMMPRIN and β-Crosslaps, especially in the RA study, so this study aims to explore the correlation between the levels of β-Crosslaps and EMMPRIN in peripheral blood and the severity of disease in postmenopausal rheumatoid arthritis patients.

Data and methods

General information

Eighty-nine postmenopausal patients with rheumatoid arthritis admitted to the rheumatology Department of our hospital from October 2019 to November 2020 were selected as the case group of this experiment, and all of them were female. According to disease activity score-DAS28[7], disease severity was divided into 35 cases in the low activity group (≤3.2), 28 cases in the moderate activity group (3.2 <DAS28≤5.1), and 26 cases in the high activity group (>1.5).

At the same time, 56 healthy postmenopausal women in physical examination center of our hospital were selected as the control group of this experiment. General data including age, BMI, mean arterial pressure, height, time of menopause, serum calcium, drinking history, smoking history and chronic diseases were collected in case group and control group.

- Age: case group (60.25±7.12) years old, control group (60.86±6.58 years old);
- BMI: case group (21.36±1.22), control group (21.68±1.21);
- Mean arterial pressure was 73.16±5.16 in the case group and 74.31±5.42 in the control group;
- Height: case group (165.43±2.78) cm, control group (166.37±2.84) cm;
- Menopause time: case group (8.69±8.12) years, control group (8.97±7.46) years;
- Serum calcium was (1.18±0.04) mmol/L in the case group and (1.19±0.03) mmol/L in the control group.
- Drinking history: 25 cases, no 120 cases;
- Smoking history: 24 cases, no 121 cases;
- Chronic diseases: 68; no 77.

Inclusion criteria:

- Postmenopausal rheumatoid arthritis was diagnosed according to the classification criteria developed by the American College of Rheumatology[8];
- All patients were ≥50 years old;
- Complete clinical data;
- The patients did not use glucocorticoid and other drugs to change the disease before enrollment;
- The patients did not use non-steroidal anti-inflammatory drugs 7 days before enrollment;
- The patients and their families voluntarily participated in this experiment and signed informed consent, which was approved by the Ethics Committee of our hospital.

Exclusion criteria:

- Patients complicated with other malignant tumors and autoimmune diseases were excluded;
- Exclude cardiovascular diseases and liver and kidney dysfunction;
- Exclude mental illness and poor mental condition;
- Exclude poor compliance;
- Exclude intermediate transfer and discharge.

Methods

Range of motion

The knee motion scale was used to test the knee motion of patients in the four groups. Medical personnel recorded the knee motion of patients in different groups, and the normal knee bend value was 120°-150°. Each patient should be measured three times, and the average value of the three times was recorded and compared.

Serum index detection

After admission, all patients were given early morning fasting venous blood of 5ml, centrifuged at 3000r/min for 10min in total. Supernatant was extracted and stored at -80°C for future use. The expression levels of β-Crosslaps and interleukin-6 (IL-6) in peripheral blood of all patients were detected by ELISA.

Determination of EMMPRIN protein expression

5ml of early morning fasting peripheral anticoagulant blood of all patients was extracted, and

Hanks solution was applied to dilute it. Neutrophils were extracted according to the instructions of cell separation and extraction kit, and the cell number was adjusted (1×10^7).

Western blot was used to extract neutrophil protein, and protein concentration was measured according to BCA kit. After electrophoresis, membrane transfer and interventional antibody treatment, the expression differences of EMMPRIN in different groups were observed.

Observation indicators

• The general data of the case group and the control group were compared, including age, BMI, mean arterial pressure, height, time of menopause, serum calcium, drinking history, smoking history, and chronic diseases.

• To study the levels of β-Crosslaps and IL-6 in peripheral blood of patients in different groups.

• The knee motion of patients in different groups was recorded and analyzed.

• The expression of EMMPRIN in different groups was observed.

• Analyze the correlation between disease severity and related factors in postmenopausal RA patients.

Statistical methods

SPSS20.0 software package was used for statistical analysis of the study data. Measurement data were expressed as standard deviation ($\bar{x}\pm s$), t-test was used for inter-group comparison, counting data was expressed as [n(%)], chi-square test was used for inter-group comparison.

Pearson correlation was used to test the correlation between the levels of β-Crosslaps and EMMPRIN in peripheral blood and the severity of disease in postmenopausal rheumatoid arthritis patients. $P<0.05$ was taken as the statistical standard.

Results

Comparison of general data between the case group and the control group

There were no significant differences between the case group and the control group in age, BMI index, mean arterial pressure, height, time of menopause, serum calcium, drinking history, smoking history, chronic diseases and other general information ($P>0.05$), which were comparable, as shown in Table 1.

General information	Case group (n=89)	Control group (n=56)	t/χ^2	*P*
Age (year)	60.25±7.12	60.86±6.58	0.541	0.588
BMI	21.36±1.22	21.68±1.21	1.542	0.125
Mean arterial pressure	73.16±5.16	74.31±5.42	1.281	0.202
Height (cm)	165.43±2.78	166.37±2.84	1.966	0.051
Period of Menopause (year)	8.69±8.12	8.97±7.46	0.208	0.835
Serum calcium (mmol/L)	1.18±0.04	1.19±0.03	1.607	0.110
Drinking History			2.281	0.130
yes	12 (48%)	13 (52%)		
no	77 (64.17%)	43 (35.83%)		
Smoking history			2.932	0.086
yes	11 (45.83%)	13 (54.17)		
no	78 (64.46%)	43 (35.54%)		
Chronic disease			1.632	0.201
yes	38 (55.88%)	30 (44.12%)		
no	51 (66.23%)	26 (33.77%)		

Table 1: Comparison of general data between the case group and the control group.

The levels of β-Crosslaps and IL-6 in peripheral blood of different groups

Compared with the control group, the levels of β-Crosslaps and IL-6 in peripheral blood of the high, medium and low activity groups were significantly increased ($P<0.05$), and the levels of β-Crosslaps and IL-6 in peripheral blood of the high activity group were significantly increased compared with the medium and low activity groups.

The difference was statistically significant ($P<0.05$), as shown in Table 2.

Group	n	β-Crosslaps (pg/ml)	IL-6 (pg/ml)
High activity group	35	$625.75\pm125.03^{*\#\triangle}$	62.77±10.23
Medium activity group	28	$576.33\pm91.34^{*\#}$	49.75±6.78
Low activity group	26	$523.46\pm82.45^{*}$	25.46±5.69
Control group	56	451.31±72.13	6.12±1.23
F		28.16	665.24
P		<0.001	<0.001

Table 2: The levels of β-Crosslaps and IL-6 in peripheral blood of different groups ($\bar{x}\pm s$).

Note: Compared with control group, $^{*}P<0.05$; Compared with low activity group, $^{\#}P<0.05$; Compared with middle activity group, $^{\triangle}P<0.05$.

Differences of knee motion in different groups

Compared with the control group, knee motion in the high, medium and low activity groups was significantly decreased, with statistically significant differences ($P<0.05$); compared with the medium and low activity groups, knee motion in the high

activity group was significantly decreased, with statistically significant differences (P<0.05), as shown in Table 3.

Group	n	Knee range of motion
High activity group	35	90.12±5.13*#△
Medium activity group	28	110.56±9.61*#
Low activity group	26	121.46±15.23*
Control group	56	135.45±12.33
F		123.27
P		<0.001

Table 3: Differences of knee motion in different groups ($\bar{x}\pm s$).

*Note: Compared with control group, *P<0.05; Compared with low activity group, #P<0.05; Compared with middle activity group, △P<0.05.*

Expression differences of EMMPRIN in different groups

Compared with the control group, EMMPRIN levels in the high, medium and low activity groups were significantly increased (P<0.05); compared with the medium and low activity groups, EMMPRIN levels in the high activity group were significantly increased (P<0.05), as shown in Table 4.

Group	n	EMMPRIN
High activity group	35	27.69±2.46*#△
Medium activity group	28	21.33±2.44*#
Low activity group	26	14.25±2.34*
Control group	56	7.45±1.42
F		745.50
P		<0.001

Table 4: Expression differences of EMMPRIN in different groups ($\bar{x}\pm s$).

*Note: Compared with control group, *P<0.05; Compared with low activity group, #P<0.05; Compared with middle activity group, △P<0.05.*

Correlation between disease severity and related factors in postmenopausal RA patients

Pearson correlation test showed that knee activity, peripheral blood β-Crosslaps, serum IL-6 and EMMPRIN were positively correlated, with statistically significant differences (P<0.05), as shown in Table 5.

Relevant factor	Severity of disease in postmenopausal RA patients	
	r	*P*
Knee range of motion	0.635	<0.001
Peripheral bloodβ-Crosslaps	0.651	<0.001
Serum IL-6	0.368	<0.001
EMMPRIN	0.386	<0.001

Table 5: Correlation between disease severity and related factors in postmenopausal RA patients.

Discussion

Postmenopausal RA is a disease of the autoimmune system, presenting with recurrent erosive arthritis. Symmetrical and slow inflammatory response of the external elbow is a typical clinical manifestation, which can lead to varying degrees of joint swelling and deformity, and even lead to dysfunction[9]. The occurrence of post-menopausal RA can affect the quality of life of patients to varying degrees. In the later stage of the disease, it can be manifested as the loss of joint function, which can lead to permanent disability of patients, and then bring serious economic burden to the society and family[10]. With the development of the disease, the patient's elbow, shoulder, wrist, and other joint activities are reduced, resulting in an increased disability rate[11]. Therefore, it is particularly important to timely diagnose and grade postmenopausal RA, carry out intervention and treatment in advance, develop an exact treatment plan, and improve the prognosis and quality of life of patients.

Clinical studies have found that the first symptom of postmenopausal RA is an inflammatory response, and inflammatory cell infiltration leads to massive formation of new blood vessels, resulting in pannus formation[12]. The appearance of pannus can cause the damage of articular cartilage and osteogenesis, and then change joint function, ultimately leading to the loss of joint function[13]. The basic change of postmenopausal RA is chronic synovitis, and there are a large number of T lymphocytes and macrophages in the synovitis of joints affected by RA, which are closely related to the autoimmune response. IL-6 is secreted by synovial macrophages and can induce protein synthesis of hepatocytes and stimulate activation and proliferation of T cells[14]. Studies have found that IL-6 plays an important role in the inflammatory process of RA and can stimulate the production of local collagenase and other inflammatory mediators to attract and chemotactic inflammatory cells and participate in the persistent inflammatory reaction of synovial joint[15]. As a product of type I collagen, β-Crosslaps can effectively evaluate the status of bone mass absorption and reflect the status of bone metabolism at different stages. Studies have confirmed that with the increasing of age, the activity of osteoblasts in patients decreases continuously, which promotes the destruction of osteoblast absorption activity, resulting in the release of a large number of β-Crosslaps into the blood, resulting in

the increase of their level, thus increasing the degree of bone absorption in patients[16]. Li Fengyi et al. found that β-Crosslaps were associated with the occurrence, development and activity of knee joint, which could aggravate the degree of bone injury and affect bone absorption and bone loss of patients with the development of the disease[17].

In this experiment, compared with the moderate and low activity groups, the levels of β-Crosslaps and IL-6 in peripheral blood of the high activity group were significantly increased, and the knee motion of the high activity group was significantly decreased ($P<0.05$). It can be concluded that the changes of RA after menopause can affect the expression of β-Crosslaps, IL-6 and other related indicators in patients, change the knee motion of patients, and aggravate the disease.

Postmenopausal RA patients mostly present with bone destruction and synovial proliferation, and most patients have gonad, kidney and nerve involvement and systemic fever and weakness[18]. EMMPRIN, as the upstream control factor of MMPs, regulates MMPs and stimulates the synthesis of MMPs in the form of autocrine, which releases MMPs into the joint cavity and peripheral blood, thereby aggravating the disease of RA patients and evaluating the disease activities of RA patients[19]. As a transmembrane protein, EMMPRIN can induce mechanistic metalloproteinases, accelerate the release of monocytes and neutrophils in peripheral blood, and thereby regulate a variety of inflammatory factors in peripheral blood, ultimately leading to local and systemic symptoms[20]. It has been found that EMMPRIN can mediate local and systemic symptoms of the body, thus accelerating the occurrence of RA[21]. In this experiment, EMMPRIN level in the high activity group was significantly higher than that in the medium and low activity groups ($P<0.05$). Therefore, the high expression of EMMPRIN in different groups can reflect the severity of patients' disease, which can change with the change of the disease.

In conclusion, β-Crosslaps, IL-6 and EMMPRIN in peripheral blood are highly expressed in postmenopausal RA patients, and the more severe the disease, the lower the knee activity and the more severe the disease.

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