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Associations of interleukin-17 and monocyte chemoattractant protein-1 with vascular lesions in patients with rheumatoid arthritis.

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Abstract

This study aimed to investigate the associations of Interleukin-17 (IL-17) and monocyte chemoattractant protein-1 (MCP-1) with vascular lesions in patients with rheumatoid arthritis (RA). Thirty RA patients (RA group) and thirty healthy subjects (control group) were enrolled in this study. In all subjects, the serum levels of rheumatoid and inflammation related indexes including rheumatoid factor (RF), erythrocyte sedimentation rate (ESR), tumor necrosis factor-α (TNF-α), highsensitivity C-reactive protein (hs-CRP), IL-17 and MCP-1 were determined. In addition, the brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) were measured. Results showed that, the levels of ES, RF, serum TNF-α, hs-CRP, IL-17 and MCP-1 in RA group were significantly higher those in control group, respectively (P<0.05). The levels of serum TNF-α, hs-CRP, IL-17 and MCP-1 in RA patients with abnormal baPWV (>1400 cm/s) were significantly higher those in RA patients with normal baPWV (≤ 1400 cm/s), respectively (P<0.05). The levels of serum hs-CRP, IL-17 and MCP-1 in RA patients with abnormal ABI (≤ 0.9) were significantly higher those in RA patients with normal ABI (>0.9), respectively (P<0.05). In 30 RA patients, hs-CRP, IL-17 and MCP-1 were the main independent risk factors of RA patients with abnormal baPWV, and IL-17 and MCP-1 were the main independent risk factors of RA patients with abnormal ABI. In conclusion, IL-17 and MCP-1 are involved in the occurrence and development of RA, and they are the reliable indicators for judging the vascular lesions in RA.

Keywords: Rheumatoid arthritis, Vascular lesions, Interleukin-17, Monocyte chemoattractant protein-1.

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Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease which is typically characterized by chronic symmetric non-suppurative arthritis. It seriously affects the health and life quality of patients. The main clinical manifestations of RA include joint involvement, weight loss and fatigue and other symptoms. For severe cases, the function of joint is seriously affected [1,2]. Vascular lesion is one of the manifestations of extra-articular lesions of RA, which is mainly characterized by vessel wall invasion of lymphocytes, neutrophils and plasma cells, intimal hyperplasia, fibrin necrosis or thrombosis [3,4]. The cardiovascular events account for 42% of the cause of death in RA patients [5]. The atherosclerosis is the main lesion in the cardiovascular events of RA [6]. Previous studies have shown that, the increase of vascular stiffness is closely related to the mortality rate of cardiovascular events [7,8]. It is found that, the arterial stiffness exists in patients with RA [9]. The early assessment of the vascular lesions in RA patients and the related early intervention are important for improving the prognosis of RA. At present it is agreed that the inflammatory response is a common pathway of atherosclerosis occurrence and progression and rupture of plaques for thrombosis formation, and a large number of inflammatory cells and inflammatory mediators are involved in this pathway [10]. Interleukin-17 (IL-17) is a potent cytokine that induces the inflammatory response. It plays an important role in a variety of autoimmune diseases. The function of IL-17 in atherosclerosis has also been extensively studied, but the conclusions are still controversial [11,12]. Monocyte chemoattractant protein-1 (MCP-1) is a specific monocyte chemoattractant factor. It can promote the formation of atherosclerosis and plays an important role in the early stage of atherosclerosis [13]. This study investigated the association of interleukin-17 and MCP-1 with vascular lesions in patients with RA. The objective was to further elucidate the mechanisms of IL-17 and MCP-1 in artery atherosclerosis in RA patients, which was conducive to early detect and change the inflammatory state of RA, and provide new ideas for the treatment of vascular lesions of RA.

Subjects and Methods

Subjects

Thirty RA patients treated in Gansu Provincial People's Hospital from June 2015 to December 2016 were investigated in this study. All patients were diagnosed with RA and enrolled according to the RA classification criteria by American College of Rheumatology in 2010. There were 17 (56.7%) males and 13 (43.3%) females. The age of patients was 43-70 years, with mean age of 57.6 ± 11.4 years. In the same period, 30 healthy subjects with physical examination in our hospital were selected as control, in which the RA was excluded by disease history inquiry, physical examination, and color Doppler examination. There were 18 (60.0%) males and 12 (40.0%) females. The age of patients was 42-68 years, with mean age of 56.3 ± 9.3 years. In all enrolled subjects, the dyslipidemia, cardiovascular disease, primary or secondary hypertension, infectious diseases, cancer and other autoimmune diseases, diabetes and renal insufficiency were excluded. This study was approved by the ethics committee of Gansu Provincial People's Hospital. Written informed consent was obtained from all participants.

Determination of inflammatory and immunology parameters

Fasting venous blood (10 mL) was collected from the subjects. After self-agglutination at room temperature, the blood was centrifuged at 256 × g for 10 min. The serum was obtained, and was stored at 4°C. The serum levels of rheumatoid factor (RF), TNF-α, hs-CRP, IL-17 and MCP-1 were determined using ELISA. The regents were provided by Fuzhou Maixin Biotechnology Development Co., Ltd. (Fuzhou, China). The experimental operation was in accordance with the manufacturer's instructions. The erythrocyte sedimentation rate (ESR) was measured using erythrocyte sedimentation rate measuring instrument.

Determination of brachial-ankle pulse wave velocity

The brachial-ankle pulse wave velocity (baPWV) of the subjects was measured using VP 2000 automatic arteriosclerosis detector (OMRON Industrial Automation (China) Co., Ltd., Tianjin, China). The patient was with supine posture, with arms on both sides of the body and palms up. The limb blood pressure cuff was tied to the upper arm and the lower limb ankle. The upper arm cuff was aligned to the brachial artery, with lower margin 2-3 cm from chelidon cross grain. The lower extremity cuff air bag was placed inside the lower limb, with lower edge 1-2 cm from the ankle in. The conductive electrode was connected to the electrocardiogram, and the baPWV was recorded. The average value of left and right baPWV values was used as the final observation result. baPWV ≤ 1400 cm/s and baPWV>1400 cm/s were regarded as normal and abnormal, respectively.

Determination of ankle-brachial index

The ankle-brachial index (ABI) of patients was determined using the method recommended by American Heart Association (AHA). The patients had supine rest for at least 15 min, and the ultrasound examination was performed. The ABI was calculated by the ratio of lateral ankle artery systolic pressure to maximum value of bilateral brachial artery systolic pressure. The lower value of bilateral measurement results was used as the final ABI. ABI>0.9 and ABI ≤ 0.9 were regarded as normal and abnormal, respectively.

Statistical analysis

All statistical analysis was carried out using SPSS 22.0 software (SPSS Inc., Chicago, IL, USA). The enumeration data were presented as number, and were compared using χ^2 test. The measurement data were presented as mean ± SD, and were compared using t test. The logistic regression analysis was performed on the risk factors of RA patients with abnormal baPWV and ABI. P<0.05 was considered as statistically significant.

Results

General data in RA and control group

The general data in RA and control group were shown in Table 1. There was no significant difference in gender, age, height, BMI, heart rate, smoking history or drinking history between two groups (P>0.05). The levels of rheumatism related indexes including ESR and RF, inflammation related indexes including TNF-α, hs-CRP, IL-17 and MCP-1 and baPWV in RA group were significantly higher than those in control group, respectively (P<0.05), and the ABI in RA group was significantly lower than that in control group (P<0.05).

Table 1. *General data in RA and control group.*

Index	RA group	Control group	P
n	30	30	
Gender (male/female, n)	17/13	18/12	>0.05
Age (years)	57.6 ± 11.4	56.3 ± 9.3	>0.05
Height (m)	154.06 ± 5.9	156.34 ± 6.7	>0.05
BMI (kg/m^2)	25.9 ± 3.9	26.1 ± 2.7	>0.05
Heart rate (bpm)	75.4 ± 8.9	77.2 ± 10.1	>0.05
Smoking history (yes/no, n)	16/14	20/10	>0.05
Drinking history (yes/no, n)	28/2	26/4	>0.05
TNF-α (ng/L)	25.5 ± 3.2	8.8 ± 1.5	<0.05
hs-CRP (mg/L)	9.3 ± 1.8	2.2 ± 0.8	<0.05
ESR (mm/h)	54.1 ± 5.9	9.5 ± 1.7	<0.05
RF (IU/ml)	32.2 ± 4.5	8.2 ± 1.4	<0.05
IL-17 (pg/ml)	26.5 ± 3.6	6.9 ± 1.1	<0.05

MCP-1 (pg/ml)	202.3 ± 32.5	91.5 ± 16.7	<0.05
baPWV (cm/s)	1480.6 ± 210.6	1240.4 ± 270.6	<0.05
ABI	0.46 ± 0.1	1.02 ± 0.2	<0.05

RA: Rheumatic Arthritis; BMI: Body Mass Index; TNF-α: Tumor Necrosis Factor-α; hs-CRP: High-Sensitivity C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; RF: Rheumatoid Factor; IL-17: Interleukin-17; MCP-1: Monocyte Chemoattractant Protein-1; baPWV: Brachial-Ankle Pulse Wave Velocity; ABI: Ankle-Brachial Index.

Comparison of rheumatoid and inflammation related indexes between RA patients with normal and abnormal baPWV

In 30 RA patients, there were 14 cases with normal baPWV (≤ 1400 cm/s) and 16 cases with abnormal baPWV (>1400 cm/s). The levels of ESR and RF had no significant difference between RA patients with normal and abnormal baPWV, respectively (P>0.05). The levels of serum TNF-α, hs-CRP, IL-17 and MCP-1 in RA patients with abnormal baPWV were significantly higher those in RA patients with normal baPWV, respectively (P<0.05) (Table 2).

Table 2. *Comparison of RA and inflammation related indexes between RA patients with normal and abnormal baPWV.*

Index	Abnormal baPWV	Normal baPWV	P
n	16	14	
TNF-α (ng/L)	28.7 ± 3.9	22.1 ± 4.3	<0.05
hs-CRP (mg/L)	12.2 ± 2.1	7.9 ± 2.2	<0.05
ESR (mm/h)	56.1 ± 6.4	52.7 ± 7.1	>0.05
RF (IU/ml)	33.5 ± 4.1	31.8 ± 5.1	>0.05
IL-17 (pg/ml)	33.3 ± 5.2	18.9 ± 2.9	<0.05
MCP-1 (pg/ml)	278.9 ± 45.1	167.2 ± 21.9	<0.05

RA: Rheumatic Arthritis; baPWV: Brachial-Ankle Pulse Wave Velocity; TNF-α: Tumor Necrosis Factor-α; hs-CRP: High-Sensitivity C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; RF: Rheumatoid Factor; IL-17: Interleukin-17; MCP-1: Monocyte Chemoattractant Protein-1.

Table 3. *Comparison of RA and inflammation related indexes between RA patients with normal and abnormal ABI.*

Index	Abnormal ABI	Normal ABI	P
n	13	17	
TNF-α (ng/L)	25.5 ± 2.2	24.8 ± 3.1	>0.05
hs-CRP (mg/L)	13.6 ± 2.7	6.8 ± 3.1	<0.05
ESR (mm/h)	55.6 ± 5.1	53.6 ± 8.3	>0.05
RF (IU/ml)	32.3 ± 5.3	31.3 ± 4.7	>0.05
IL-17 (pg/ml)	34.6 ± 4.5	17.8 ± 3.5	<0.05
MCP-1 (pg/ml)	269.1 ± 38.8	176.4 ± 29.5	<0.05

RA: Rheumatic Arthritis; ABI: Ankle-Brachial Index; TNF-α: Tumor Necrosis Factor-α; hs-CRP: High-Sensitivity C-Reactive Protein; ESR: Erythrocyte Sedimentation Rate; RF: Rheumatoid Factor; IL-17: Interleukin-17; MCP-1: Monocyte Chemoattractant Protein-1.

Comparison of rheumatoid and inflammation related indexes between RA patients with normal and abnormal ABI

In 30 RA patients, there were 17 cases with normal ABI (>0.9) and 13 cases with abnormal ABI (≤ 0.9s). The levels of TNF-α, ESR and RF had no significant difference between RA patients with normal and abnormal ABI, respectively (P>0.05). The levels of serum hs-CRP, IL-17 and MCP-1 in RA patients with abnormal ABI were significantly higher those in RA patients with normal ABI, respectively (P<0.05) (Table 3).

The logistic regression analysis was performed on the risk factors of RA patients with abnormal baPWV and with abnormal ABI, respectively. Results showed that, hs-CRP, IL-17 and MCP-1 were the main independent risk factors of RA patients with abnormal baPWV (Table 4), and IL-17 and MCP-1 were the main independent risk factors of RA patients with abnormal ABI (Table 5).

Table 4. *Logistic regression analysis on risk factors of RA with abnormal baPWV.*

Variable	B	χ^2	OR	95% CI	P
IL-17	0.183	4.041	2.356	1.088-6.472	0.021
MCP-1	1.174	5.648	3.069	2.018-7.993	0.007
hs-CRP	1.253	6.341	3.409	2.384-9.205	0.002

RA: Rheumatic Arthritis; baPWV: Brachial-Ankle Pulse Wave Velocity; IL-17: Interleukin-17; MCP-1: Monocyte Chemoattractant Protein-1; hs-CRP: High-Sensitivity C-Reactive Protein.

Table 5. *Logistic regression analysis on risk factors of RA with abnormal ABI.*

Variable	β	χ^2	OR	95% CI	P
IL-17	1.019	3.104	2.782	1.021-8.032	0.017
MCP-1	1.053	5.021	2.883	1.101-6.452	0.023

RA: Rheumatic Arthritis; ABI: Ankle-Brachial Index; IL-17: Interleukin-17; MCP-1: Monocyte Chemoattractant Protein-1.

Discussion

IL-17 is mainly produced by helper T cells. It can promote the elevation of vascular endothelial growth factors. It is confirmed that, the expression level of IL-17 is increased in human or mouse vascular disease [14,15]. Therefore, it is believed that the occurrence of vascular lesions has great relationship with the vascular endothelial growth factors and the autoimmune vasculitis. IL-17 can participate in the process of vascular lesions through inflammatory changes in various layers, exerting the regulatory and controlling actions. It can strengthen the role of T cells, activate the Rho/RhoA kinase pathway, which promotes the increase of vascular endothelial growth factors [16]. Therefore, IL-17 may have a causal

relationship with vascular endothelial growth factors. This study compared the serum IL-17 level between RA patients and healthy controls. Results showed that, the level of IL-17 in RA group was significantly higher those in control group, respectively ($P<0.05$). This indicates that, IL-17 is involved in the pathogenesis of RA.

MCP-1 is a member of the subfamily of CC chemokine. It is a key cytokine mediating the inflammatory response. MCP-1 belongs to the chemokine superfamily, and acts as a chemoattractant protein specifically for monocytes. MCP-1 can combine with CCR2 receptor on the surface of monocytes, through which the monocytes enter the tunica intima, and become macrophages, thereby promoting the increase of vascular endothelial growth factors [17]. Therefore, MCP-1 has great effect on vascular lesions. In addition, MCP-1 can promote the accumulation of macrophages and neutrophil in the arterial wall to cause the inflammation, macrophages devouring the lipid to form the foam cells, and proliferation and migration of vascular smooth muscle cells to cause the atherosclerotic plaque [18]. MCP-1 can also stimulate the fiber cells and upregulate the TGF-β expression, thereby increasing the formation of collagen fibers [19]. Results of this study showed that, the level of MCP-1 in RA group was significantly higher than those in control group, respectively ($P<0.05$). This indicates that, IL-17 is also involved in the pathogenesis of RA.

Vascular wall lesions are the basis of the occurrence and development of cardiovascular and cerebrovascular diseases. The change of arterial stiffness occurs earlier than the change in the arterial structure [7]. Only the early detection and finding of arterial stiffness change, followed by monitoring and intervention, can effectively prevent the occurrence and development of cardiovascular and cerebrovascular diseases. As the indicators of atherosclerosis, the roles of baPWV and ABI are attracting more and more attention. BaPWV can well reflect the arterial compliance [20], and ABI can reflect the degree of atherosclerosis [21]. It is found that, the elevation of baPWV and the decrease of ABI are significantly related to cardiovascular and cerebrovascular diseases. This study had compared the rheumatoid and inflammation related indexes between RA patients with normal and abnormal baPWV, and between RA patients with normal and abnormal ABI. Results showed that, the levels of serum IL-17 and MCP-1 in RA patients with abnormal baPWV (>1400 cm/s) were significantly higher those in RA patients with normal baPWV (≤ 1400 cm/s), respectively ($P<0.05$), and those in RA patients with abnormal ABI (≤ 0.9) were significantly higher those in RA patients with normal ABI (>0.9), respectively ($P<0.05$). The results of logistic regression analysis showed that, IL-17 and MCP-1 were the main independent risk factors of RA patients with abnormal baPWV and abnormal ABI. This has further verified the roles of IL-17 and MCP-1 in predicting the vascular lesions in RA patients.

In this study, the logistic regression analysis showed that, in 30 RA patients, the levels of serum IL-17 and MCP-1 were positively correlated. Therefore, it can be inferred that, II-17 can induce MCP-1 to mediate the infiltration and migration of neutrophils and monocytes to inflammatory sites, leading to vascular lesions. Nevertheless, the signaling pathways between II-17 and MCP-1 and their biological effects cannot be determined for the time being, and further studies and analyses are needed. In conclusion, IL-17 and MCP-1 are involved in the occurrence and development of RA, and they are the reliable indicators for judging the vascular lesions in RA. This study has provided a basis for further elucidating the mechanisms of IL-17 and MCP-1 in vascular lesions in RA patients, which is conducive to early detect and change the inflammatory state of RA for treatment of vascular lesions of RA. This study still has some limitations. The sample size of this study is relatively small. Larger sample size will make the results more convincing. In our next studies, the sample size should be further increased for obtaining more satisfactory opinions. In addition, it is reported that, the asymmetric dimethylarginine (ADMA) is involved in the pathogenesis of rheumatism, and it can be used as a marker of endothelial dysfunction and cardiovascular risk in patients with systemic rheumatic diseases [22]. This study has not investigated the relation of plasma ADMA level with vascular lesions in RA, which is also a limitation of this study. In our related studies, this should be considered.

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