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Original article

Intervention of integrative medicine treatment has impact on serum levels of ET-1, TNF-α, MLT in RA-CVD

Meng Chen [a], Zhenbin Li [b,*], Zheng Zhang [a], Yong Du [a], Yingjie Zhang [a], Minghua Xu [a], Caixia Sun [a]

[a] Department of Rheumatology, Affiliated Hospital of Hebei University, Baoding 071000, PR China
[b] Department of Rheumatology and Immunology, Bethune International Peace Hospital of PLA, Shijiazhuang 050000, PR China

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ABSTRACT

Rheumatoid arthritis (RA) is a systemic autoimmune disease that can destroy peripheral joints. However, very little is known regarding specific biological marker for RA in Chinese patients. In this study, we determined the serum biomarkers and clinical features of RA-CVD. We also evaluated the short-term efficacy of routine RA treatment combined with integrative medicine treatment on RA-CVD. We found that anti-cyclic citrullinated peptide (CCP) and disease activity score in 28 joints (DAS28) are associated with risks of cardiovascular disease (CVD) in RA. And, melatonin (MLT) may play a negative regulatory role in cardiovascular damage in patients with RA. Furthermore, endothelin (ET-1) and inflammatory markers may be subclinical cardiovascular damages in RA. Moreover, of the 17 patients with RA-CVD, test results of ET-1, TNF-α and OSCAR after integrative medicine treatment were significantly decreased than before treatment. Collectively, our results provide a therapeutic potential of integrative medicine to the treatment of RA-CVD.

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder that primarily affects the peripheral joints. It is characterized by chronic inflammation, progressivity and destruction of joints. Synovitis and pannus are also known to be associated with its pathology. In RA patients, the slowly progressive articular cartilage and bone destruction leads to joint deformity and eventually loss of function. In addition to the joints, other tissues and organs are involved in, such as heart, lungs, kidneys and other important internal organizations because of vasculitis. RA thus appears has a considerable effect on patients' quality of life and causes economic burden. Previous research shows that RA caused shorter life expectancy by about 5–10 years (Minaur et al., 2004). Recent studies showed that around 42% death of RA resulted from cardiovascular diseases (CVD) and 34% of RA patients found plaque in carotid artery (Seriolo et al., 2003). However, it is still unclear about the relationship between RA and CVD after the correction of blood lipids level, systolic blood pressure, smoking, diabetes, body mass index, age, sex and other known cardiovascular risk factors in RA patients and the general population (Frostegard, 2005). Therefore, traditional risk factors are not the sole explanation for high CVD incidence in RA patients. Recent studies showed that the CVD incidence caused by RA is similar to that caused by diabetes (Inmaculabadel et al., 2005). However, very little is known regarding the specific biological markers of the CVD incidence in RA patients. Furthermore, the lack of specific biological markers resulting in a number of cases missed in the RA patients with CVD complication.

It has been widely accepted that patients with RA has higher mortality rate compared with the general population (Dadoun et al., 2013). A meta-analysis showed CVD is the reason for the excessive 50% mortality rate of RA patients from 1970 to 2005 (Avina-Zubieta et al., 2008). And, anti-CCP antibody were detected in the patients with excessive mortality rate (Symmons and Gabriel, 2011; Kerola et al., 2012).

Inflammation has been known as the pathologic basis of CVD which supports that RA patients with chronic non-bacterial inflammation and immune dysfunction are more prone to develop CVD. The clinical data of China shows that the characteristics of patients with CVD including: (1) arrhythmia; (2) ECG ST-T abnormality; (3) myocardial damage; (4) pericardial effusion; (5) valve diseases; (6) pulmonary hypertension; (7) abnormal echocardiograms with abnormalities of cardiac structure such as atrial or ventricular

* Corresponding author.
E-mail address: mvznrh@sina.com (Z. Li).

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intracavitary thrombus. Therefore, patients with the above characteristics are identified as RA-CVD group. Previous study showed that CRP level may be correlated with high CVD incidence (Emerging et al., 2012). However, it is still unclear about the role of CRP in the pathogenesis of CVD. It was revealed that CRP level is associated with CVD risk factors (obesity, hypertension, hyperlipemia, low level of high density lipoprotein-cholesterol (HDL-C)) in the general population without RA (Grad and Danenberg, 2013). And, it remained unclear that whether the high CVD incident is related to CRP associated risk factors or RA (Grad and Danenberg, 2013).

TNF-α antagonist was used to treat CVD in the RA patients. In a meta-analysis, the CVD incidence decreased 31% (RR, 0.69; 95%CI, 0.53–0.89) (Barnabe et al., 2011). In a random sampling study using placebo as control group, meta-analysis showed the TNF-α antagonist treatment did not have significant decrease in CVD incidence (RR, 0.85; 95%CI, 0.28–2.59) Barnabe et al. (2011). In another meta-analysis, TNF-α antagonist treatment reduced the CVD incidence compared with DMARDS (HR, 0.39; 95%CI, 0.19–0.82) treatment in RA patients by CORRONA data (Greenberg et al., 2011). However, there was no remarkable decrease of CVD incidence in RA patients between TNF-α antagonist and DMARDs treatment in Sweden (Ljung et al., 2012).

Recently, the correlation of RA and CVD received more research attention but the studies associated with Chinese populations are very limited. To make clear about the specific clinical indicators and clinical characteristics of RA patients with RA-CVD in Chinese populations, we analyzed the clinical indicators (CRP, ESR, DAS28, anti-CCP antibody, MIF, ET-1, MLT, OSCAR, VA and TG) in RA and RA-CVD patients as well as health control (NC) groups. Furthermore, we analyzed the impact of integrative medicine treatment for 17 patients from RA-CVD group in order to obtain clinical evidence to the integrative medicine treatment for RA-CVD patients.

2. Material and methods

2.1. Patient material

In this study, the patients analyzed were from the clinical department of rheumatism and Immunology of Bethune International Peace Hospital of the People's Liberation Army, China between April 2014 and December 2014 and endorsed by the American College of Rheumatology (ACR) 1987 criteria (Lightwood and Glantz, 1997). Of the 58 patients, 24 were male and 34 were female. According to the results of myocardial repolarization, twelve-lead ECG and ultrasonic cardiogram, 58 patients were divided into RA and RA-CVD group according to previous studies (Geroulakos et al., 1994; Borhani et al., 1996). RA group includes 31 patients (male = 18, female = 13, median age = 46.52 ± 8.89 years old), and RA-CVD group includes 27 patients (male = 16, female = 11, median age = 49.13 ± 12.80 years old).

The normal control group (NC) analyzed were from the same hospital between October 2014 and November 2014 includes 27 patients without RA (male = 15, female = 12, median age = 45.18 ± 15.33 years old). 17 patients of RA-CVD group (male = 10, female = 7, age: 18–70) were selected to use normal integrative medicine. After 2 weeks of treatment, we detected changes in clinical parameters and peripheral MIF, TNF-α, ET-1, MLT, OSCAR, ESR, CRP, UA and TG levels. Patients' age comparison is presented in Table 1.

Table 1
General comparison of the three groups.

Groups	N	Age (year)	P
RA	31	49.13±12.80	0.566
RA with CVD	27	46.52±8.89	
Control	26	45.18±15.33	

Note: P > 0.05, there was no significant difference in age among the diseases and the health ones.

2.2. Peripheral venous blood serum analysis

Immediately after collection, peripheral venous blood samples from RA groups and RA- CVD groups for serum preparation was distributed onto tubes with coagulation-activating reagents. After clotting, serum was separated and frozen on site. Samples were finally aliquoted into cryotubes (Biocoen, Beijing, China) and then frozen immediately for storage at −80 °C for subsequent analysis.

Concentrations of TNF-α, MIF, MLT, ET-1, and OSCAR in the serum of peripheral venous blood samples were assayed using human ELISA kits (TNF-a [R&D System, Minneapolis, MN, USA], MIF [R&D System], MLT [MyBiosource, San Diego, CA], ET-1 [R&D System], and OSCAR [R&D System]), according to the manufacturers' instructions. All samples were assayed in duplicate. Protein concentrations are expressed as pg/ml based on relevant standard curves.

2.3. Statistical analysis

All data were analyzed with SPSS 16.0 software. For the normal distribution, measurement data were presented as means ± SD ($\bar{x}$ ± s). Student's t test was used to analyze the difference between two groups, and one-way analysis of variance (ANOVA) was used to determine any significant differences between the three or more independent (unrelated) groups. If the measurement variable is not normally distributed, data were presented as median (25%, 75%) performed with SPSS using rank sum test.

All experiments were performed at least 3 independent experiments and P < 0.05 was considered as significant.

3. Result

3.1. CRP, ESR, DAS28 and anti-CCP antibody levels in patients between RA and RA-CVD

The aim of this prospective study was to compare the clinical value of the ESR and CRP between RA and RA-CVD groups which are common laboratory measurements of infection and tissue injury in clinical practice of RA.

For CRP, we observed that level of RA group is lower than RA-CVR groups, but again, the differences were not statistically different (Fig. 1A) (P > .05).

We further explored the value of ESR level of in RA and RA-CVD groups. ESR of RA is higher than RV-CVD group but there was no significant differences between RA and RA-CVR groups were investigated (Fig. 1B) (P > .05).

However, the diagnostic testing of DAS28 showed that DAS28 level was significantly up-regulated in RA-CVD group compared with RA group (Fig. 1C) (P < .05).

The outcomes of diagnosis also comprised anti-CCP antibody level. The anti-CCP antibody level in RA group was lower than RA-CVD group and the difference between these 2 groups was significant (Fig. 1D) (P < .05).

3.2. MIF, TNF-α, ET-1, MLT and OSCAR levels in patients with RA, RA-CVD and in NC groups

The MIF for the RA group were 4.26 ± 1.56 pg/mL compared with 4.22 ± 1.90 pg/mL and 4.01 ± 0.90 pg/mL in RA-CVD group

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