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.

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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

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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.

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

Table 1
General comparison of the three groups.

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

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

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

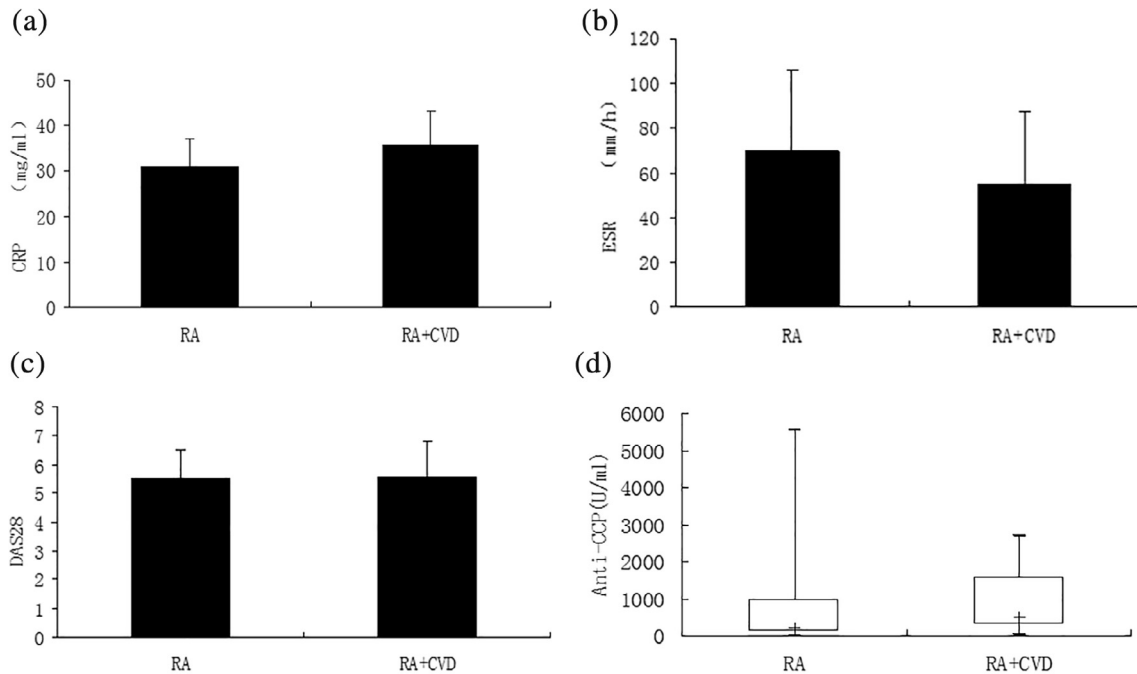(a)

(b)

(c)

(d)

Fig. 1. Comparisons of CRP (A), ESR (B), DAS28 (C) and Anti-CDDP (D) related RA between the two groups.

and NC group (Fig. 2A). We did not observe significant difference of MIF in these 3 groups (P > .05, Table 2-1). The median of TNF-α in RA group was 303.44 (236.80, 486.89) µg/L compared with 272.20 (215.42, 263.59) µg/L and 265.72 (206.82, 306.11) µg/L in RA-CVD and NC groups (Fig. 2B). However, TNF-α level only has significant difference between RA and NC group (P < .05, Table 2-1).

Furthermore, the ET-1, MLT and OSCAR of RA group were 3.23 ± 1.78 ng/L, 206.25 (159.57, 252.30) pg/mL and 115.93 (91.62, 146.13) ng/mL, respectively, compared with 3.26 ± 2.04 ng/L, 176.28 (141.47, 192.77) pg/mL and 207.17 (157.09, 252.30) pg/mL of RA-CVD group (Table 2-2) (Fig. 2C, D, F). Of the health group, the ET-1, MLT and OSCAR were 2.40 ± 0.56 ng/L, 207.17 (157.09, 252.30) pg/mL and 124.74 (100.24, 147.74) ng/Ml, respectively (Fig. 2C, D, F) (Table 2-2). For ET-1, we observed a significant difference between RA and NC groups as well as RA-CVD and health groups (Fig. 2C) (P < .05, Table 2-1). Of MLT, we investigated a significant difference within RA, RA-CVD and NC groups (Fig. 2F) (P < .05, Table 2-2). However, there was no significant difference in RA, RA-CVD and NC groups for OSCAR (Fig. 9) (P > .05, Table 2-2).

3.3. ET-1, MIF, MLT, TNF-α, OSCAR, ESR, CRP, UA and TG levels in patients of RA-CVD group after 2 weeks of integrative medicine treatment

To detect whether RA clinical conditions were improved in RA-CVD after 2 weeks of integrative medicine treatment, we randomly chose 17 patients from RA-CVD group to determine the ET-1, MIF, MLT, TNF-α, OSCAR, ESR, CRP, UA and TG levels before and after treatment.

Of the 17 patients with RA-CVD, test results of TNF-α, OSCAR and ET-1 after integrative medicine treatment were significantly decreased than before treatment (Fig. 3A, B and I) (P < .05, Table 3-1). However, the MIF and MLT levels were down-regulated than before but not reaching the statistic difference (Fig. 3C, D) (P > .05, Table 3-1).

Of the ESR, CRP, UA and TG levels in the 17 RA-CVD patients, we observed that ESR and CRP levels were significantly decreased after integrative medicine treatment than treatment before (Fig. 3E, F)

(P < .05, Table 3-2). However, UA and TG levels trended to decrease after treatment than before treatment but there was no significant difference (Fig. G, H) (P > .05, Table 3-2).

4. Discussion

RA is a systemic autoimmune disease and a common chronic inflammatory lesion of the synovial tissue of the joints. In addition to the joints, other tissues or organs are involved, such as heart, lungs, kidneys and other important internal organizations. Recent studies have shown that patients diagnosed with RA increases the risk of cardiovascular damage. And, patients with RA have increased risk for death from CVD. It was demonstrated that the prevalence between CVD incidence and RA is not same in different countries. For example, the CVD incidence because of RA is 16% in Sweden (World Health Organization Study Group). However, previous studies have not suggested that traditional CVD risk factors were associated with the high CVD incidence in RA patients (Morand et al., 2006).

In 2009, Peters MJ et al. have reported that RA and diabetes are independent risk factors for CVD incidence (Peters et al., 2009). In a brief epidemiological overview of Rincon et al., it showed that the CVD incidence of RA patients was 3.96 times (95% confidence interval: 1.86–8.43) higher than the general population (Rincon et al., 2001). After appropriate correction of the traditional risk factors such as systolic blood pressure, cholesterol, smoking, body mass index and blood sugar, the CVD incidence was still as high as 3.17 times (95% CI: 1.33–6.36) compared to the general population (Rincon et al., 2001). Clearly, it suggests that there are other unknown related risk factors to the increased CVD incidence in RA patients.

In our study, we aimed to determine whether the intervention of integrative medicine treatment has effect on the RA-CVD. In order to determine the effects of the integrative medicine, we evaluated the biomarkers (CRP, ESR, DAS28, Anti-CCP, MIF, TNF-α, ET-1, MLT, OSCAR, UA and traditional factors) related to the RA-CVD. We found that DAS28 score is significantly different between RA and RA-CVD groups. This result suggests that inflammation index
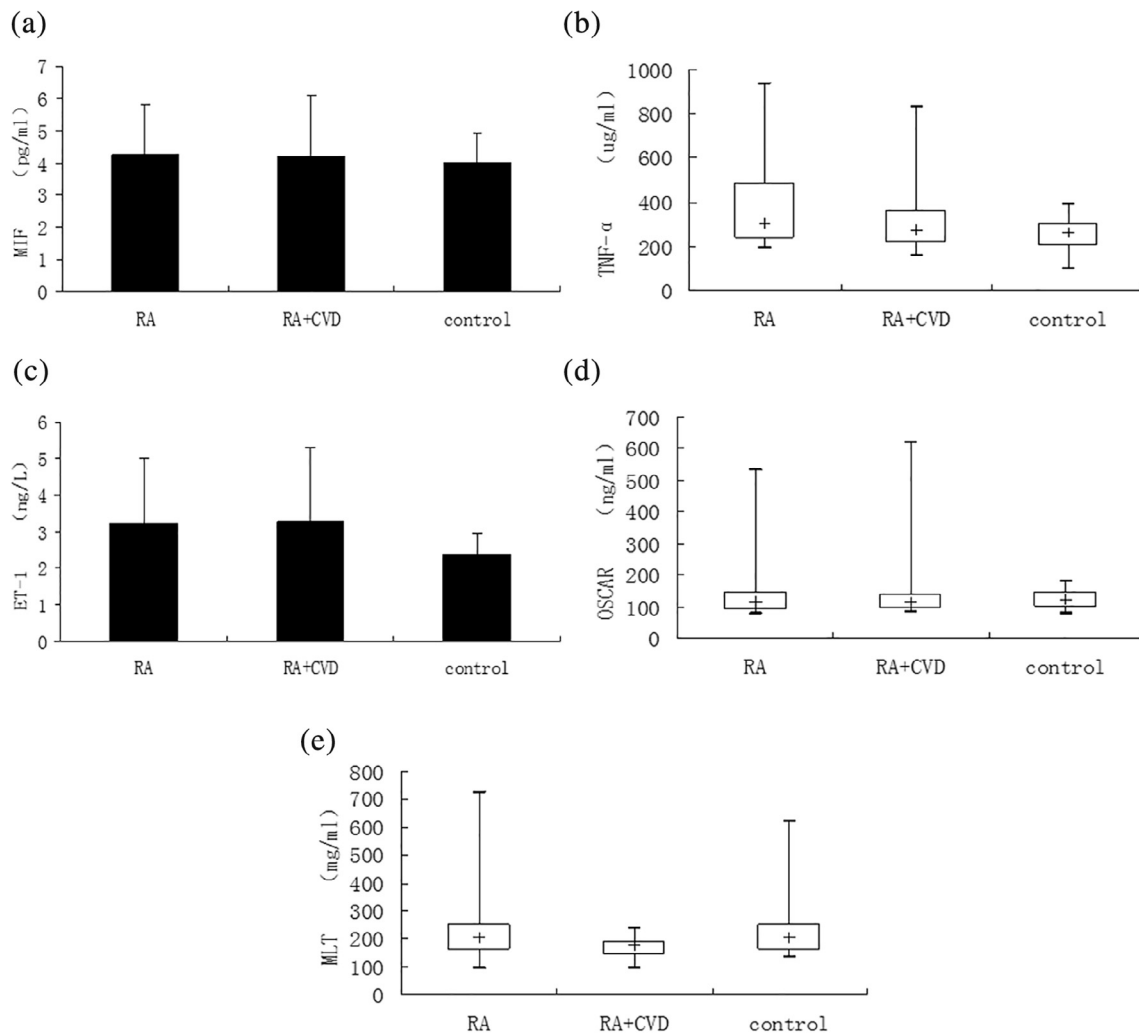
(a)

(b)

(c)

(d)

(e)

Fig. 2. Comparisons of MIF (A), TNF-α (B), ET-1 (C), OSCAR (D), ET-1 (E) related RA among the group.

Table 2-1
Comparisons of the New factors related RA among the groups.

Groups	N	MIF (Pg/mL)	TNF-α (Ug/mL)
RA	21	4.26±1.56	303.44（236.80，486.89）
RA+CVD	12	4.22±1.90	272.20（215.42，263.59）○
Control	26	4.01±0.90 □	265.72（206.82，306.11）●

Note: □ P > 0.05, there was no significant difference in MIF among the groups control, RA and RA with cardiovascular complications. Pairwise comparisons of MIF among the three groups, P > 0.05, there was no significant difference in MIF between any two groups.
● P > 0.05, there was no significant difference in TNF-α among the groups control, RA and RA with cardiovascular complications.
● vs the group RA with cardiovascular complications, P > 0.05.
● vs the group RA, P < 0.05. ○ vs the level of the group RA, P > 0.05, there was no significant difference in TNF-α between the groups RA and RA with cardiovascular complications.

Table 2-2
Comparisons of the New factors related RA among the groups.

| Groups	N	ET-1 (ng/L)	MLT (mg/mL)	OSCAR (ng/mL)
RA	21	3.23±1.78	206.25（159.57，252.30）	115.93（91.62，146.13）
RA with CVD	12	3.26±2.04	176.28（141.47，192.77）	113.31（95.07，137.71）
Control	26	2.40±0.56	207.17（157.09，252.30）	124.74（100.24，147.74）

Note: There was no significant difference in ET-1, MLT and OSCAR among the groups control, RA and RA with cardiovascular complications. Comparisons between the group control and the group RA with cardiovascular complications in ET-1 and MLT, P < 0.05. Comparisons between the group control and the group RA in ET-1. P < 0.05. Comparisons between the group RA and the group RA with cardiovascular complications in MLT, P < 0.05.

(ALI) may have been related to RA-CVD. This is consistent with the observation of Conzales-Gay MA and Dessein PH (Guillevin and Dorner, 2007). Anti-TNF-α treatment has been approved to decrease carotid intima-media thickness (cIMT) in RA group compared with the health control group by Del Porto F group. In this study, we confirmed that there was significant difference in TNF-α between RA and health control groups but not between the RA and RA-CVD groups. Our results indicate that TNF-α is one of the inflammation factors involved in the RA progression.

MIF level was found to increase in the initial stage of RA and activate various inflammatory cytokines. In the present study,
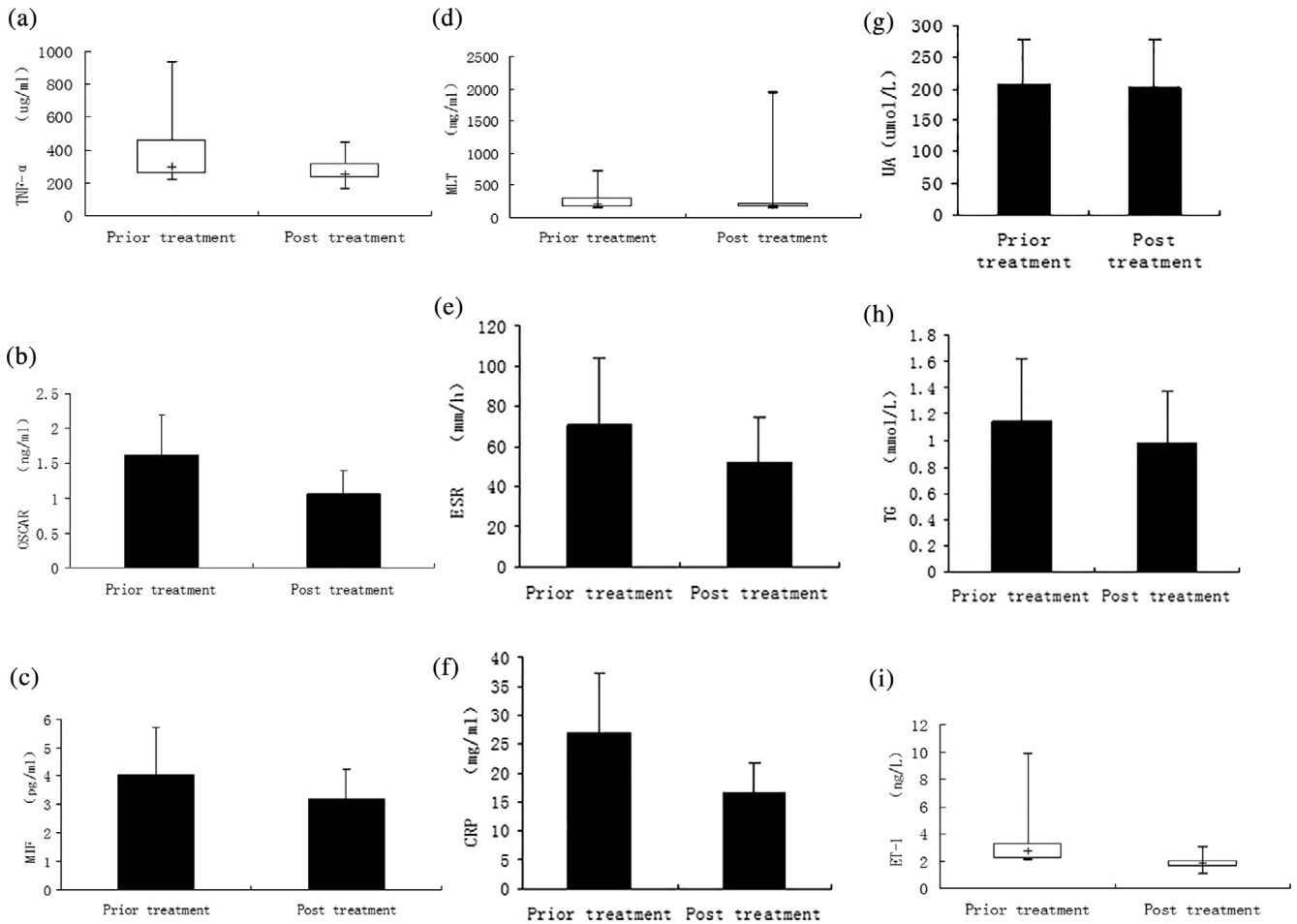
Fig. 3. Comparisons of TNF-α (A), OSCAR (B), ET-1(I) MIF (C), MLT (D), ESR (E), CRP (F), UA (G) and the traditional factors (H) related RA in the patients treated with integrative medicine.

Table 3-1

Comparisons of the new factors related RA in the patients treated with Integrative Medicine.

Group	ET-1	MIF	MLT	TNF-α	OSCAR
Prior treatment	2.71 (2.21, 3.33)	4.04±1.66	206.64 (171.50, 309.59)	330.37 (258.83, 463.20)	1.62±1.19
Post treatment	1.80 (1.59, 2.02)	3.19±1.05	193.27 (158.54, 233.06)	252.70 (231.03, 314.52)	1.07±2.40

Note: There was no significant difference in MIF, MLT and OSCAR between the group before and after treatment, $P > 0.05$. There was significant difference in ET-1, and TNF-α between the group before and after treatment, $P < 0.05$.

Table 3-2

Comparisons of the traditional factors related RA in the patients treated with Integrative Medicine.

Group	ESR	CRP	UA	TG
Prior treatment	71.00±33.26	26.89±20.30	207.94±70.46	1.15±0.47
Post treatment	51.29±22.84	16.79±13.94	202.24±77.04	0.98±0.40

Note: There was no significant difference in UA and TG between the group before and after treatment, $P > 0.05$. There was significant difference in ESR and CRP between the group before and after treatment, $P < 0.05$.

MIF level in the serum of RA-CVD group is remarkably up-regulated in comparison with health control group but not with RA group but has no significant difference between RA and health control groups. Abnormal ET-1 results in vascular remodeling and effects blood flow. In our study, there was statistically significant higher in RA group compared with health control group. Similarly, ET-1 level in RA-CVD group was significantly higher than health control groups. Our results confirmed that ET-1 is an important regulator in the pathogenesis of RA. MLT acts as a direct scavenger of free radicals and stabilization of biological rhythms. In our study, we found that MLT level in RA-CVD group was significantly decreased than both RA and health control groups. This result indicates that MLT is involved in the regulatory network in RA patients to protect against CVD.

It was found that there is large aggregate of osteoclast in the surface of joint bone destruction of RA patients. In our study, OSCAR level was not significantly different in RA, RA-CVD and health control groups. This leaves us with an interesting direction to test whether OSCAR is one of the risk factors for CVD incidence related to RA patients by increasing the sample size.

Combination of Methotrexate (MTX) and leflunomide (LEF) has been approved to have therapeutic effects on RA and CVD by improving blood flow. In our study, we use integrative medicine treatment for RA-CVD patients. We found ESR and CRP levels in RA-CVD patients decreased after treatment and the difference was statistically significant. Moreover, TG and VA levels also

down-regulated after treatment but did not reach statistical significance. It indicates that drugs may also inhibit the inflammation in RA besides the improvement of blood flow. However, since there were only 17 RA-CVD patients involved in our study, more data are needed to confirm this observation.

Taken together, our results found that ESR, CRP levels have no significant different between RA and RA-CVD groups. In addition, MLT level in RA-CVD group was higher than RA group but lower that health control group. And, ET-1 level in RA and RA-CVD groups were remarkably increased than health control group. Moreover, ESR and CRP levels in RA-CVD patients significantly decreased after integrative medicine treatment. All these results suggest that anti-CCP antibody level and DAS28 may be related to the CVD incidence in RA patients. RA patients may have subclinical cardiovascular damage and MLI may play a negative regulatory role. Furthermore, integrative medicine treatment may be considered as a novel therapy of RA.

Conflicts of interest

None.

Human and animal rights and informed consent

In this study, written informed consent was obtained from all patients. And, all aspects of this study were approved Bethune International Peace Hospital's Ethics Committee. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals and approved by our institutional ethical guidelines for animal experiments.

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