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Association of homocysteine with immunological-inflammatory and metabolic laboratory markers and factors in relation to hyperhomocysteinaemia in rheumatoid arthritis

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ABSTRACT

Objective. *The increase of homocysteine (Hcy) is an independent risk factor related to the development of atherosclerosis (AS) and the cardiovascular disease (CVD) in patients with rheumatoid arthritis (RA). Cardiovascular disease is a leading cause of mortality in RA. The aim of the study was to determine its relationship with homocysteine and the clinical immunological-inflammatory and metabolic laboratory markers and evaluate the factors in relation to hyperhomocysteinaemia in RA.*

Methods. *Analysis of total serum homocysteine (Hcy) concentrations was carried out in fifty patients with RA compared with 50 matched health controls. In patients with RA, numerous immunological-inflammatory and metabolic laboratory makers included, folate, vitamin B12 complement (i.e. C3 and C4), C-reactive protein (CRP), Rheumatoid factor (RF), anticyclic citrullinated peptide (anti-CCP) antibody, cystatin C (CysC), triglycerides, cholesterol and total leukocyte count. We also assessed imaging makers, common carotid intima-media thickness (IMT) and disease activity makers such as Disease Activity Score in 28 joints (DAS28).*

Results. *Median concentration of Hcy was significantly greater in patients with RA than in controls: 9.09 (5.38–33.91) vs. 7.45 (4.89–25.77) μmol/L (p<0.001). In RA patients, homocysteine was inversely associated with folate (rho=-0.672, p<0.0001), vitamin B12 (rho=-0.424, p=0.002), C3 (rho=-0.612, p<0.0001), C4 (rho=-0.323, p=0.022), correlate with CRP (rho=0.342, p=0.015), CysC (rho=0.430, p=0.002), but not with anti-CCP antibody (rho=0.205, p=0.152), RF (rho=0.214, p=0.135), triglycerides (rho=-0.107, p=0.459), cholesterol (rho=0.160, p=0.268), total leukocyte count (rho=-0.157, p=0.276), IMT (rho=0.134, p=0.156), DAS28 (rho=0.211, p=0.148). There was association between hyperomocysteinaemia in RA with vitamin B12 (p=0.037), folate (p<0.001), and CRP (p=0.018). There was no association with male gender, anti-CCP antibody, RF, IMT and DAS28.*

Conclusion. *Results from this study suggested that Hcy concentration was increased in RA patients and associated with RA-related immunological-inflammatory and metabolic laboratory markers, including folate, vitamin B12, CRP, CysC, C3, C4. Early determination of Hcy and correlated clinical laboratory markers may be useful to evaluate RA patients with high cardiovascular risk.*

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by erosive synovitis that involves peripheral joint destruction and physical disability (1-2). An increased mortality due to accelerated atherosclerosis and cardiovascular disease (CVD) in patients with RA has been reported in several studies (3-6), although the underlying pathogenic mechanisms have not been identified but are known to involve inflammation, methylation-dependent DNA methylation Pathway, impaired fibrinolysis and endothelial dysfunction (7-10). The present study revealed that cardiovascular disease risk predicition scores sush as the Framingham Risk Score (FRS), the Systematic Coronary Risk Evaluation (SCORE), are correlated with vascular function and morphology, which accurately reflects early atherosclerosis changes in RA patients (11). The morbidity of cardiovascular disease (CVD) in RA remains greater than that of the general population after consideration of traditional cardiovascular risks, sush as hypertension, dyslipidaemia, obesity, diabetes mellitus, cigarette smoking (12). However, the cause of atherosclerosis in RA is unclear, many factors contribute to RA-associated AS. One potential contributor related to AS in RA patients is homocysteine (Hcy) (13).

Hcy is defined a sulphur-containing amino acid formed during the metabolism of methionine (14). Mild hyperhomocysteinaemia (HHcy) is defined on the basis of plasma concentration ranging between 15 and 30 μmol/L (15). HHcy has been regarded as an independent risk factor in AS and other vascular disease. It has been demonstrated that in the general population

Table I. Comparison of clinical characteristics between patients with rheumatoid arthritis (RA) and controls.

Characteristic	RA (n=50)	Controls (n=50)	*p*-value
Female, n (%)	39 (78%)	39 (78%)	NS
Age (years), median (range)	48 (18-73)	49 (19-70)	NS
Disease duration (years), median (range)	8.5 (0.1-25)		
BMI, kg/m^2	27.0 (24.8-33.5)	26.2 (23.4-31.5)	NS
Morning stiffness, min (range)	15 (0-180)		
Smoking current, n (%)	10 (20%)	11 (22%)	NS
Hypertension, n (%)	14 (28%)		
Diabetes mellitus, n (%)	5 (10%)		
Triglycerides (mmol/L), Median (range)	1.27 (0.36-3.66)	1.16 (0.40-3.39)	NS
Totalcholesterol (mmol/L), Median (range)	4.89 (2.94-7.57)	4.75 (2.88-7.06)	NS
Homocysteine (µmol/L), Median (range)	9.09 (5.38-33.91)	7.45 (4.89-25.77)	<0.001
C-reative protein (mg/L), Median (range)	27.6 (0.9-200)	3.04 (0.6-149)	<0.001
DAS28 score, Median (range)	4.53 (2.66-5.98)		
Medications, n (%)			
NSAIDs	35 (70%)		
DMARDs	27 (54%)		
GCs	22 (44%)		

BMI: body mass index; DAS28: Disease Activity Score in 28 joints; NSAID: non-steroidal anti-inflammatory drug; DMARD: disease-modifying anti-rheumatic drug; GC: glucocorticosteroid; Comparisons between quantitative variables were made using the Mann-Whitney U-test. Comparisons between proportions were made using the χ^2-test.

Table II. Factors associated with Hcy concentrations in RA patients (n=50).

Continuous variables	rho	*p*-value
Folate	-0.672	<0.0001
Vitamin B12	-0.424	0.002
CRP	0.342	0.015
Anti-CCP antibody	0.205	0.152
RF	0.214	0.135
CysC	0.430	0.002
C3	-0.612	<0.0001
C4	-0.323	0.022
Triglycerides	-0.107	0.459
Cholesterol	0.160	0.268
Total leukocyte count	-0.157	0.276
IMT	0.134	0.156
DAS28	0.211	0.148

Spearman rank correlation coefficient statistic.

Table III. Clinical markers associated with hyperhomocysteinaemia (>15µmol/L) in RA patients.

Characterstics	Homocystein ≤15µmol (n=37)	Homocysteine >15µmol (n=13)	*p*-value
Male, n (%)	6 (16%)	5 (38%)	0.095
Folate (nmol/L), Median (range)	12.2 (4.4-38.5)	4.8 (1.4-8.4)	<0.001
Vit. B12 (pmol/L), Median (range)	281 (137-1476)	190 (80-1476)	0.037
CRP (mg/L), Median (range)	22.7 (0.9-200)	67.3 (3.4-190)	0.018
RF (IU/ml), Median (range)	47.5 (11.1-10500)	69 (11.1-6710)	0.632
Anti-CCP antibody (U/ml), Median (range)	36.4 (0.5-816.1)	174.9 (0.5-254.5)	0.111
IMT (mm), Median (rang)	0.62 (0.20-1.48)	0.65 (0.22-1.33)	0.355
DAS28, Median (range)	4.47 (2.66-5.78)	4.66 (2.98-5.98)	0.298

Comparisons between quantitative variables were made using the Mann-Whitney U-test. Comparisons between proportions were made using the χ^2-test.

the risk of ischaemic heart disease increased by 32%, deep vein thrombosis increased by 60%, stroke increased by 59% for a 5µmol/L increase in Hcy (16). Increased levels of Hcy has been reported in previous studies of patients with RA (17).

We assessed Hcy as a cardiovascular risk factor in patients with RA. Increased intima-media thickness (IMT) indicating AS has also been described in RA (18). We went on to analyse immumological-inflammatory and metabolic makers that may be linked to the pathogenesis of vascular damage in RA patients. Among immunological makers, anti-CCP antibody, RF, C3, C4 were analysed and inflammatory makers such as serum CRP, total leukocyte count were measured. Among metabolic markers, levels of folate, viamin B12, cystatin C (CysC), triglycerides, cholesterol were assessed and their relationship determined with homocysteine and clinical laboratory markers in RA.

Materials and methods

Study subjects

We studied 50 patients with RA who were recruited from a rheumatology clinic in the Second Affliated Hospital Harbin Medical University. The patients met the 1987 American College of Rheumatology (ACR) criteria for RA and not taken folate, vitamin B in three months. We also studied 50 sex- and age-.matched subjects as a control group that were recruited in the Health Check Centre. We excluded pregnant patients, hepatic and thyroid disease, current infectious disease, or renal failure (serum creatine ≥117mmol/L). Current use of medications was determined by the medical records provided by patients with RA. Control subjects did not have RA or any other inflammatory disease.

Physical and laboratory tests

All subjects were interviewed with regard to age, sex, duration of morning stiffness, medical history, smoking, diabetes, hypertension, numbers of swollen and painful joints. Height and weight were measured. In patients with RA, disease activity was measured by

the Disease Activity Score based on the numbers of the swollen and the painful joints and the erythrocyte sedimentation rate (ERS). The IMT measurements were taken by a duplex ultrasound system (HP Sonos 5500, 10MHz linear array transducer). IMT values were expressed in mm. Blood was collected after an overnight fast. Plasma Hcy, folate, vitamin B12, Anti-CCP antibody were determined by fluorescent polarisation immunoassay (FPIA; Abbot), serum IgM RF, CRP, C3 and C4 were assessed by immunoturbidimetric assay (Siemens BNII). Total cholesterol, triglyceride and CysC were measured by colorimetry (Roche P800).

Statistical analysis

Comparisons of parametric variables were by *t*-tests and non-parametric variables were expressed by the Mann-Whitney U-test. Comparisons between proportions were made using the χ^2-test. Correlations between variables were analysed by spearman correlation coefficient. *P*-values less than 0.05 were considered significant.

Results

Clinical characteristics of patients with RA and control groups

Data for patients with RA and controls are presented in Table I. Age, sex, BMI and smoking were similar between the two groups. The median disease duration was 8.5 years in patients with RA. The prevalence of hypertension was 28% in patients with RA. Serum concentrations of total cholesterol as well as triglyceride levels were similar among patients and controls. Median Hcy were higher in patients than in controls, 9.09 (5.38–33.91) *versus* 7.45 (4.89–25.77) μmol/L ($p<0.001$). Median CRP levels were significantly higher in RA patients than in controls, 27.6 (0.9–200) *versus* 3.04 (0.6–149)mg/L.

Association between Hcy and clinical markers in RA patients

The correlations between Hcy and immunological-inflammatory and metabolic laboratory markers in RA patients (n=50) are shown in Table II. Hcy concentrations inversely correlated with folate (rho=-0.672, $p<0.0001$), vitamin B12 (rho=-0.424, $p=0.002$), C3 (rho=-0.612, $p<0.0001$), C4 (rho=-0.323, $p=0.022$) and markers of inflammation such as CRP (rho=0.342, $p=0.015$) and were significantly correlated with CysC (rho=0.430, $p=0.002$). Hcy concentrations were not associated with anti-CCP antibody ($p=0.152$), RF ($p=0.135$), triglyceride ($p=0.459$), cholesterol ($p=0.268$), total leukocyte count ($p=0.276$), IMT ($p=0.156$), DAS28 ($p=0.148$).

Clinical features associated with hyperhomocysteinaemia in patients with RA

According to Hcy levels, we divided patients with RA into low (≤15μmol/L; n=37) and high (>15μmol/L; n=13) groups. In the analysis, the variables associated with HHcy were folate ($p<0.001$), vitamin B12 ($p=0.037$), levels of CRP ($p=0.018$). There was no corrletion between HHcy and male gender, RF, anti-CCP antibody, IMT and disease activity such as DAS28. (Table III).

Discussion

This study confirms that patients with RA maintain higher levels of Hcy compared with matched healthy controls. In our patients, an inverse correlation existed between Hcy and folate, vitamin B12, which leads us to conclude that Hcy concentrations in the RA patients studied depend on folate, B-vitamin. Folate supplementation decreased the levels of Hcy and protected against potential cardiovascular risks (19). In addition to the folate and B-vitamin treatments, the use of the drugs have long been found to be a crucial factor for the elevated Hcy levels in patients with RA. It has been proposed that an effect of low-dose methotrexate (MTX) treatment leads to increased plasma Hcy levels (20). In contrast, the RA patients who were treated with MTX presented low Hcy levels (21). Nevertheless, the different effect of MTX on Hcy levels may be in relation to the genetic variability. The immunological-inflammatory factors may be involved in the develop of AS and pathogenesis of vascular damage in RA (22). Inflammtory makers such as CRP, as well as total leukocyte counts, immunological markers anti-CCP antibody, RF, C3, C4, were analysed in RA patients. Our data showed an association between Hcy and CRP, C3, C4. The systemic inflammatory response may be involved in the development of accelerated atherosclerosis in RA patients (23). The present analysis revealed that CRP concentrations were not only inversely related to TNF-related apoptosis-inducing ligand (TRAIL) which is lower in patients with RA than controls (24) but, strikingly have also been found to be associated with endothelial dysfunction and increased mortality of CVD in patients with RA (25). The inverse correlation between Hcy level and complement (C3 and C4) in our cohort for the first time. C3, C4 play a crucial role in the pathogenesis and progression of RA. Increased Hcy may be involved in the autoimmune response as a pro-inflammatory and immumomodulating molecule (26). There was no correlation between Hcy and anti-CCP antibody. We have not found any studies on the relation between anti-CCP production and the development of atherosclerosis in RA. In addition, the metabolic markers including CysC, triglycerides, cholesterol were also assessed in these patients. Elevated blood concentrations of metabolic markers CysC were found in parallel to the increased Hcy concentrations. CysC has been proposed as a novel marker of renal function and predictor of cardiovascular risk in the general population. CysC might be influenced by chronic inflammation in RA patients (27). Also, CysC was associated with atherosclerosis markers such as CRP in coronary artery disease patients (28). It has been found that CysC was higher in RA patients and significantly correlated with Hcy (29). These findings which were presented in our analysis proved that metabolic makers of CVD in patients with RA may be useful for the management and prevention in patients. In this study, some disease-associated variables, such as DAS28, were not correlated with Hcy. On the contrary, Wallberg-Jonsson *et al*. reported an association between Hcy and accumulated disease activity (30). Regarding

imaging data, Hcy was not associated with IMT. IMT was significantly greater in RA compared to control subjects (22). Hole *et al*. anticipated that plaque progression was statistically significant associated with the levels of changed Hcy with RA patients in the 5.5 years follow-up study (31).

At least one of every five patients with RA had HHcy and factors associated with HHcy include, folate, vitamin B12, CRP. In the analysis, there no correlation between HHcy and male gender with RA patients in the north of China. Lopez-Olivo *et al*. (32) reported the risk of HHcy in male was 3.1 fold increased than that of the female patients in Mexico. Folate, vitamin B12 concentrations showed a tendency toward lower levels in the group with HHcy compared with to the other without. Immuno-inflammatory activation could be the reason for the development of HHcy in patients with RA (33). B-vitamin depleted in the course of chronic immune activation and seemed particularly relevant in the Hcy metabolism (26). The occurrence of HHcy paralleled greater concentrations of inflammatory markers CRP (p=0.018) suggesting that the aptitude of HHcy enhances the inflammatory process.

In summary, Hcy is increased in RA compared with control subjects. Hcy is robustly associated with the mediators of immunological-inflammtory and metabolic makers such as CRP, C3, C4, CysC, folate and vitamin B12. The suppression of homocysteine can prevent cardiovascular complications during the progress of RA.

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