Original Paper

Vitamin Status as Predictors in Rheumatoid Arthritis

RADU RAZVAN MITITELU[1], MANUELA VIOLETA BACANOIU[2], ANA-MARIA BUGA[3], VLAD PADUREANU[4], ANDREEA BARBULESCU[5]

[1]Department of Microbiology, University of Medicine and Pharmacy of Craiova, Romania

[2]Department of Physical Therapy and Sports Medicine, University of Craiova, Romania

[3]Department of Biochemistry, University of Medicine and Pharmacy of Craiova, Romania

[4]Department of Internal Medicine, University of Medicine and Pharmacy of Craiova, Romania

[5]Department of Pharmacology, University of Medicine and Pharmacy of Craiova, Romania

ABSTRACT: Musculoskeletal disorders are the leading cause of long term disability in EU with a significant impact on health care system and with increased social and economic costs. Despite of recent advances in Rheumatoid arthritis (RA) research field, here is still lacking of specific biomarkers that can be used in order to distinguish between different RA patterns and the clinical criteria are still the main tool used only for classification of diseases. Our hypothesis is that the vitamin deficiency associated with chronic inflammation can lead to a mild increase in Hcy level in blood that can act as predictor of increased risk of complication in RA patients. The aim of our study was to identify a correlation between level of Hcy in peripheral blood samples collected from RA patients and to establish if the Hcy level can be validate as potential predictive biomarker in RA patients treated with different DMARDs. Our findings suggest that Hcy level in plasma and CRP are independent predictors of chronic inflammatory status and are useful biomarkers in order to estimate the risk of complication in RA patients. To our knowledge to date, studies before had a controversial findings regarding the efficiency of folate and B12 vitamins supplements on decreasing the cardiovascular events risk. We showed that the folic acid and B12 supplements are important.

KEYWORDS: *Rheumatoid arthritis, homocysteine, vitamin status, inflammation.*

Introduction

Rheumatoid arthritis (RA) is the most common autoimmune inflammatory disease that affect the joint and other organs or tissues in an extra-articular fashion [1,2].

RA is a heterogeneous disease that affect around 1% of population worldwide with a decreased incidence across the European Union areas, such as Southern and Western part of Europe [3,4].

The disease distribution according with age and sex is an important factor that affect the prevalence of diseases worldwide.

However, a general age-specific RA prevalence pattern show as an increased incidence in older age and in women compared with man [5,6].

As many other autoimmune diseases, the etiology of RA is still not fully understood.

According to existing evidence, it has been associated with triggers that come from an interaction between different environmental and genetic factors [7,8].

Despite of recent advances in RA research field, here is still lacking of specific biomarkers that can be used in order to distinguish between different RA patterns and the clinical criteria are still the main tool used only for classification of diseases.

The main biomarkers associated with RA diseases prognostics are the rheumatoid factor (RF) and anticitrullinated protein antibody (ACPA), but only 70% of RA patients has an increased plasma concentration of these biomarkers [9].

Studies before showed that besides the main biomarkers here still we need to identify biomarkers that can be easy to perform and are specific for different type of trigger factors of the immune system.

Oxidative stress biomarkers and lipid peroxidation was described as potential biomarkers that are associated with RA diseases progression.

A long time increase in concentration of these biomarkers are the main triggers of chronic inflammation that can increase the joint destruction and promote the extra-articular damage with unfavourable functional outcome [10-12].

Recent studies showed that the myeloperoxidase activity is increased in RA patients as hallmark of oxidative damage of the tissue [13].

Also, increased myeloperoxidase activity leads to lipid metabolic pathway alteration.

An increased lipid peroxidation and alteration of lipid profile in RA patient that can be a useful

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biomarker in order to appreciate disease progression and prognostic [11,14].

Other metabolic changes due to pro-inflammatory cytokine overproduction, such as protein changes (e.g. carbonylation or carbamylation) or metabolomic changes in lipid metabolic pathways, were described as potential predictors in autoimmune diseases [10,15,16].

Studies before showed that the carbamylated protein (e.g. albumin or vimentin) act as target antigens in RA patients [17,18].

Growing of evidence suggest that the atherosclerosis and RA diseases share a common inflammatory pattern that lead to cardiovascular comorbidities in RA patients due to autoimmune mediated endothelial dysfunction and chronic inflammation [20,21].

In this light, recent data show that the homocysteine level (Hcy) is a mirror tool of inflammation in diseases that are associated with chronic inflammation (e.g. cardiovascular diseases, diabetes or neurological disease) [20-22].

Homocysteine (Hcy) is a sulphur-containing amino acid that received an increased research interest in the last decade, due to growing evidence that show an involvement in alteration of vascular function.

However, despite of increasing research interest, the potential role of Hcy level as diseases predictor is still not fully understood.

Our hypothesis is that the vitamin deficiency associated with chronic inflammation can lead to a mild increase in Hcy level in blood that can act as predictor of increased risk of complication in RA patients.

The aim of our study was to identify a correlation between level of Hcy in peripheral blood samples collected from RA patients and to establish if the Hcy level can be validate as potential predictive biomarker in RA patients treated with different DMARDs.

Material and Methods

Ethical Issue

This study was approved by the Academic and Scientific Ethics and Deontology Committee of the University of Medicine and Pharmacy in Craiova (Registration No. 32174/2019) according to European Union Guidelines (Declaration of Helsinki).

All the patients signed an information and acceptance form to be included in the present study.

Patients and Sample selection

A number of 27 patients with RA, over 18 years old, that signed an acceptance form were included in this study.

The participants were divided in three groups as follow:

i) group 1 include seronegative RA patients (n=8) with average in age 52±19.7, treated with conventional synthetic Disease Modifying Drugs (csDMARDS);

ii) group 2 include seropositive RA patients (n=15) treated with conventional synthetic Disease Modifying Drugs (csDMARDS) and age mean of 62.5±9.01;

iii) group 3 include seropositive RA patients (n=7) with age mean of 50±2.8, treated with biological DMARDS (bDMARDS).

The severity of RA was establish using the Disease Activity Scale (DAS28) according with 2010 ACR/EULAR criteria [8].

Hcy level was assessed in plasma obtained from EDTA blood sample between 8 and 10 am after 12 hour fasting.

The sample were centrifuged at 3000rpm (Eppendorf centrifuge) and analysed using automatic analyser (ACL-Top 500; Instrumentation Laboratory-Werfen Group, Barcelona, Spain).

The Hcy plasma level is expressed as micromole/litre.

C-reactive protein (CRP) level was measured in serum obtained from venous blood sample after centrifugation, by immunoturbidimetric method automated device (Cobas e4000 series, Roche Diagnostics, Germany).

Vitamin status was evaluated by measuring the serum level of cobalamin and folic acid by chemiluminescence method (CLIA) using automated system Arhitect I1000 (Abbott Laboratories, USA)

Statistical analysis

Statistical analysis was performed with GraphPad Prism version 5.0 (GraphPad software, San Diego, USA).

Descriptive statistics were used to patients' characteristics using percentages for categorical variables and mean±standard deviation (SD) and p-values less than 0.05 were considered statistically significant.

Results

Hcy level associated with disease severity

We found that in our RA study groups, the quantitative measurement tools of disease activity (DAS28) (DAS28 mean=4.68±1.05) was significant correlated with Hcy levels (Hcy mean=11±2.53) in plasma of group 1 (p=0.034) (Figure 1).

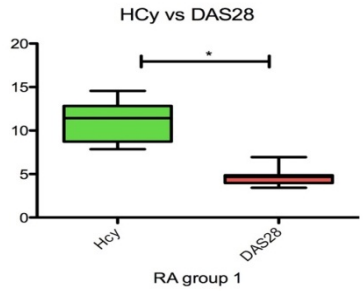

Figure 1. Hcy plasma level vs DAS28 in study group 1 (p< 0.05).

In our study group 2 and 3, the results did not show any significant correlation of Hcy levels in plasma with DAS28 (Figure 2).

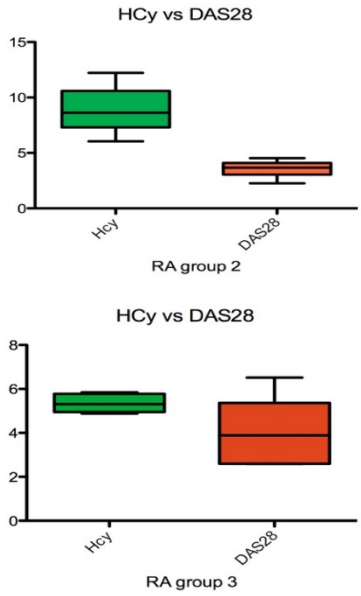

Figure 2. Hcy plasma level vs DAS28 in study group 2 and 3 (p>0.05).

Inflammatory status associated vs Hcy level

In order to analyse the correlation between inflammatory status and Hcy level in plasma of RA patients included in this study, we measured the CRP and Hcy level.

We found that the Hcy plasma level was decreased in RA group 3 (5.35±0.42) compare with group 1 and 2 (11.00±2.53 and 8.90±1.74; p<0.05) but we did not find a significant correlation with CRP level in plasma (Figure 3).

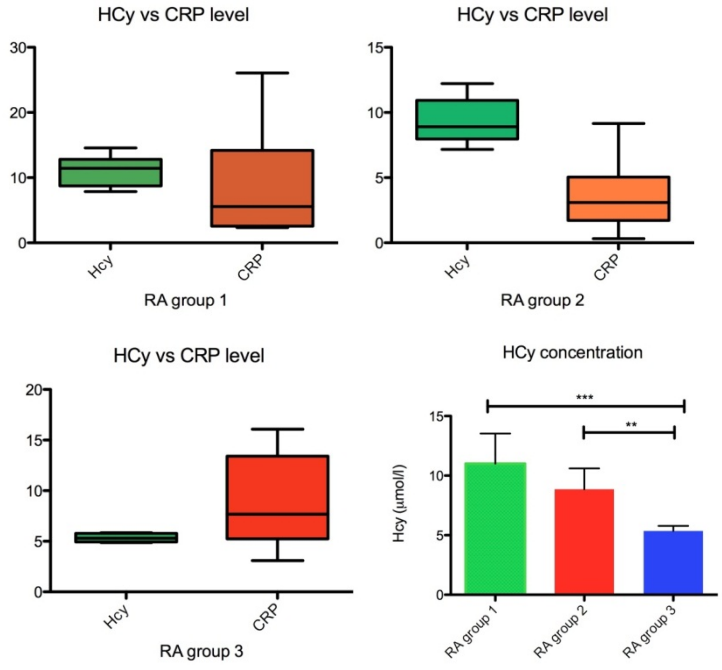

Figure 3. Hcy plasma level in RA study group 1, 2 and 3.

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Vitamin status associated vs. Hcy level

In order to asses the vitamin status and the impact of vitamin status in Hcy plasma level we measured the cobalamin and folic acid level in the blood of RA patients.

Our results show a significant difference in cobalamin level between the study groups 1 and 3 ($p < 0.05$), but not a significant correlation in folic acid level (Figure 4).

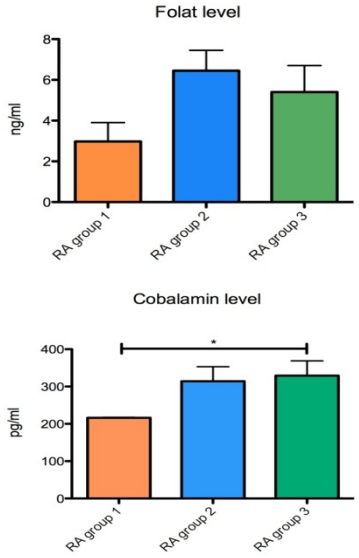

Figure 4. Folate and cobalamin serum level in RA study group 1, 2 and 3 (p >0.05).

Discussion

In the absence of a right time and right therapeutic option, RA has an increased risk of long term disability, complications and mortality.

The right therapeutic option decision is very complex and need to be correlated with a specific pattern of diseases and other potential comorbidities.

Today, we have different possibilities that can be used in a multimodal approach such as different pharmacological drugs and also non-pharmacological interventions (diet, physical exercise, nutraceuticals).

However, the main objectives are as main targets the control of inflammation and the improvement of functional outcome and the quality of life in RA patients.

For short period of time, glucocorticoids (GC) are the firs line choice for RA patients, but these are not the right choice for a long-time diseases control due to the increased risk of side effects.

Nowadays, in order to have a better long-time control, the different type of DMARDS are available.

The European League Against Rheumatism (EULAR) establish an algorithm of therapeutic management in RA diseases, but this decision algorithm takes into account only pharmacological approach and here is still needed a multidisciplinary decision [23].

Study before suggested that the seropositive RA patients with high activity diseases has an increased risk of therapeutic failure [24,25].

In addition, some of DMARDS has an important interaction with vitamin status that should be not neglected in the management of RA diseases [26,27].

In this light, our objective in this study was to understand the complex interaction between inflammation, Hcy level and vitamin status associated with different DMARDS therapy and with diseases severity.

We found that the Hcy level in plasma is increased according with disease severity for seronegative RA ($p=0.034$) (Figure 1).

We did not find a direct correlation in our study group between disease severity indicator DAS28 and Hcy plasma level for RA patients treated with csDMARDS or bDMARDS ($p>0.05$), and this can be explained by the small number of patients included in our study (Figure 2).

According with previous studies we found that the Hcy level has an increased trend in group 1 and group 2 [27,28].

Interestingly, in our study group 3, treated with bDMARDS display a reduced level of Hcy in plasma compared with other groups.

Homocysteine is a sulphur-containing aminoacid that promote a pro-inflammatory status, both in vitro and in vivo studies [28,29].

We found that the Hcy level in plasma of RA patients did not correlate with inflammatory biomarker CRP in RA patients (Figure 3).

These findings are in agreement with other studies before [30].

In addition, in our study group we found a decreased trend of cobalamin and folic acid in response to an increased level of Hcy.

We found a significant difference in cobalamin level between seronegative RA patients (group 1) and RA patients treated with bDMARDS (group 3), but we did not find a significant difference between other study groups.

However, our study is limited by a small number of patients and further studies on a large cohort must be performed in order to validate this pilot study results.

Conclusions

Our findings suggest that Hcy level in plasma and CRP are independent predictors of chronic inflammatory status and are useful biomarkers in order to estimate the risk of complication in RA patients.

To our knowledge to date, studies before had a controversial findings regarding the efficiency of folate and B12 vitamins supplements on decreasing the cardiovascular events risk.

We showed that the folic acid and B12 supplements are important in order to lowering the Hcy level and should be included as metabolic biomarkers together with Hcy in order to predict the RA diseases outcome.

Conflict of interests

None to declare.

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Corresponding Author: Buga Ana-Maria, Department of Biochemistry, University of Medicine and Pharmacy of Craiova, Petru Rareş nr. 2, Craiova, Dolj, Romania, Postal Code 200349, e-mail: ana.buga@umfcv.ro