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RESEARCH ARTICLE

Relationship between Plasma Levels of Homocysteine and Pro-Inflammatory Cytokines in Patients with Rheumatoid Arthritis

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ABSTRACT

Objective: The aim of the study was to investigate levels of homocysteine (Hcy), folate, vitamin B12, interleukin-6 (IL-6) and Tumor necrosis factor-alpha (TNF-α) and to assess relationship between Hcy levels and inflammatory mediators including IL-6 and TNF-α in untreated patients with rheumatoid arthritis (RA).

Methods: The study included 55 women including 30 newly diagnosed and untreated female RA patients and 25 age-matched healthy individuals as controls.

Results: Erythrocyte sedimentation rate (ESH), C-reactive protein (CRP), IL-6 and TNF-α levels were significantly higher in RA patients than controls ($p<0.05$). Homocysteine levels were higher in RA group when compared to controls; however, folate and vitamin B12 levels were not different between groups ($p>0.05$). There were no significant correlations between Hcy levels and acute phase reactants or inflammatory mediators ($p>0.05$).

Conclusion: Both mediators such as IL-6 and TNF-α and hyper-homocysteinemia are associated with atherosclerotic cardiovascular diseases. There was no significant correlation between Hcy levels and inflammatory mediators, although levels of these molecules were elevated in RA. Both inflammatory mediators and Hcy may cause an increase in atherosclerotic cardiovascular disorders through diverse pathways. *J Clin Exp Invest 2016; 7 (2): 163-167*

Key words: Rheumatoid arthritis, IL-6, homocysteine, TNF- α

Romatoid Artrit Hastalarında Plazma Homosistein ve Proinflamatuvar Sitokin Seviyeleri Arasındaki İlişki

ÖZET

Amaç: Bu çalışmanın amacı tedavi edilmemiş romatoid artrit (RA) hastalarında Hcy, folat, vitamin B12, IL-6, Tümör nekrozis faktör alfa (TNF-α) seviyelerini ve homosistein (Hcy) seviyeleri ile inflamatuar mediatörler olan interlökin-6 (IL-6) ile TNF-α arasındaki ilişkiyi araştırmaktır.

Yöntemler: Çalışmaya yeni tanı almış ve tedavi başlanmamış 30 kadın RA hastası ve kontrol grubu olarak yaş eşleştirmeli 25 sağlıklı kadın olmak üzere 55 kadın dahil edilmiştir.

Bulgular: Eritrosit sedimentasyon hızı (ESH), C-reaktif protein (CRP), IL-6 ve TNF-α seviyeleri RA hastalarında sağlıklı kontrol grubundan daha yüksekti ($p>0,05$). Homosistein seviyeleri RA grubunda sağlıklı kontrol grubundan daha yüksek olmasına ($p<0,05$) ragmen; folat ve vitamin B12 seviyeleri her iki grupta farksız bulundu ($p>0,05$). Homosistein seviyeleri ve akut faz reaktanları ve inflamatuar mediatörler arasında korelasyon yoktu ($p>0,05$.

Sonuç: Hem IL-6, TNF-α gibi mediatörler, hem de hiperhomosisteinemi aterosklerotik kardiyovasküler hastalıklarla ilişkilidir. RA'da bu üç molekül artmasına rağmen Hcy seviyeleri ve inflamatuar mediatörler arasında ilişki yoktu. Hem inflamatuar mediatörler hem de Hcy RA hastalarında aterosklerotik kardiyovasküler hastalıkları farklı yolaklar aracılığı ile arttırıyor olabilir.

Anahtar Kelimeler: Romatoid artrit, IL-6, homosistein, TNF-α

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic disorder with unknown etiology, which is characterized by involvement of hand joints and deformation [1-3]. Although it is a systemic, inflammatory disorder with ambiguous borders, target tissue is inflamed synovium in RA physiopathology. Tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1) and interleukin-6 (IL-6) play a pivotal role in the onset and maintenance of inflammatory response [4,5]. Primary functions of TNF-α include production of acute phase proteins, proliferation and differentiation of hematopoietic cells, cachexia, bone resorption, shock and tissue injury, and it contribute to inflammation by inducing fever. Serum IL-6 concentration was found to be correlated to radiological joint damage and disease activity [5-7].

Increased atherosclerosis in RA has become increasingly studied topic in recent years. It is known that risk for ischemic heart disease is increased through inflammation as well humoral and cellular immune mechanisms. In patients with RA, cardiovascular mortality and morbidity are increased due to increased atherosclerotic heart disease [8-11]. These cytokines playing a pivotal role in the development of RA also play a key role in atherosclerosis.

High cardiovascular disease-related mortality rate worldwide prompts investigators to evaluate reasons for increased mortality. Homocysteine is one of the popular risk factors in this topic. In epidemiological studies, it was shown that hyper-homocysteinemia is an independent risk factor for coronary heart disease, peripheral vascular disorder and stroke and that an independent predictor for cardiovascular mortality in atherosclerotic patients [12-18].

In this prospective study, it was aimed to investigate relationship between serum Hcy levels and pro-inflammatory cytokines including IL-6 and TNF-α which play an important role in the RA pathogenesis.

METHODS

The study included 55 women including 30 newly diagnosed and untreated female RA patients who were followed in Rheumatology clinic of Teaching and Research Hospital of Pamukkale University, Medicine School, and 25 healthy individuals. In all participants, age, gender, smoking status, alcohol and coffee consumption and menopausal status were questioned. Inclusion criteria were as follows:

*Fulfilling American College of Rheumatology/ European League against Rheumatism (ACR/EULAR) Rheumatoid Arthritis classification criteria [19],

* No hypertension, hyperlipidemia, cardiovascular disease, previous cerebrovascular disease, thromboembolic event,

*Renal and hepatic function tests within normal range,

*Non-smoker or no alcohol consumption,

*Being volunteer to participate and providing informed consent,

*No current therapy for rheumatoid arthritis including steroids,

After 12-hours fasting, blood samples were drawn at sitting position between 08:00 and 10:00 AM. Blood samples for Hcy measurements were drawn into EDTA tubes while blood samples for other tests were drawn into test tubes with no anticoagulant. Plasma Hcy, IL-6 and TNF-α measurements were performed by using Immulite One immunoassay system.

Statistical analysis

Kolmogorov-Smirnov test was used to assess normality of parameters studied in both patient and control group. Numeric variables are presented as arithmetic mean ± standard deviation (±SD). Independent sample t test was used to assess difference in parametric variables between groups and Mann-Whitney U Test was used to assess difference in nonparametric variables between groups. The relationships among homocysteine, IL-6 and TNF-α were assessed by using Spearman correlation analysis.

RESULTS

Mean age was 51.70 ± 13.25 years (25-77 years) in the patient group whereas 48.92 ± 9.81 years (26-63 years) in the control group. Mean Hcy level was 7.80±1.98 μmol/L in the control group whereas 13.40±2.00 μmol/L in the patient group. Serum IL-6 level was 2.49±0.78 pg/mL in the control group whereas 6.89±6.38 pg/mL in the patient group ($p<0.001$). Serum TNF-α level was 6.46 pg/mL in the control group whereas 11.68±11.43 pg/mL in the patient group ($p=0.045$) (Table 1).

A weak but significant negative correlation was detected between Hcy and vitamin B12 levels ($r=-0.402$; $p=0.028$; Table 2). In the patient group, no significant correlation was detected between Hcy and folic acid

levels (r=-0.312; p=0.063). No significant correlation was found between tHcy and CRP, IL-6, TNF-α, or ESR. It was found that there was a significant, moderate, positive correlation between serum IL-6 and CRP levels (r=0.506; p=0.004). It was found that there was a weak but significant positive correlation was detected between IL-6 and ESR (r=0.488; p=0.006). No significant correlation was found between IL-6 and tHcy, TNF-α, vitamin B12 and folic acid. There was no significant correlation between TNF-α and ESR(r=0.360; p=0.05) or CRP level (r=0.354 p=0.055).

Table 1. Results in the patient and control groups

Parameters	Patient Group (n=30)	Control Group (n=25)	p
Age, years[1]	51.7±13.25	48.92±9.81	0.395
Homocysteine, µmol/L[1]	13.40 ± 2	7.8 ± 1.98	0.002
Vitamin B12, pg/mL[2]	301.43±107.49	315.8±94.03	0.626
Folic acid, ng/mL[2]	9.45±2.74	11.27±6.45	0.203
IL-6, pg/mL[2]	6.89±6.38	2.49±0.78	<0.001
TNF-α, pg/mL[2]	11.68±11.43	6.46±2.33	0.045

[1]Independent Samples t Test; [2]Mann-Whitney U Test

Table 2. Cross-correlations among Hcy, vitamin B12, folic acid, interleukin-6, tumor necrosis factor-alpha, C-reactive protein and erythrocyte sedimentation rate

	Hcy, µmol/L	Vitamin B_{12}, pg/mL	Folic acid, ng/mL	IL-6, pg/mL	TNF-α, pg/mL	CRP, mg/dL
Vitamin B_{12}	-0.402*					
Folic acid	-0.312	0.123				
IL-6, pg/mL	0.326	-0.350	-0.264			
TNF-α, pg/mL	-0.057	0.152	-0.150	0.270		
CRP, mg/dL	-0.017	0.028	-0.150	0.507**	0.354	
ESR, mm/h	0.045	-0.324	-0.186	0.488**	0.360	0.563**

IL-1: Interleukin-1, IL-6: Interleukin-6, TNF-α: Tumor necrosis factor-alpha, FA: Folic acid, Hcy: Homocysteine, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate

Values represent correlation coefficients. *: Correlation is significant at level of <0.05. **: Correlation is significant at level of < 0.01

DISCUSSION

Rheumatoid arthritis (RA) is systemic disease characterized by chronic inflammation of hand and foot joints [9]. It is most common form of chronic inflammatory polyarthritis and extra-articular findings and symptoms are almost always present in RA.

Hyperhomocysteinemia, defined as elevation of serum total Hcy, is a commonly seen, strong risk factor for arterial thromboembolism in coronary, cerebral and peripheral vessels and venous thromboembolism [20-24]. Atherogenic and thrombotic effects of Hcy are consequence of direct endothelial injury caused by hydrogen peroxide that generated from O_2 via a reaction chain catalyzed by Hcy itself. [20-24]. In experimental studies, it has been shown that elevated Hcy levels causes detachment of endothelial cells from endothelium; that increases albumin permeability in the endothelial cell layers; that enhances hydrogen peroxide generation; and that decreases cellular NAD4 (nicotinamide adenine dinucleotide dehydrogenase subunit 4) levels [20]. In addition, it was also shown that it inhibits deoxyribonucleic acid (DNA) synthesis by causing breakdown in DNA chain within endothelial cells, resulting in attenuated repair capability of cells.

In RA, there is increased mortality and morbidity as cardiovascular diseases being leading cause of death [10,11,25-29]. Several etiopathogenetic mechanisms are implied in increased cardiovascular mortality in RA. Inflammation observed in RA increases risk for atherosclerosis, presumably, by influencing on cholesterol metabolism [30]. Moreover, drugs used in therapy may also contribute to atherosclerosis. Steroids are primary drugs in the treatment of RA. Hyperlipidemia, hypertension and obesity can develop due to corticosteroids, all which are known risk factors for atherosclerosis.

TNF-α, IL-1 and IL-6 have a pivotal role in the onset and maintenance of inflammatory response in RA [4]. Main functions attributed to TNF-α can be summarized as production of acute phase proteins, proliferation and differentiation of hematopoietic cells, cachexia, bone resorption, shock, tissue injury and contribution to inflammation by inducing fever [4]. Serum IL-6 concentration was found to be corre-

lated to radiological joint damage and disease activity [6]. Atherosclerosis is accelerated in RA due to several factors including increased pro-inflammatory cytokines, circulating immune complexes, dyslipidemia, elevated Hcy level as well as folic acid and vitamin B12 depletion [29]. Previous studies indicate a graded association between hyper-homocysteinemia and cardiovascular diseases, suggesting a plausible role in the pathogenesis of atherosclerosis for Hcy [31,32]. Pyridoxal phosphate deficiency is common in patients with RA, which may be the reason for abnormal Hcy metabolism seen in RA [31,32]. This can be due to either RA itself or treatment employed. Although IL-6, TNF-α and Hcy were found to be increased in RA patients, no significant correlation was detected between Hcy level and inflammatory mediators in our study.

There are many studies on this topic in the literature. In two recent studies, serum Hcy levels were found to be increased in patients with RA [31,32]. In these studies, IL-6 and TNF- α levels weren't studied. However, Yang et al. [31] found correlations between Hcy and CRP, anti-CCP antibody, rheumatoid factor and disease activity (DAS28 score). In both studies, it was found that Hcy plays a significant role in the development of premature atherosclerosis in RA. Thus, authors suggested that Hcy can be used in the assessment of cardiovascular risk in patients with RA. In our study, serum Hcy levels were found to be increased in patients with RA compared to healthy controls. However, we failed to identify an association between Hcy and IL-6 or TNF-α, pro-inflammatory cytokines playing significant role in the pathogenesis of RA.

In patients with RA, circulatory disease-related mortality rate ranges from 1.13 to 5.25 [33]. The role of homocysteine in atherosclerosis is emphasized in epidemiological studies [34]. In patients with RA, concurrent vitamin deficiencies as well as drugs used in treatment may also have role in impaired Hcy metabolism. Methotrexate is the gold standard therapy in RA. It is a folic acid antagonist; thus, influences on some metabolic pathways including homocysteine-methionine cycle. Insufficient folate availability in addition to inhibition of dihydrofolat reductase can negatively affect this cycle. However, hyperhomocysteinemia observed in RA can result from disease itself independent from effects of drugs used in the treatment [35,36]. In a study, it was suggested that RA have direct effect on elevation of Hcy [35]. Tiftikçi et al. found increased homocysteine levels in patients with RA [7]. However, the study doesn't reflect direct effect of RA on Hcy levels since the patients included were receiving methotrexate therapy [7]. Our study included patients with RA who were not receiving treatment. Thus, we think that Hcy levels detected in our study resulted from disease itself.

Our study has some limitations. Sample size was limited since we included only patients with RA who weren't receiving treatment. RA patients receiving treatment could be included as a third group, which might provide information direct effect of disease itself and drugs. However, the aim was to investigate the relationship between Hcy levels and pro-inflammatory cytokines in the present study.

Both inflammatory mediators and Hcy may cause an increase in atherosclerotic cardiovascular disorders through distinct pathways. However, controlling inflammation alone is insufficient since there are several factors involved in atherosclerosis that presumably contributes through diverse pathways. On the other hand, increased Hcy level related to agents used to suppress inflammation such as methotrexate or sulphasalazine also contribute to atherosclerosis [36]. Thus, folic acid replacement should be provided to prevent methotrexate-related hyper-homocysteinemia; additional risk factors should be reviewed; the patient should be closely monitored and lifestyle modifications should be implemented.

Declaration of Conflicting Interests: The authors declare that they have no conflict of interest.

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