Serum Levels of Homocysteine in Young Psoriasis Patients Naïve for Conventional Systemic and Biologic Therapy

Maja Tolušić Levak1,2, Vera Plužarić3, Ivana Krajina1, Marija Šola1,3, Stana Tokić4, Marija Delaš Aždajić5, Hrvoje Brajčić6, Ivanka Muršić1, Mirna Šitum5,7, Martina Mihaljić1,8

1 Department of Dermatology and Venereology, University Hospital Osijek, Osijek, Croatia
2 Department of Histology and Embryology, Faculty of Medicine Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
3 Department of Dermatology and Venereology, Faculty of Medicine Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
4 Department of Medical Chemistry, Biochemistry and Clinical Chemistry, Faculty of Medicine Josip Juraj Strossmayer University of Osijek, Osijek, Croatia
5 Department of Dermatology and Venereology, Sestre milosrdnice University Hospital Center, Zagreb, Croatia
6 Department of Infectiology and Dermatovenereology, General Hospital Dubrovnik, Dubrovnik, Croatia
7 Department of Dermatology and Venereology, Faculty of Dental Medicine University of Zagreb, Zagreb, Croatia
8 Department of Physiology and immunology, Faculty of Medicine Josip Juraj Strossmayer University of Osijek

ABSTRACT:
Introduction: Psoriasis is an immune-mediated chronic inflammatory disease, affecting approximately 1–3% of the population worldwide. Psoriasis patients are more likely to be diagnosed with cardiovascular diseases and hyperhomocysteinemia; however, it remains elusive whether serum homocysteine levels correlate to disease activity and duration of disease. The aim of this study was to investigate serum levels of homocysteine in young patients with plaque psoriasis naïve for conventional systemic and biologic therapy. An additional aim was to determine correlation of homocysteine levels with disease severity, inflammation, folic acid and vitamin B12 supplies.

Materials and methods: 26 subjects were enrolled to participate in this case-control study, including 13 adult psoriatic patients naïve for systemic therapy, without comorbidities, malignancies and infectious diseases, and 13 healthy unrelated, age and sex-matched volunteers. The disease severity and life quality were assessed using standardized tools – Psoriasis Area and Severity Index (PASI), Dermatology Life Quality Index (DLQI), respectively. Venous blood was collected and processed for analysis of differential blood count (DBC), erythrocyte sedimentation rate (ESR), C reactive protein (hsCRP), serum levels of homocysteine, vitamin B12 and folic acid in the routine clinical laboratory.

Results: Studied cohort consisted of young participants with average age around 35 years. According to the PASI index, disease severity ranged from mild (2.10) to moderate (15.2). There was no significant difference in hsCRP and DBC levels between the groups. Psoriasis patients had significantly higher levels of homocysteine compared to healthy subjects, but there was no evidence of hyperhomocysteinemia related to psoriasis. All subjects had normal serum levels of vitamin B12 and folic acid. A moderate negative correlation was found between plasma homocysteine level and vitamin B12 and folic acid. Furthermore, homocysteine levels did not correlate to hsCRP, total leukocytes, and thrombocytes count, but did significantly positively correlate to ESR.

Conclusions: The risk of cardiovascular diseases should be considered among all psoriasis patients, regardless of age and disease severity, but larger prospective controlled studies are needed to estimate the role of homocysteine in cardiovascular morbidity and mortality in psoriatic patients.

KEYWORDS: psoriasis, homocysteine, folic acid, cardiovascular disease
Homocysteine is a sulfur-containing amino acid, displaying elevated homocysteine levels might contribute to the cardiovascular diseases is lately extensively studied, the exact underlying mechanism, as well as other cardiovascular events, is noticeably in 20 and 50 years old increased in patients with severe psoriasis, especially those between 20 and 50 years old. As suggested in some previously published studies, elevated serum homocysteine levels might contribute to the cardiovascular risk in psoriatic patients. Homocysteine is a sulfur-containing amino acid, displaying an atherogenic and prothrombotic features. It is produced in the metabolism of the essential amino acid methionine and can be catalyzed by re-methylation to methionine in the presence of co-factors folic acid and vitamin B12 or by trans-sulfuration to cysteine in the presence of cofactor vitamin B6. Moreover, homocysteine promotes endothelial dysfunction and, consequently, atherosclerosis by reducing the bioavailability of nitric oxide due to inhibition of nitric oxide synthase (eNOS) and oxidative stress, provokes endothelial cell damage, and leads to a state of chronic inflammation of the endothelium. A meta-analysis of 24 studies showed that psoriatic patients have higher homocysteine serum levels, a greater prevalence of hyper-homocysteinemia and, conversely, have lower serum folate levels compared to controls. Yet, the two groups did not significantly differ in vitamin B12 serum levels. Rapid keratinocyte proliferation with high mitotic activity of basal cells, along with intestinal malabsorption caused by microscopic inflammatory changes of the intestinal mucosa, might contribute to folate deficiency in patients with psoriasis. Since folic acid is a cofactor in the catabolism of homocysteine, the
decrease in folate serum levels is therefore one of the causes for hyperhomocysteinemia.\(^\text{23,24}\)

However, due to inconsistent results from several studies, it still remains unclear whether a correlation between homocysteine levels, disease activity and disease duration exists or not.\(^\text{8,9,19-22}\)

Another potential link between homocysteine and psoriasis lies in epigenetic changes of human leukocyte antigen (HLA) DRB1, a major histocompatibility complex (MHC) II protein.\(^\text{23,24}\)

Provided that the expression of HLA-DRB1 on antigen presenting cells (APCs) can be altered by aberrant methylation status of the gene, these methylation changes can affect the APCs capability of antigen presentation to T lymphocytes, the main role of HLA-DRB1.\(^\text{23}\)

Indeed, hypomethylation of the promoter region of HLA-DRB1 was found in the epidermis of psoriatic lesions when compared to psoriatic non-lesioned skin, whereas the mean methylation rate of HLA-DRB1 in lesioned skin negatively correlated to Psoriasis Area and Severity Index (PASI) score, suggesting that aberrant methylation of this region might play a role in the pathogenesis of psoriasis.\(^\text{23}\)

Given the fact that homocysteine, as an intermediate, plays an important role in the methylation cycle, the majority of published data supports the claim that high homocysteine serum levels are associated with global DNA hypomethylation in humans. Since the role of homocysteine in the abnormal methylation pattern seen in psoriatic lesions remains unclear, there is a need for further research to elucidate this.\(^\text{23,25}\)

The aim of this study was to investigate serum levels of homocysteine in patients with vulgar psoriasis naïve for conventional systemic and biologic therapy. An additional aim was to determine correlation of homocysteine levels with disease severity, inflammation, and folic acid and vitamin B12 supplies.

MATERIALS AND METHODS

Subjects

This was a case-control study, including 13 psoriatic patients with clinically diagnosed plaque psoriasis and 13 healthy unrelated, age- and sex-matched control subjects. Subjects were recruited among the patients admitted at the Department of Dermatology and Venerology of the University Hospital Osijek (Croatia). At the time of enrollment, psoriasis patients had a clinical active and histopathological confirmed disease. The disease severity and life quality were assessed using standardized tools – the Psoriasis Area Severity Index (PASI) and Dermatology Life Quality Index (DLQI), respectively.\(^\text{26,27}\)

Exclusion criteria for this study were: (1) ongoing systemic therapy for psoriasis (including immunomodulatory therapy, cytokine, photochemotherapy and phototherapy), (2) concomitant rheumatic and autoimmune diseases (i.e. spondyloarthropathy, rheumatoid arthritis), (3) malignant and infectious diseases, and (4) age (under 18 years). Anthropometric and demographic data, such as body height, body weight, age, gender and parity were assessed using a questionnaire.

The study was reviewed and approved by the Ethical Committee of the University Hospital Osijek (number: R2-9042 / 2018) and the Ethics Committee of the Faculty of Medicine University of Osijek (number: 2158-61-07-18-135). The study was conducted in accordance with Declaration of Helsinki, and all participants signed written informed consent prior inclusion into the study.

Methods

For the purpose of determining differential blood count, C-reactive protein (hs-CRP), erythrocyte sedimentation rate (ESR), serum levels of homocysteine, vitamin B12 and folic acid, venous blood was collected in the morning, after a minimum of 8-hours fasting period. Samples intended for homocysteine level analysis were immediately put on ice. All samples were delivered to the laboratory at the Clinical Department for Laboratory Diagnostics (University Hospital Osijek, Osijek, Croatia) and analyzed within 1 hour. The normal laboratory range for plasma homocysteine was 0–14 µmol/l, and hyperhomocysteinemia was defined as a homocysteine concentration > 14 µmol/l.\(^\text{28}\)

Statistical analysis

All results are reported as the mean ± standard deviation (SD). The Shapiro–Wilk test was used to test the normality of data distribution. Next, data were compared by Student’s t-test or the Mann-Whitney rank-sum test for normally distributed data and data that did not follow a normal distribution pattern, respectively. The correlations between serum levels of homocysteine and other assessed parameters were determined by Pearson’s correlation tests. \(p < 0.05\) was considered statistically significant. SigmaPlot, version 11.2 (Systat Software, Inc., Chicago, IL, USA) was used for statistical analysis.

Results

The present study included 26 subjects, of which 13 participants were psoriasis patients with an active disease, and the remaining 13 participants were age- and sex- matched healthy controls. Their demographic and clinical data are shown in Table 1. The average age of enrolled subjects in both groups was around 35. Disease severity in psoriasis patients was assessed using the PASI index and ranged from 2.10 to 15.2. The rate of smokers among psoriasis patients and healthy controls was 46.2% and 23.1%, respectively. C-reactive protein levels and differential blood count did not vary significantly between the groups (Table 1).

Psoriasis patients had significantly higher serum levels of homocysteine compared to healthy subjects (\(p=0.021\); Table 1 and Figure 1), however, there was no evidence of hyperhomocysteinemia related to psoriasis. All included subjects had serum homocysteine levels within the normal reference range except one psoriasis patient who presented with slightly elevated level of homocysteine (15.5 µmol/L), (Table 1).

All subjects had normal serum levels of vitamin B12 and
folic acid (Table 1). The mean plasma levels of either vitamin B12 (318.2±103.2 pmol/L in psoriasis patients versus 291.0±141.6 pmol/L in healthy subjects, p=0.370), or folic acid (13.7±4.72 nmol/L in psoriasis patients versus 15.6±5.96 nmol/L in healthy subjects, p=0.550) were not statistically significantly different between two groups. A moderate negative correlation was found between plasma homocysteine level and vitamin B12 (r=-0.603, p=0.004) and folic acid (r=-0.638, p=0.002). Furthermore, homocysteine levels did not correlate to hsCRP (p=0.638), total leukocytes (p=0.203) and thrombocytes count (p=0.291), but did significantly positively correlate to sedimentation rate (r=0.410, p=0.0159).

Psoriasis patients included in this study had mild to moderate disease severity. Mean PASI score was 9.16±3.91, ranging from 2.10 to 15.2.

### Table 1: Comparison of serum parameters between psoriasis patients and healthy controls

| Parameter (unit; laboratory referent values) | Psoriasis patients | Healthy controls | t test (p value) |
|---------------------------------------------|--------------------|-----------------|----------------|
| **CRP (mg/l; <5.0)**                        | 1.86±2.03 (0.30, 7.80) | 1.41±1.09 (0.12, 3.20) | 0.488 |
| **Leukocytes (x10e9 /L; 3.40-9.70)**        | 6.77±1.67 (3.7, 9.0) | 6.18±1.83 (4.50, 10.4) | 0.402 |
| **Erythrocytes (x10e12/L; 3.86-5.08)**      | 4.84±0.43 (4.08, 5.34) | 5.00±0.36 (4.47, 5.73) | 0.342 |
| **Thrombocytes (x10e9/L; 158.0-424.0)**     | 271.4±58.8 (214.0, 416.0) | 254.1±58.1 (145.0, 391.0) | 0.458 |
| **Sedimentation rate (mm/3.6 KS; 4.00-24.0)** | 5.45±1.69 (3.00, 8.00) | 5.58±4.83 (1.00, 15.0) | 0.511 |
| **Homocysteine (umol/l)**                   | 9.99±2.30 (7.10, 15.5) | 8.03±1.36 (6.10, 10.5) | 0.021* |
| **Folic acid (nmol/L; 7.0-64.0)**           | 13.7±4.72 (7.0, 19.7) | 15.6±5.96 (8.00, 21.8) | 0.370 |
| **Vitamin B12 (pmol/L; 138.0-652.0)**       | 318.2±103.2 (216.0, 602.0) | 291.0±141.6 (136.0, 479.0) | 0.550 |

Data are presented as mean±SD (min, max). The differences were tested by Student's t-test or Mann-Whitney rank-sum test, where appropriate. **p<0.05 was considered significant.
Psoriasis is considered to be an independent risk factor for cardiovascular morbidity and mortality, but the exact underlying mechanisms have not yet been uncovered. Some recent studies suggested that elevated serum homocysteine levels in psoriatic patients might contribute to the cardiovascular risk in psoriatic patients, due to their atherogenic and prothrombotic effect. In the present study, young psoriasis patients naïve for systemic and biologic therapy presented with significantly higher serum levels of homocysteine compared to the healthy subjects, but still their serum homocysteine levels were within normal reference range. In addition, all participants had normal serum levels of B12 vitamin and folic acid. These results partly correspond to previous studies that are included in the meta-analysis performed by Tsai TY et al. The authors reported significantly higher serum homocysteine level in 18 out of 24 studies, a higher prevalence of hyperhomocysteinaemia in seven studies, a lower serum folate level in 14 studies, and no difference in serum vitamin B12 levels between psoriasis patients and the healthy controls. Although serum levels of folic acid between two groups in our research showed no significant difference, we found a moderate negative correlation between the serum homocysteine level and vitamin B12, as well as between plasma homocysteine level and folic acid level. Similar results were found in previous research concerning negative correlation between serum homocysteine levels and folic acid levels, but none of the previous studies showed significant correlation between the serum levels of homocysteine and B12 vitamin. Negative correlation between homocysteine and folic acid as well as homocysteine and B12 vitamin could be related to the fast epidermal overgrowth and higher mitotic basal cell activity of the psoriasis patients. An additional reason for the higher homocysteine levels among psoriasis patients could be the reduced absorption of folic acid and B12 vitamin in the intestine due to micro damage of the intestinal villi that is caused by long term chronic inflammation in psoriatic patients. Furthermore, normal folate levels observed in our study, which is contrast to previous reports, might be explained by younger age (35 years old) of subjects and shorter exposure to systemic inflammation. Additionally, homocysteine levels did not correlate to hsCRP and total leukocytes count, but did significantly positively correlate to sedimentation rate. Consistent to previous reports, we failed to find direct correlation between current disease severity and serum homocysteine levels. These results suggest that hyperhomocysteinemia and cardiovascular comorbidity rise as a result of prolonged exposure to systemic inflammation.

**Conclusion**
Based on the results of the present study, homocysteine has...
the potential as a biomarker of cardiovascular risk in psoriasis patients, primarily during long term follow-up. Single measurements of serum homocysteine levels in population of young psoriasis patients are likely to fall within the normal reference range, and thus could not be used for prediction of cardiovascular risk. In addition, etiology of cardiovascular diseases is complex, including genetic, environmental and other factors thus large prospective controlled studies are needed to overcome immense heterogeneity among them. Here we would like to emphasize

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