Methotrexate effect on biochemical indices of psoriasis patients depends on MTHFR gene polymorphism

O. M. Fedota1, L. V. Roschenyuk2,3, T. V. Tyzhnenko4, N. G. Puzik1,3, V. M. Vorontsov4, P. P. Ryzko1

1V.N. Karazin Kharkiv National University, Ukraine;
2Kharkiv Regional Clinical Skin and Venereal Diseases Dispensary №1, Ukraine;
3Kharkiv National Medical University, Ukraine;
e-mail: tyzhnenko@ukr.net

Received: 13 June 2019; Accepted: 29 November 2019

Methotrexate (MTX) is the immunosuppressive anti-inflammatory drug and the antagonist of the enzyme dihydrofolate reductase. Pharmacogenomic studies and clinical evidences suggest that altered response to MTX in patients with different diseases is associated with polymorphisms of genes that regulate folate metabolism. The purpose of the article was to analyze the methotrexate effect on the biochemical indices of psoriasis patients depending on methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms. Effects of two single-nucleotide polymorphisms, C677T and A1298C, were studied. An increase of alanine aminotransferase and aspartate aminotransferase activity above the normal level in the patients with both MTHFR gene polymorphisms after methotrexate intake was observed. In patients with CC, TT, CT genotypes for C677T polymorphism and AA genotype for A1298C polymorphism of MTHFR gene, significant differences in alpha-amylase activity before and after treatment with methotrexate were detected. Analysis of the biochemical indices of patients with arthropathic and vulgaris psoriasis showed that the positive effect of MTX treatment could be associated with wild-type alleles in both polymorphisms of MTHFR gene, while the ineffectiveness of methotrexate was associated with the diheterozygous genotype. The largest number of smokers was found within the CCAA genotype group (37.5%), while no smokers were observed within TTAA patients and most of CCAA patients. The data obtained testify the utility of the individual approach to the psoriasis patients therapy taking into account genetic background.

Keywords: psoriasis, folate cycle, MTHFR gene, C677T, A1298C, single-nucleotide polymorphism (SNP), methotrexate, smoking.

Individualization of pharmacotherapy based on the patients’ genetic background allows to increase the effectiveness of treatment and reduce costs [1, 2]. Psoriasis is a heterogeneous chronic painful, disfiguring disease. Joints deformations and disability are the most prominent extracutaneous manifestations of this condition [3]. Currently, psoriasis is considered as a systemic disease affecting multiple organs. Cardiovascular, neurological and mental disorders could be observed in psoriasis patients [4]. The use of drugs of different pharmacological groups could be accompanied by a various response of patients to therapy, including serious complications depending on the symptoms, organ function, gender, age, diet, genotype, and others [5, 6].

Methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes in the folate and homocysteine metabolic pathways. Polymorphisms of MTHFR (OMIM 607093) gene lead to decreased activity of enzyme, lower efficiency of the homocysteine-methionine cycle and hyperhomocysteinemia. MTHFR gene polymorphisms effects are not only detected in patients with cardiovascular, nervous, mental, dermatological, reproductive, oncological, chromosomal and other...
diseases but are also shown to depend on smoking, coffee and alcohol consumption, diet features, physical activity [7, 8]. Therefore, multifactorial pathology could be considered in terms of psoriasis, reflecting the interaction between genetic and environmental factors.

One of the main objectives of psoriasis pharmacotherapy is to find treatment options that quickly and firmly suppress and prevent further progression of the disease. Methotrexate (MTX) is the drug with anti-inflammatory and immunosuppressive action and an antagonist of the enzyme dihydrofolate reductase, that blocks the synthesis of tetrahydrofolate and therefore prevents the synthesis of purines and pyrimidines [9, 10]. Pharmacogenomic studies and clinical evidences suggest that altered response to MTX in patients with different diseases is associated with polymorphisms of folate metabolism genes [11].

Methylenetetrahydrofolate reductase is known as an enzyme that mediates MTX pharmacokinetics [12]. The interconnection between MTHFR gene polymorphisms (OMIM 607093) responses to MTX treatment and adverse reactions to MTX was discovered in patients with rheumatoid arthritis [13]. MTX was effective without any adverse effects in psoriasis patients with positive presumed 677C-1298A SNP haplotypes. At the same time no association between SNPs in FPGS, GGH and MTHFR genes and either MTX efficacy or toxicity in patients with psoriasis in the UK were shown. The assumption about the high risk of adverse effects development after MTX administration in carriers of the 677T allele of the MTHFR gene was not confirmed in patients with psoriasis arthritis [14]. According to the results of researches in Algeria, association between the 677T allele, methotrexate-induced toxicity and adverse effects was detected, while the C allele, in contrast, had a protective effect against MTX toxicity in patients with rheumatoid arthritis. A response to the treatment (good or moderate) was observed more often in A1298 allele carriers than in carriers of 1298C allele associated with a reduced response [15]. Generally, research results evaluating the role of individual SNPs in response to MTX were ambiguous. This could be attributed to different study designs, insufficient statistical power and folate supplementation, MTX dose, duration and route of administration and concurrent therapies [15]. Moreover, it is known that alleles and genotypes frequencies distribution of folate metabolism genes showed ethnic and geographical patterns, so studies of one-carbon metabolism genes are actually carried out in each population. Pharmacogenetic studies on methotrexate therapy in the Ukrainian population enable the development of an effective and targeted therapeutic strategy.

The purpose of the article was to analyze methotrexate effect on the biochemical parameters of psoriasis patients depending on methylenetetrahydrofolate reductase gene (MTHFR) polymorphisms and to evaluate the response to therapy.

Materials and Methods

The study included a retrospective analysis of the medical history of 41 patients with psoriasis. Patients age ranged from 19 to 77 years, mean age was 46.27±1.78 years. All participants had signed the informed consent before the study. Screening included standardized questionnaires for personal data and clinical measurements such as age, sex, medical and family history of psoriasis. The liver function tests were carried out in all patients at the beginning and at the end of methotrexate treatment. Genotyping of patients according to the C677T and A1298C polymorphisms was carried out by PCR-RFLP and allele-specific PCR [16, 17]. The data were accumulated on clinical observations of adverse and favorable reactions in patients with psoriasis who were on methotrexate treatment [18]. The data distribution was tested for normality before further statistical analyses by the Kolmogorov-Smirnov and Shapiro-Wilk tests. Wilcoxon test was used for determination of difference between the means of two groups before and after methotrexate treatment. Kruskal-Wallis test value was calculated in order to establish significant differences between continuous dependent variable by a categorical independent variable. Biochemical parameters were presented as mean ± standard error of mean (M±SEM). All statistical tests were two-tailed and a probability (P) value of 5% or less was considered statistically significant.

Results and Discussion

We evaluated the relation of MTHFR gene polymorphisms with the main biochemical metabolic parameters before/after taking methotrexate.

Stratification of psoriatic patients by genotype according to C677T of MTHFR gene showed the prevalence of heterozygotes CT (25 persons) and homozygotes CC (12 persons) compared to TT homozygotes (4 persons). A similar situation was observed when considered genotypes of A1298C polymorphism in MTHFR gene, where the prevalence
of AA homozygotes and heterozygotes AC was observed in comparison with the CC homozygotes (29, 10 and 0 patients, respectively). The most prevalent genotype of C677T was CT in the groups of patients with vulgaris psoriasis (83.33%) and arthropathic psoriasis (51.72%). The most prevalent genotype of A1298C SNP was AA in the group of patients with arthropathic psoriasis (85.19%), while in vulgaris psoriasis patients group the frequencies of homo- and heterozygotes of A1298C SNP in MTHFR gene were equal.

Among arthropathic psoriasis patients with SNP C677T, those with CT genotype had the lowest alanine aminotransferase (ALT) activity compared to CC and TT genotypes. The level of activity before and after methotrexate treatment by patients with CT genotype was within the normal range, as opposed to persons with CC and TT genotypes. The same trend was noted for aspartate aminotransferase (AST) activity (Table 1). A significant change in the parameters of α-amylase activity and urea level was noted for all three genotypes, and there were differences between the genotypes. The deviations from the normal range were noted for creatinine in TT genotype, total protein in CC genotype, and total cholesterol in CT genotype (Table 1).

Psoriasis vulgaris patients with CC genotype had reduced levels of α-amylase activity (19.74±8.58)

**Table 1. Biochemical characteristics of arthropathic psoriasis patients depending on C667T of MTHFR genotypes before and after methotrexate therapy**

| Parameter (normal range) | Before/After MTX treatment | CC, N = 10 | CT, N = 15 | TT, N = 4 | P (Kruskal-Wallis test) |
|--------------------------|---------------------------|-----------|-----------|----------|------------------------|
| ALT, (0.10–0.68 mmol/lh) | Before                    | 0.62 ± 0.08 | 0.57 ± 0.05 | 0.98 ± 0.25* | 0.032805 |
|                          | After                     | 0.77 ± 0.08 | 0.69 ± 0.09 | 1.42 ± 0.06* | 0.000654 |
|                          | P (Wilcoxon test)         | 0.202623   | **0.026757** | 0.144128  | –                      |
| AST, (0.10–0.45 mmol/lh) | Before                    | 0.40 ± 0.05 | 0.35±0.03 | 0.53 ± 0.09 | 0.134523 |
|                          | After                     | 0.45 ± 0.07 | 0.41 ± 0.06 | 0.72 ± 0.04 | 0.035220 |
|                          | P (Wilcoxon test)         | 0.386271   | 0.255989  | 0.144128  | –                      |
| Bilirubin, (2–17 μmol/l) | Before                    | 16.86 ± 0.84 | 16.56 ± 0.47 | 16.97 ± 0.81 | 0.912228 |
|                          | After                     | 17.23 ± 0.85 | 17.15 ± 0.36 | 18.36 ± 0.38 | 0.523244 |
|                          | P (Wilcoxon test)         | 0.444587   | 0.232980  | 0.144128  | –                      |
| α-Amylase, (25–125 IU/l) | Before                    | 29.59 ± 3.08 | 29.81 ± 3.53 | 27.81 ± 1.13 | 0.953281 |
|                          | After                     | 21.20 ± 2.98 | 23.07 ± 1.88 | 30.70 ± 0.25 | 0.129310 |
|                          | P (Wilcoxon test)         | **0.036659** | **0.010594** | **0.067890** | – |
| Urea, (1.70–8.30 mmol/l) | Before                    | 4.85 ± 0.31 | 4.59 ± 0.25 | 5.60 ± 0.36 | 0.182647 |
|                          | After                     | 5.65 ± 0.32 | 5.37 ± 0.25 | 6.43 ± 0.13 | 0.148415 |
|                          | P (Wilcoxon test)         | **0.021825** | **0.008985** | **0.067890** | – |
| Creatinine, (44–115 μmol/l) | Before                | 48.45 ± 10.67 | 50.07 ± 8.12 | 33.26 ± 19.16 | 0.662706 |
|                          | After                     | 62.12 ± 7.22 | 63.22 ± 4.72 | 68.95 ± 0.89 | 0.826595 |
|                          | P (Wilcoxon test)         | 0.284504   | 0.111770  | **0.067890** | – |
| Total protein, (65.0–85.0 g/l) | Before             | 80.24 ± 2.13 | 76.89 ± 0.96 | 78.63 ± 1.34 | 0.259128 |
|                          | After                     | 76.58 ± 1.37 | 77.63 ± 0.85 | 79.43 ± 1.52 | 0.428144 |
|                          | P (Wilcoxon test)         | **0.036659** | **0.649563** | 1.000000  | – |
| Total cholesterol, (3–6.26 mmol/l) | Before | 5.20 ± 0.26 | 5.16 ± 0.09 | 6.14 ± 0.41* | 0.027209 |
|                          | After                     | 5.19 ± 0.29 | 5.75 ± 0.28 | 6.61 ± 0.07 | 0.055638 |
|                          | P (Wilcoxon test)         | 0.798860   | **0.060894** | 0.273323* | – |

Note: *Significant difference revealed with Kruskal-Wallis test in the biochemical parameters in TT group compared to CC and CT groups with C667T polymorphism of MTHFR gene.
Certain pharmacotherapeutics were reported to in- clearance of circulating amylase in the blood [21]. Kidney function is a crucial modifier that affects the most cases was within the normal range or lower. psoriasis patients after methotrexate treatment in homocysteine level and decreasing folic acid level in homocysteine remyelation, thereby increasing the serious complication of methotrexate treatment, the study of patients with various forms of psoriasis showed that the decrease of therapeutic effect occurred in persons with CTAC genotype of C667T and A1298C polymorphisms of MTHFR gene. The genotypes distribution of the smokers was represented as CTAA (37.5%), CTAC (25.0%) > CTAC (0.63±0.06/0.83±0.10) > CCAC (0.53±0.02/0.65±0.03). Patients with genotypes TTAA and CCAA showed the highest rates and dynamics of ALT – 0.98±0.25/1.42±0.06 and 0.65±0.08/0.85±0.10, which was probably one of the smoking prevention factors in these persons. The differences in the gene pool indicate both specific risks and a unique system of genetic markers for each ethnic group, which could be considered while forming risk groups and choosing a treatment strategy. Our study demonstrated that patients with wild-type alleles of MTHFR gene responded well to methotrexate treatment. The study of MTHFR gene polymorphisms association with the biochemical parameters of response to methotrexate in patients with various forms of psoriasis showed that the decrease of therapeutic effect occurred in persons with CTAC genotype of C667T and A1298C polymorphisms of MTHFR gene. The genotypes distribution of the smokers was represented as CTAA (37.5%), CTAC (25.0%), CCAC (12.5%), CCAA (6.25%) > TTAA (0%), the largest number of smokers were found within CCAA genotype group. TTAA patients and most of CCAA patients did not smoke. The distribution of genotypes and ALT parameters in smokers before/after taking methotrexate was: CTAA (0.63±0.06/0.83±0.10) > CTAC (0.57±0.05/0.60±0.02) > CCAC (0.53±0.02/0.65±0.03). Patients with genotypes TTAA and CCAA showed the highest rates and dynamics of ALT – 0.98±0.25/1.42±0.06 and 0.65±0.08/0.85±0.10, which was probably one of the smoking prevention factors in these persons. The differences in the gene pool indicate both specific risks and a unique system of genetic markers for each ethnic group, which could be considered while forming risk groups and choosing a treatment strategy.

Our analysis showed that among the examined group of patients, 81.3% of persons were smokers or had a long history of smoking in the past. The smoker experience ranged from four to several decades. The distribution of genotypes could be represented as CTAA (37.5%) > CTAC (25.0%) > CCAC (12.5%) > CCAA (6.25%) > TTAA (0%), the largest number of smokers were found within CTAA genotype group. TTAA patients and most of CCAA patients did not smoke. The distribution of genotypes and ALT parameters in smokers before/after taking methotrexate was: CTAA (0.63±0.06/0.83±0.10) > CTAC (0.57±0.05/0.60±0.02) > CCAC (0.53±0.02/0.65±0.03). Patients with genotypes TTAA and CCAA showed the highest rates and dynamics of ALT – 0.98±0.25/1.42±0.06 and 0.65±0.08/0.85±0.10, which was probably one of the smoking prevention factors in these persons. The differences in the gene pool indicate both specific risks and a unique system of genetic markers for each ethnic group, which could be considered while forming risk groups and choosing a treatment strategy.

Our study demonstrated that patients with wild-type alleles of MTHFR gene responded well to methotrexate treatment. The study of MTHFR gene polymorphisms association with the biochemical parameters of response to methotrexate in patients with various forms of psoriasis showed that the decrease of therapeutic effect occurred in persons with CTAC genotype of C667T and A1298C polymorphisms of MTHFR gene. The genotypes distribution of the smokers was represented as CTAA (37.5%), CTAC (25.0%), CCAC (12.5%), CCAA (6.25%), TTAA (0%), the largest number of smokers were found within CCAA genotype group, while TTAA patients and most of CCAA patients did not smoke. The data obtained in our research will allow the development of pharmacotherapy in dermatology, which consists in predicting the effectiveness of therapy for psoriasis patients with methotrexate, taking into account genetic background.

and creatinine level (33.39±33.31), whereas patients with CT genotype had increased bilirubin level (17.40±0.64). After taking methotrexate, patients with both genotypes showed an increase in the ALT and AST activity above the normal limit: ALT – 0.93±0.25 (CC), 0.81±0.12, AST – 0.49±0.20 (CC), 0.51±0.09 (CT).

Positive effect of C677T and A1298C in MTHFR genotypes on the biochemical parameters of arthropathic psoriasis patients followed the pattern CTAC > CTAA > CCAC > CCAA > TTAA (Table 3). A similar trend was observed for the biochemical parameters of response to methotrexate in patients with various forms of psoriasis showed that the distribution of genotypes and ALT parameters in smokers before/after taking methotrexate was: CTAA (0.63±0.06/0.83±0.10) > CTAC (0.57±0.05/0.60±0.02) > CCAC (0.53±0.02/0.65±0.03). Patients with genotypes TTAA and CCAA showed the highest rates and dynamics of ALT – 0.98±0.25/1.42±0.06 and 0.65±0.08/0.85±0.10, which was probably one of the smoking prevention factors in these persons. The differences in the gene pool indicate both specific risks and a unique system of genetic markers for each ethnic group, which could be considered while forming risk groups and choosing a treatment strategy.

Our analysis showed that among the examined group of patients, 81.3% of persons were smokers or had a long history of smoking in the past. The smoker experience ranged from four to several decades. The distribution of genotypes could be represented as CTAA (37.5%) > CTAC (25.0%) > CCAC (12.5%) > CCAA (6.25%) > TTAA (0%), the largest number of smokers were found within CTAA genotype group. TTAA patients and most of CCAA patients did not smoke. The distribution of genotypes and ALT parameters in smokers before/after taking methotrexate was: CTAA (0.63±0.06/0.83±0.10) > CTAC (0.57±0.05/0.60±0.02) > CCAC (0.53±0.02/0.65±0.03). Patients with genotypes TTAA and CCAA showed the highest rates and dynamics of ALT – 0.98±0.25/1.42±0.06 and 0.65±0.08/0.85±0.10, which was probably one of the smoking prevention factors in these persons. The differences in the gene pool indicate both specific risks and a unique system of genetic markers for each ethnic group, which could be considered while forming risk groups and choosing a treatment strategy.

Our study demonstrated that patients with wild-type alleles of MTHFR gene responded well to methotrexate treatment. The study of MTHFR gene polymorphisms association with the biochemical parameters of response to methotrexate in patients with various forms of psoriasis showed that the decrease of therapeutic effect occurred in persons with CTAC genotype of C667T and A1298C polymorphisms of MTHFR gene. The genotypes distribution of the smokers was represented as CTAA (37.5%), CTAC (25.0%), CCAC (12.5%), CCAA (6.25%), TTAA (0%), the largest number of smokers were found within CCAA genotype group, while TTAA patients and most of CCAA patients did not smoke. The data obtained in our research will allow the development of pharmacotherapy in dermatology, which consists in predicting the effectiveness of therapy for psoriasis patients with methotrexate, taking into account genetic background.
| Parameter (normal range) | Before/After MTX treatment | Arthropatic psoriasis |   | Vulgaris psoriasis |   |
|-------------------------|---------------------------|----------------------|---|-------------------|---|
|                         |                           | AA, N = 23           | AC, N = 4 | \(P\) (Kruskal-Wallis test) | AA, N = 6 (5) | AC, N = 6 | \(P\) (Kruskal-Wallis test) |
| ALT, (0.10–0.68 mmol/lh) | Before                    | 0.70 ± 0.06          | 0.49 ± 0.02 | 0.196633          | 0.64 ± 0.12 (6) | 0.60 ± 0.07 | 0.708877 |
|                         | After                     | 0.89 ± 0.08          | 0.58 ± 0.02 | 0.136329          | 1.07 ± 0.17 (5) | 0.63 ± 0.03 | \textbf{0.019021*} |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.023457}    | \textbf{0.067890} | –                | \textbf{0.043115} | 0.753153 | –                |
| AST, (0.10–0.45 mmol/lh) | Before                    | 0.42 ± 0.03          | 0.31 ± 0.05 | 0.185613          | 0.47 ± 0.12 (6) | 0.36 ± 0.04 | 0.425681 |
|                         | After                     | 0.51 ± 0.05          | 0.37 ± 0.05 | 0.240694          | 0.68 ± 0.13 (5) | 0.37 ± 0.03 | 0.026205 |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.094363}    | 0.715001 | –                | 0.138012 | 1.000000 | –                |
| Bilirubin, (2–17 μmol/l) | Before                    | 16.85 ± 0.47         | 16.61 ± 0.44 | 0.840299          | 15.84 ± 0.59 (6) | 16.37 ± 0.71 | 0.715244 |
|                         | After                     | 17.62 ± 0.42         | 16.02 ± 0.26 | 0.130607          | 17.32 ± 0.82 (5) | 17.23 ± 0.77 | 0.937676 |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.048046}    | 0.144128 | –                | \textbf{0.043115} | 0.043115 | –                |
| \(\alpha\)-Amylase, (25–125 IU/l) | Before                    | 30.61 ± 2.53         | 26.52 ± 2.59 | 0.518451          | 17.88 ± 3.66 (6) | 30.55 ± 4.45 | 0.063222* |
|                         | After                     | 23.93 ± 1.69         | 26.78 ± 1.75 | 0.500812          | 16.21 ± 1.84 (5) | 27.38 ± 2.03 | 0.003103* |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.011589}    | 1.000000 | –                | 0.892738 | 0.345448 | –                |
| Urea, (1.7–8.3 mmol/l)  | Before                    | 4.77 ± 0.22          | 5.28 ± 0.18 | 0.358733          | 5.39 ± 0.45 (6) | 5.58 ± 0.34 | 0.569564 |
|                         | After                     | 5.77 ± 0.20          | 5.08 ± 0.24 | 0.179695          | 6.13 ± 0.79 (5) | 5.69 ± 0.40 | 0.613588 |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.000099}    | 1.000000 | –                | 0.224917 | 0.463072 | –                |
| Creatinine, (44–115 µmol/l) | Before                    | 44.70 ± 7.00         | 51.35 ± 17.16 | 0.718925          | 27.29 ± 17.11 (5) | 68.82 ± 1.20 | \textbf{0.025247*} |
|                         | After                     | 62.45 ± 4.29         | 67.35 ± 2.00 | 0.643963          | 65.68 ± 18.19 (5) | 67.63 ± 1.66 | 0.908392 |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.030815}    | 0.273323 | –                | 0.043115 | 0.600180 | –                |
| Total protein, (65–85 g/l) | Before                    | 79.39 ± 0.97         | 74.58 ± 2.56 | \textbf{0.069956} | 78.68 ± 2.35 (6) | 78.08 ± 1.97 | 0.877892 |
|                         | After                     | 77.76 ± 0.79         | 77.65 ± 1.39 | 0.957849          | 76.38 ± 4.56 (5) | 78.48 ± 1.72 | 0.653648 |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.068017}    | 0.465209 | –                | 0.500185 | 0.916512 | –                |
| Total cholesterol (3–6.26 mmol/l) | Before                    | 5.34 ± 0.15          | 5.47 ± 0.17 | 0.724562          | 5.57 ± 0.26 (6) | 5.54 ± 0.31 | 0.951776 |
|                         | After                     | 5.84 ± 0.22          | 5.39 ± 0.32 | 0.421453          | 5.79 ± 0.32 (5) | 5.59 ± 0.21 | 0.604549 |
|                         | \(P\) (Wilcoxon test)    | \textbf{0.025385}    | 0.715001 | –                | 0.500185 | 0.753153 | –                |

Note: *significant difference revealed with Kruskal-Wallis test in the biochemical parameters in AA group compared to AC group with A1298C polymorphism of \textit{MTHFR} gene.
### Table 3. Biochemical characteristics of arthropathic psoriasis patients, depending on C667T and A1298C of MTHFR genotypes before and after methotrexate therapy

| Parameter (normal range) | Before / After MTX treatment | CCAA, N = 8 | CC-AC, N = 1 | CTA A, N = 11 | CTAC, N = 3 | TTAA, N = 4 | P (Kruskal-Wallis test) |
|--------------------------|-----------------------------|-------------|--------------|--------------|-------------|-------------|---------------------|
| ALT, (0.10–0.68 mmol/lh) | Before                      | 0.67 ± 0.09 | 0.51         | 0.61 ± 0.06  | 0.48 ± 0.02  | 0.98 ± 0.25  | **0.076170***       |
|                          | After                       | 0.81 ± 0.10 | 0.62         | 0.75 ± 0.11  | 0.57 ± 0.02  | 1.42 ± 0.06  | **0.002458***       |
|                          | P (Wilcoxon test)           | 0.326990    | –            | **0.091162** | 0.108810     | 0.144128     | –                   |
| AST, (0.10–0.45 mmol/lh) | Before                      | 0.42 ± 0.06 | 0.39         | 0.39 ± 0.04  | 0.28 ± 0.07  | 0.53 ± 0.09  | 0.218441           |
|                          | After                       | 0.49 ± 0.08 | 0.45         | 0.44 ± 0.07  | 0.34 ± 0.06  | 0.72 ± 0.04  | **0.083798***       |
|                          | P (Wilcoxon test)           | 0.441209    | –            | 0.533695     | 1.000000     | 0.144128     | –                   |
| Bilirubin, (2–17 μmol/l) | Before                      | 16.86 ± 1.06| 17.21        | 16.80 ± 0.60 | 16.41 ± 0.56 | 16.97 ± 0.81 | 0.993676           |
|                          | After                       | 17.36 ± 1.06| 16.01        | 17.54 ± 0.43 | 16.02 ± 0.37 | 18.36 ± 0.38 | 0.401926           |
|                          | P (Wilcoxon test)           | 0.400815    | –            | 0.247747     | 0.285050     | **0.067890**    | –                   |
| α-Amylase, (25–125 IU/l) | Before                      | 31.68 ± 3.47| 19.41        | 30.85 ± 4.78 | 28.89 ± 1.47 | 27.81 ± 1.13 | 0.876998           |
|                          | After                       | 22.15 ± 3.58| 22.19        | 22.76 ± 2.13 | 28.31 ± 1.21 | 30.70 ± 0.25 | 0.217387           |
|                          | P (Wilcoxon test)           | **0.035693**| –            | **0.016369** | 0.592980     | **0.067890**    | –                   |
| Urea, (1.7–8.3 mmol/l)   | Before                      | 4.77 ± 0.38 | 5.50         | 4.47 ± 0.32  | 5.20 ± 0.24  | 5.60 ± 0.36  | 0.193251           |
|                          | After                       | 5.76 ± 0.36 | 4.43         | 5.53 ± 0.30  | 5.30 ± 0.14  | 6.43 ± 0.13  | 0.234411           |
|                          | P (Wilcoxon test)           | **0.017291**| –            | **0.007646** | 0.285050     | **0.067890**    | –                   |
| Creatinine, (44–115 μmol/l) | Before                  | 51.80 ± 11.46| 0.09        | 43.71 ± 10.50| 68.43 ± 2.28 | 33.26 ± 19.16 | 0.876707           |
|                          | After                      | 60.96 ± 9.06| 61.90        | 61.16 ± 6.40 | 69.17 ± 1.19 | 68.95 ± 0.89 | 0.862838           |
|                          | P (Wilcoxon test)           | 0.674424    | –            | 0.130666     | 0.592980     | **0.067890**    | –                   |
| Total protein, (65.0–85.0 g/l) | Before                | 80.99 ± 2.48| 81.80        | 78.50 ± 0.82 | 72.17 ± 1.22 | 78.63 ± 1.34 | 0.100133           |
|                          | After                      | 77.48 ± 1.55| 74.10        | 77.35 ± 1.13 | 78.83 ± 1.03 | 79.43 ± 1.52 | 0.782961           |
|                          | P (Wilcoxon test)           | **0.068704**| –            | 0.247747     | 0.108810     | 1.000000     | –                   |
| Total cholesterol, (3–6.26 mmol/l) | Before                | 5.19 ± 0.32 | 5.74         | 5.16 ± 0.09  | 5.38 ± 0.20  | 6.14 ± 0.41  | **0.069186***      |
|                          | After                      | 5.33 ± 0.34 | 4.78         | 5.92 ± 0.35  | 5.59 ± 0.35  | 6.61 ± 0.07  | 0.126416           |
|                          | P (Wilcoxon test)           | 0.674424    | –            | **0.032855** | 1.000000     | 0.273323     | –                   |

Note: *significant differences revealed with Kruskal-Wallis test in the biochemical parameters in TTAA group compared to CCAA, CTA, and CTAC groups with C667T and A1298C polymorphisms of MTHFR gene.
Table 4. Biochemical characteristics of psoriasis vulgaris patients depending on C667T and A1298C of MTHFR genotypes before and after methotrexate therapy

| Parameter (normal range) | Before / After MTX treatment | CCAA, N = 1 | CCAC, N = 1 | CTAA, N = 5 (4) | CTAC, N = 5 | P (Kruskal-Wallis test) |
|--------------------------|------------------------------|-------------|-------------|-----------------|-------------|------------------------|
| ALT, (0.10–0.68 mmol/lh) | Before                       | 0.52        | 0.54        | 0.66 ± 0.14 (5) | 0.61 ± 0.08 | 0.910531               |
|                          | After                        | 1.18        | 0.68        | 1.05 ± 0.21 (4) | 0.62 ± 0.03 | 0.170531               |
|                          | P (Wilcoxon test)            | –           | –           | 0.067890        | 0.892738    | –                      |
| AST, (0.10–0.45 mmol/lh) | Before                       | 0.33        | 0.40        | 0.49 ± 0.14 (5) | 0.36 ± 0.05 | 0.793056               |
|                          | After                        | 0.69        | 0.29        | 0.68 ± 0.16 (4) | 0.38 ± 0.03 | 0.215047               |
|                          | P (Wilcoxon test)            | –           | –           | 0.273323        | 0.589639    | –                      |
| Bilirubin, (2–17 μmol/l) | Before                       | 15.54       | 15.20       | 15.90 ± 0.72 (5) | 16.60 ± 0.82 | 0.870154               |
|                          | After                        | 16.01       | 17.40       | 17.65 ± 0.96 (4) | 17.20 ± 0.94 | 0.907968               |
|                          | P (Wilcoxon test)            | –           | –           | 0.067890        | 0.067890    | –                      |
| α-Amylase, (25–125 IU/l) | Before                       | 26.57       | 45.49       | 16.14 ± 3.94 (5) | 27.56 ± 4.03 | 0.084393*              |
|                          | After                        | 11.16       | 28.31       | 17.48 ± 1.73 (4) | 27.19 ± 2.47 | 0.029363*              |
|                          | P (Wilcoxon test)            | –           | –           | 0.465209        | 0.685831    | –                      |
| Urea, (1.7–8.3 mmol/l)   | Before                       | 4.43        | 6.42        | 5.58 ± 0.50 (5) | 5.41 ± 0.36 | 0.603039               |
|                          | After                        | 6.81        | 5.61        | 5.96 ± 0.99 (4) | 5.70 ± 0.49 | 0.922523               |
|                          | P (Wilcoxon test)            | –           | –           | 0.465209        | 0.138012    | –                      |
| Creatinine, (44–115 μmol/l) | Before                  | 0.09        | 66.70       | 34.09 ± 20.27 (4) | 69.24 ± 1.38 | 0.130466               |
|                          | After                        | 109.30      | 60.40       | 54.77 ± 18.79 (4) | 69.08 ± 1.00 | 0.335440               |
|                          | P (Wilcoxon test)            | –           | –           | 0.067890        | 0.892738    | –                      |
| Total protein, (65–85 g/l) | Before                 | 80.70       | 74.80       | 78.28 ± 2.84 (5) | 78.74 ± 2.28 | 0.914165               |
|                          | After                        | 81.30       | 70.80       | 75.15 ± 5.66 (4) | 80.02 ± 0.96 | 0.605001               |
|                          | P (Wilcoxon test)            | –           | –           | 0.465209        | 0.685831    | –                      |
| Total cholesterol, (3–6.26 mmol/l) | Before        | 5.39        | 4.11        | 5.60 ± 0.32 (5) | 5.83 ± 0.15 | 0.159201               |
|                          | After                        | 5.61        | 5.12        | 5.83 ± 0.41 (4) | 5.68 ± 0.24 | 0.818710               |
|                          | P (Wilcoxon test)            | –           | –           | 0.715001        | 0.224917    | –                      |

Note: *significant difference revealed with Kruskal-Wallis in the biochemical parameters in CTAC group compared to CTAA group with n C667T and A1298C polymorphisms of MTHFR gene.
ВПЛИВ МЕТОТРЕКСАТУ НА БІОХІМІЧНІ ПОКАЗНИКИ ХВОРИХ НА ПСОРИАЗ ЗАЛІЖНО ВІД ГЕНОТИПІВ ГЕНА MTHFR

O. M. Fedota¹, L. V. Roschenyuk², V. M. Voroncov³, P. P. Ryzko³

¹Харківський національний університет імені В. Н. Каразина, Україна; ²Харківський обласний клінічний шкірно-венерологічний диспансер №1, Україна; ³Харківський національний медичний університет, Україна; e-mail: tyzhnенко@ukr.net

Метотрексат (МTX) є протизапальним та імуносупресивним препаратом, а також антагоністом ензиму дигідрофолатредуктази. Клінічні дані свідчать, що відмінності у відповіді пацієнтів із різними хворобами на МTX пов’язані з поліморфними варіантами генів, що регулюють метаболізм фолату. Метою роботи був аналіз впливу метотрексату на біохімічні показники хворих на псоріаз від поліморфними варіантами гена MTHFR. Досліджено ефекти двох поліморфних варіантів генів: МТХФР: C677T і A1298C. Після прийому метотрексату в пацієнтів із різними генотипами за обома поліморфними варіантами гена MTHFR: C677Т і А1298С, було виявлено значні відмінності в рівнях α-амілази до та після лікування метотрексатом. Аналіз біохімічних показників хворих на артропатичний та вульгарний псоріаз показав, що позитивний вплив лікування метотрексату пов’язаний з наличністю алей дикого типу за обома поліморфними варіантами, а неефективність метотрексату за дослідженими поліморфізмами пов’язана з дигетерозиготним генотипом. Найбільшу кількість курців виявлено в групі з СТАА генотипом (37,5%), тоді як серед пацієнтів з ТTAА та більшості хворих з ССАА генотипами — курці не виявлено. Одержані дані можуть бути використані для персоналізованої терапії хворих на псоріаз із урахуванням генетичних особливостей пацієнтів.

Ключові слова: псоріаз, фолатний цикл, гена MTHFR, С677Т, А1298С, однонуклеотидний поліморфізм (ОНП), метотрексат.

References
1. Zhylkova IS, Sotnik NN, Yegunkova OV, Feskov OM, Fedota OM. Analysis of single nucleotide polymorphisms G919A and A2039G of gene FSHR in infertile men. Cytol Genet. 2018; 52(2): 132-138.
2. Abulezz R, Alhamdan H, Khan MA. Use of a strategic plan for the clinical pharmacy section in a tertiary care center. J Basic Clin Pharma. 2018; 9(3): 289-293.
3. Global report on psoriasis [Electronic resource]. Publications of the World Health Organization / WHO Library Cataloguing-in-Publication Data, 2016. 48 p. Regime of access : http://www.who.int.
4. Raaby L, Ahlehoff O, de Thurah A. Psoriasis and cardiovascular events: updating the evidence. Arch Dermatol Res. 2017; 309(3): 225-228.
5. Fedota O, Roschenyuk L, Tyzhnenko T, Merenkova I, Vorontsov V. Pharmacogenetic effects of methotrexate (MTX) in Ukrainian patients depending on the MTHFR genotypes (clinical cases). Georgian Med News. 2018; (279): 111-117.
6. Gossec L, Smolen JS, Ramiro S, de Wit M, Cutolo M, Dougados M, Emery P, Landewé R, Oliver S, Aletaha D, Betteridge N, Braun J, Burmester G, Cañete JD, Damjanov N, FitzGerald O, Haglund E, Helliwell P, Kvien TK, Lories R, Luger T, Maccarone M, Marzo-Ortega H, Mcgonagle D, McInnes IB, Olivieri I, Pavelka K, Schett G, Sieper J, van den Bosch F, Veale DJ, Wollenhaupt J, Zink A, van der Heijde D. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. Ann Rheum Dis. 2016;75(3): 499-510.
7. Fedota O, Roshchenyuk L, Sadovnychenko I, Merenkova I, Gontar I, Vorontsov V. Analysis of one-carbon metabolism genes and epidermal differentiation complex in patients with ichthyosis vulgaris. Georgian Med News. 2017; (264): 90-97. (In Russian).
8. Xia LZ, Liu Y, Xu XZ, Jiang PC, Ma G, Bu XF, Zhang YJ, Yu F, Xu KS, Li H. Methylenetetrahydrofolate reductase C677T
and A1298C polymorphisms and gastric cancer susceptibility. *World J Gastroenterol.* 2014; 20(32): 11429-11438.

9. Gonen N, Assaraf YG. Antifolates in cancer therapy: structure, activity and mechanisms of drug resistance. *Drug Resist Updat.* 2012;15(4): 183-210.

10. Lopez-Olivo MA, Siddhanamatha HR, Shea B, Tugwell P, Wells GA, Suarez-Almazor ME. Methotrexate for treating rheumatoid arthritis. *Cochrane Database Syst Rev.* 2014; (6): CD000957.

11. Castaldo P, Magi S, Nasti AA, Arcangeli S, Lariccia V, Alesi N, Tocchini M, Amoroso S. Clinical pharmacogenetics of methotrexate. *Curr Drug Metab.* 2011; 12(3): 278-286.

12. Umerez M, Gutierrez-Camino Á, Muñoz-Maldonado C, Martin-Guerreo I, Garcia-Orad A. MTHFR polymorphisms in childhood acute lymphoblastic leukemia: influence on methotrexate therapy. *Pharmgenomics Pers Med.* 2017; 10: 69-78.

13. Hirotaka D, Akemi T, Masahiko M. Analysis of single nucleotide polymorphisms of methylenetetrahydrofolate reductase in Japanese psoriasis patients. *Bull Yamaguchi Med School.* 2010; 57(3-4): 41-48.

14. Warren RB, Smith RL, Campalani E, Eyre S, Smith CH, Barker JN, Worthington J, Griffiths CE. Outcomes of methotrexate therapy for psoriasis and relationship to genetic polymorphisms. *Br J Dermatol.* 2009; 160(2): 438-441.

15. Berkani LM, Rahal F, Allam I, Mouaki Benani S, Laadjouz A, Djidjik R. Association of MTHFR C677T and A1298C gene polymorphisms with methotrexate efficiency and toxicity in Algerian rheumatoid arthritis patients. *Heliyon.* 2017; 3(11): e00467.

16. Fedota AM, Ryzhko PP, Roshenyuk LV, Vorontsov VM, Solodyankin AS, Solodyankina YeS. C677T polymorphism of MTHFR gene in psoriasis patients. *Bull Karazin Kharkiv Nat Univ. Ser Biol.* 2010; 12(920): 37-41.

17. Bagheri M, Rad IA, Omrani MD, Nanbakshh F. C677T and A1298C Mutations in the Methylene tetrahydrofolate Reductase Gene in Patients with Recurrent Abortion from the Iranian Azeri Turkish. *Int J Fertil Steril.* 2010; 4(3): 134-139.

18. Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) medical aid. Psoriasis, including psoriatic arthropathies. Kyiv: Publishing House "KIM", 2016. 68 p.

19. Lv S, Fan H, Li J, Yang H, Huang J, Shu X, Zhang L, Xu Y, Li X, Zuo J, Xiao C. Genetic Polymorphisms of TYMS, MTHFR, ATIC, MTR, and MTRR Are Related to the Outcome of Methotrexate Therapy for Rheumatoid Arthritis in a Chinese Population. *Front Pharmacol.* 2018; 9: 1390.

20. Saviola G, Abdi-Ali L, Sacco S, Comini L, Plewnia K, Rossi M, Orrico A. Complete clinical and functional recovery following low-dose methotrexate related paraparesis in a patient with compound c.1298A>C AND c.677C>T MTHFR polymorphism: A case report. *Medicine (Baltimore).* 2018; 97(49): e13350.

21. Nakajima K, Nemoto T, Muneyuki T, Kakei M, Fuchigami H, Munakata H. Low serum amylase in association with metabolic syndrome and diabetes: A community-based study. *Cardiovasc Diabetol.* 2011; 10: 34.

22. Tokuyama H, Kawamura H, Fujimoto M, Kobayashi K, Nieda M, Okazawa T, Takemoto M, Shimada F. A low-grade increase of serum pancreatic exocrine enzyme levels by dipeptidyl peptidase-4 inhibitor in patients with type 2 diabetes. *Diabetes Res Clin Pract.* 2013; 100(3): e66-9.

23. Zappacosta B, Graziano M, Persichilli S, Di Castelnuovo A, Mastroiacovo P, Iacoviello L. 5,10-Methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C polymorphisms: genotype frequency and association with homocysteine and folate levels in middle-southern Italian adults. *Cell Biochem Funct.* 2014; 32(1): 1-4.

24. Welzel TM, Katki HA, Sakoda LC, Evans AA, London WT, Chen G, O'Broin S, Shen FM, Lin WY, McGlynn KA. Blood folate levels and risk of liver damage and hepatocellular carcinoma in a prospective high-risk cohort. *Cancer Epidemiol Biomarkers Prev.* 2007; 16(6): 1279-1282.

25. Haj Mouhamed D, Ezzaher A, Neffati F, Douki W, Najjar MF. Effect of cigarette smoking on plasma homocysteine concentrations. *Clin Chem Lab Med.* 2011; 49(3): 479-483.