Original Article

# RELATIONSHIP BETWEEN MTHFR GENE rs1801133 AND rs1801131 POLYMORPHISMS WITH DISEASE SEVERITY OF COVID-19 AND HOMOCYSTEIN LEVELS IN UZBEK PATIENTS

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## Abstract

As a result of endothelial cell alteration and cytokine storm induced by SARS-CoV-2, the causative agent of COVID-19, hypercoagulopathy occurs during the course of the disease. Unfortunately, as a result of this, patients suffering from COVID-19 are suffering from severe and fatal conditions due to the occurrence of thrombosis in vital organs. Therefore, the study of the MTHFR gene A1298C (rs 1801131), C677T (rs 1801133) polymorphisms in the Uzbek population and the significance of the C (rs 1801131) and T (rs 1801133) minor alleles of this gene in the pathogenesis of COVID-19 and in patients with hyperhomocysteinemia It is important to assess the extent of the disease of COVID-19. By identifying this, it is possible to prevent various serious complications that can be caused by COVID-19 by selecting those who have a tendency to develop the disease in a severe form, and by carrying out special prophylactic and therapeutic procedures.

**Keywords:** MTHFR, polymorphism, rs 1801131, rs 1801133, wild allele, minor allele folate cycle, hyperhomocysteinemia, endothelial dysfunction.

## INTRODUCTION

The coronavirus infection (COVID-19) is a new infectious disease with severe complications that is rapidly spreading around the world. In December 2019, an epidemic outbreak of an unknown infection was observed in Wuhan, and the development of pneumonia in a large number of patients caused an emergency in the Chinese health system [7]. The Chinese Center for Disease Control and Prevention examined swabs from patients' throat swabs and confirmed that the case was caused by a new type of beta-coronavirus [6]. The new virus was called SARS-CoV-2 (severe acute respiratory syndrome coronavirus) [8].

COVID-19 is a systemic disease characterized by hyperimmune inflammation, renin-angiotensin-aldosterone system imbalance, endothelial dysfunction, and severe vasculopathy (thrombotic microangiopathy and intravascular coagulopathy) [9].

The SARS-CoV-2 life cycle consists of 5 stages. In the first step, as mentioned above, SARS-CoV-2 binds to the ACE2 receptor of the host cell with the help of its S protein, and in the second step, with the help of other intracellular proteins, in particular, cathepsins and TMPRSS2 , it enters the cell by endocytosis or membrane fusion (penetration) . These receptors are abundantly secreted in the lungs, intestines, liver, heart, vascular endothelium, testis, kidneys, and brain. For this reason, various disorders of these organs occur in the disease of COVID-19 [10, 11]. The virus entering the host cell undergoes proteolytic changes with the help of its C protein, especially cathepsin, furin and other enzymes, and viral RNA is released from its nucleocapsid. Viral (+)RNA is translated by host cell enzymes and expresses nonstructural proteins and replication/transcription complex (third stage - biosynthesis). In the fourth step, through them, it replicates again, and in the last step, the host leaves the cell [12, 13].

Studies have shown that SARS-CoV-2 binds to ACE2 in the vascular endothelium and, as a result of its cytopathic effect, causes massive apoptosis and necrosis of endothelial cells, as well as endothelial dysfunction induced by the cytokine storm in the pathogenesis of COVID-19 - hypercoagulopathy, thrombophilia and thrombosis in vessels. causes an increase in blood vessel permeability, microcirculation disorders. These changes cause endothelial dysfunction and the development of local or systemic vasculitis [ 14, 15 ].

When talking about the hemostasis system, it makes sense to talk about the folate cycle. As a result of folate cycle deficiency, hyperhomocysteinemia - hypercoagulopathy has been proven by many studies [16, 17].

Folic acid enters the body through the alimentary tract in the form of polyglutamate and is broken down by the enzyme glutamate carboxypeptidase II (GCPII) into monoglutamates for absorption through SLC19A1 and PCFT transporters in enterocytes. Inside the cell, folic acid is re-deconjugated for storage. Folic acids stored in the form of folate polyglutamate within the cell must be converted back to dihydrofolate (DHF) to enter the folate cycle. Then, dihydrofolate is reduced back to tetrahydrofolate (THF) (using NADPH with the enzyme dehydrofolate reductase), tetrahydrofolate is transformed into 5,10-methylenetetrahydrofolate (5, 10-methyleneTHF) by the enzyme Serine-hydroxymethyltransferase. And finally, 5, 10-methyleneTHF is converted into the active form - 5-methyltetrahydrofolate by the enzyme MTHFR (methylenetetrahydrofolate reductase). The next steps in the metabolism of folate and homocysteine continue with the formation of methionine from 5-methyltetrahydrofolate, giving homocysteine its methyl group (-CH3). As a result, 5-methyltetrahydrofolate itself becomes tetrahydrofolate. This process is controlled by the enzyme MTR (methionine synthase), and B12 plays a role as a cofactor in this case. But after a certain time, MTR becomes inactivated as a result of oxidation of B12. In this case, the enzyme MTRR (methionine synthase reductase) returns V12 to an active state. Methionine formed from homocysteine is converted to S-adenosylmethionine (SAM) by the enzyme methionine adenosyltransferase. SAM is important in the organism as a universal methyl group donor, in the synthesis of nucleotides, epigenome production and its maintenance, synthesis of neurotransmitters, phospholipids, hormones [ 1, 2, 3, 4] (see Figure 1) .

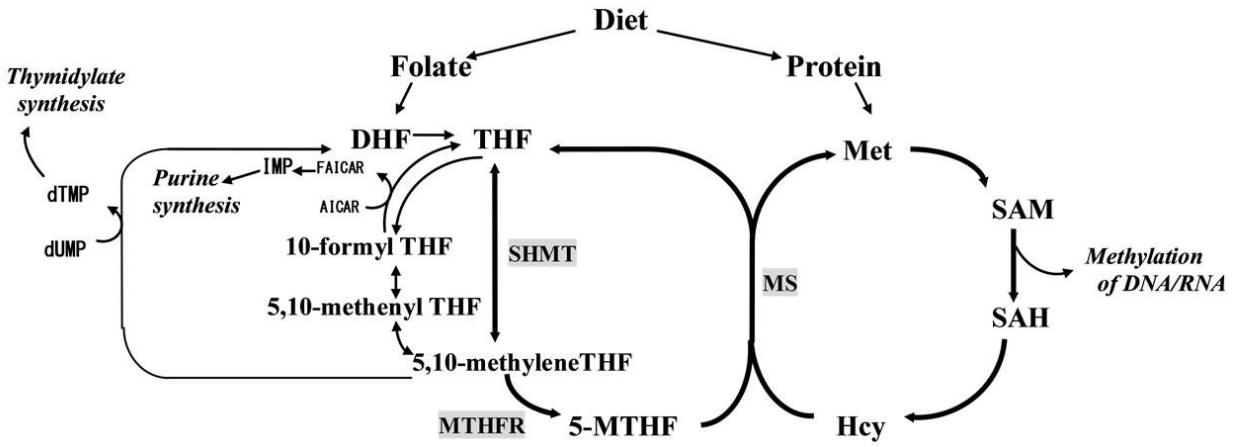


Figure 1. The folate and methionine cycle. DHF = dihydrofolate; THF = tetrahydrofolate; 5-MTHF = 5-methyltetrahydrofolate; 5,10-methyleneTHF = 5,10-methylenetetrahydrofolate; 5,10-Methenyl THF = 5,10-Methenyltetrahydrofolate; 10-formyl THF = 10-formyltetrahydrofolate; Hcy = homocysteine; Met = methionine; SAM = s-adenosyl methionine; SAH = s-adenosyl homocysteine; dUMP = deoxyuridine monophosphate; dTMP = deoxythymidine monophosphate; AICAR = 5-amino-4-imidazole carboxamide ribonucleotide; FAICAR = 5-formamidoimidazole-4-carboxamide ribonucleotide; IMP = inosine monophosphate; SHMT = serine hydroxymethyltransferase; MTHFR = methylenetetrahydrofolate reductase; MS = methionine synthase [5] .

## Material and methods.

In the scientific study, 80 patients were examined and they formed the main group. Based on the clinical symptoms of COVID-19 and the severity of the disease, the patients of the main group were divided into: Group I - 20 mild cases, Group II - 26 cases of moderate severity, and Group III - 34 cases of severe cases. When dividing the patients into groups in this order, we used the 8th revision of the manual of the Ministry of Health of the Republic of Uzbekistan entitled "TEMPORARY RECOMMENDATIONS ON THE TREATMENT OF PATIENTS WITH CORONAVIRUS INFECTION"

(https://ssv.uz/uploads/documentation/823b672dcd7e9a5f0fde55dba1cc18ab.pdf ). The purpose of dividing the groups in this order was to determine the relationship between the tested gene polymorphisms and the severity of the disease.

In a clinical study, thrombophilia genes were examined in the blood of 80 patients with coronavirus infection. In the detection of MTHFR gene A1298C (rs 1801131), C677T (rs 1801133) polymorphisms in the venous blood of patients, nucleotide sequencing was carried out using polymerase chain reaction in a DT-Lite 48 amplifier, using DNA-technology (Russia) reagents. Similarly, the amount of homocysteine in the plasma of all group patients and healthy subjects was checked (by the IFA method in the "Human" (Germany) reagent) and the correlation between their genotypes and the amount of homocysteine was checked.

Statistical processing of research results . The $\chi$ 2 criterion was used to evaluate genotypes meeting taking into account the Hardy-Weinberg equilibrium and to compare the level of distribution of genotypes and alleles. If the $\chi$ 2 criterion was used to confirm the presence of a predisposition to the studied pathology through the association of alleles and genotypes, the pathogenetic significance of alleles and genotypes in the studied disease was confirmed by the relative risk (RR) and extimolar ratio (OR) with a 95% confidence interval (95% CI:). A level of $p < 0.05$ was considered statistically significant. Statistical data processing was performed using Statistica 6.1 (StatSoft, USA).

## Results.

The results of distribution of genotypes of alleles of rs1801133 polymorphism in MTHFR gene are presented in Table 1.

Table 1 Distribution frequency of alleles and genotypes of MTHFR 677 C>T (rs1801133) gene polymorphism

| G groups | Alleles are variable | | | | Distribution of genotypes is average | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | C | | T | | C/C | | C/T | | T/T | |
| | n | % | n | % | n | % | n | % | n | % |
| Main group, (n = 80) | 123 | 76,88 | 37 | 23.13 | 48 | 60 | 27 | 33.75 | 5 | 6.25 |
| First group, (n = 20) | 38 | 95 | 2 | 5 | 18 | 90 | 2 | 10 | 0 | 0 |
| Second group, (n = 26) | 40 | 76.92 | 12 | 23.08 | 14 | 53.85 | 12 | 46.15 | 0 | 0 |
| Third group, (n = 34) | 45 | 66.18 | 23 | 33.82 | 16 | 47.06 | 13 | 38,24 | 5 | 14.71 |
| Control group, (n=20) | 38 | 95 | 2 | 5 | 18 | 90 | 2 | 10 | 0 | 0 |

In this context, the indicators obtained on the distribution of genotypes in the MTHFR gene 677 C>T (rs1801133) polymorphism were checked based on the Hardy-Weinberg law. As a result, the empirical indicators obtained from the main and control groups did not reveal a statistically reliable shift compared to the theoretical indicators determined by the Hardy-Weinberg law ( $\chi$ 2 <3.84; R>0.05). (See Table 2).

Table 2 Comparison of empirical-observed results with theoretical-expected results calculated by Hardy-Weinberg law in MTHFR gene 677 C>T (rs1801133) polymorphism

| Main group | | | | |
|---|---|---|---|---|
| Alleles | Distribution of alleles | | | |
| C | 0.779 | | | |
| T | 0.231 | | | |
| Genotypes | Distribution of genotypes | $\chi^2$ | p | df |

| | Observed | Expected | | | |
|---|---|---|---|---|---|
| C / C | 0.60 | 0.59 | | | |
| C / T | 0.3375 | 0.3555 | | | |
| T / T | 0.0625 | 0.0535 | | | |
| General | 1 | 1 | 0.20 | 0.90 | 1 |

| Control group | | | | | |
|---|---|---|---|---|---|
| Alleles | Distribution of alleles | | | | |
| C | 0.95 | | | | |
| T | 0.05 | | | | |
| Genotypes | Distribution of genotypes | | $\chi^2$ | p | df |
| | Observed | Expected | | | |
| C / C | 0.90 | 0.90 | | | |
| C / T | 0.1 | 0.095 | | | |
| T / T | 0.0 | 0.05 | | | |
| General | 1 | 1 | 0.055 | 0.97 | 1 |

Interestingly, the study of the frequency of distribution of alleles and genotypes of the MTHFR 677 C>T (rs1801133) polymorphism showed that the C/T heterozygous genotype was detected in only 2 (10%) patients with mild coronavirus infection (this indicator is equal to the result obtained from the control group subjects). Therefore, genetic predisposition to thrombophilia was not confirmed by $\chi$ 2, RR and OR with 95% confidence interval (95% CI): $\chi$ 2 =0.0; R=0.99; OR=1.0, 95% CI 0 (see Table 3).

Table 3. Allele and Genotype Prevalence Differences of MTHFR 677 C>T (rs1801133) Gene Polymorphism in Mild COVID-19

| Alleles and genotypes | Number of alleles and genotypes | | | | $\chi^2$ | P | RR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group 1 | | Control group | | | | | | | |
| | N | % | N | % | | | | | | |
| C | 38 | 95.0 | 38 | 95.0 | 0.0 | p = 0.99 | 1.0 | 0.14 - 7.17 | 1.0 | 0 - 0 |
| T | 2 | 5.0 | 2 | 5.0 | 0.0 | p = 0.99 | 1.0 | 0.14 - 7.17 | 1.0 | 0 - 0 |
| C/C | 18 | 90.0 | 18 | 90.0 | 0.0 | p = 0.99 | 1.0 | 0.13 - 7.57 | 1.0 | 0 - 0 |
| C/T | 2 | 10.0 | 2 | 10.0 | 0.0 | p = 0.99 | 1.0 | 0.13 - 7.57 | 1.0 | 0 - 0 |

C, T alleles and C/C, C/T, T/T genotypes in moderate-severe COVID -19 were as follows: 76.92%, 23.08% and 53.85%, 46.15%, respectively. 0% MTHFR 677 C>T (rs1801133) in moderate-to-severe COVID-19. It was found that the occurrence of the C/T genotype in the gene is reliably high, and according to the extimolar ratio, the S/T heterozygous genotype increases the risk of developing a moderate form of COVID-19 by 7.7 times ( 95% CI 1.69-35.12 ), and this result is statistically significant ($\chi$ 2 =7.0; R=0.01) . On the other hand, C/C wild genotype was found to have a protective effect on the development of moderate form of COVID-19 (OR=0.1; 95%CI: 0.03-0.59; $\chi$ 2 =7.0; R=0.01 ) ( Table 4 ).

Table 4. Moderately severe COVID-19 MTHFR 677 C>T (rs1801133) gene polymorphism allele and genotype distribution frequency differences

| Alleles and genotypes | Number of alleles and genotypes | | | | $\chi^2$ | P | RR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group II | | Control group | | | | | | | |
| | N | % | n | % | | | | | | |
| C | 40 | 76.9 | 38 | 95.0 | 5.7 | p = 0.025 | 0.8 | 0.45 - 1.47 | 0.2 | 0.04 - 0.73 |
| T | 12 | 23.1 | 2 | 5.0 | 5.7 | p = 0.025 | 1.2 | 0.1 - 15.88 | 5.7 | 1.37 - 23.71 |
| C/C | 14 | 53.8 | 18 | 90.0 | 7.0 | p = 0.01 | 0.6 | 0.25 - 1.44 | 0.1 | 0.03 - 0.59 |
| C/T | 12 | 46.2 | 2 | 10.0 | 7.0 | p = 0.01 | 4.6 | 1.92 - 11.09 | 7.7 | 1.69 - 35.12 |

C, T alleles and C/C, C/T, T/T genotypes in severe COVID -19 was as follows: 66.18%, 33.82% and 47.06%, 38.24%, 14 .71%. MTHFR 677 C>T (rs1801133) When analyzing the differences in the distribution frequency of alleles and genotypes in the polymorphism of the gene, a correct correlation was found between the severe course of COVID-19 and the occurrence of the heterozygous C/T genotype: $\chi$ 2 =5.0; R=0.05; OR=5.6, 95% CI 1.24–25.09. This indicates that the minor allele and heterozygous genotypes are factors that induce the severe course of COVID-19. On the other hand, normal homozygous genotype C/C showed a protective effect in the development of severe form of COVID-19 (OR=0.1; 95%CI: 0.02-0.42; $\chi$ 2 =10.0; R=0.01) ( Table 5 ).

Table 5. Allele and Genotype Prevalence Differences of MTHFR 677 C>T (rs1801133) Gene Polymorphism in Severe COVID-19

| Alleles and genotypes | Number of alleles and genotypes | | | | $\chi^2$ | P | RR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group III | | Control group | | | | | | | |
| | n | % | n | % | | | | | | |
| C | 45 | 66.2 | 38 | 95.0 | 11.8 | p = 0.01 | 0.7 | 0.44 - 1.09 | 0.1 | 0.03 - 0.38 |
| T | 23 | 33.8 | 2 | 5.0 | 11.8 | p = 0.01 | 1.4 | 0.1 - 20.22 | 9.7 | 2.65 - 35.6 |
| C/C | 16 | 47.1 | 18 | 90.0 | 10.0 | p = 0.01 | 0.5 | 0.25 - 1.11 | 0.1 | 0.02 - 0.42 |
| C/T | 13 | 38.2 | 2 | 10.0 | 5.0 | p = 0.05 | 3.8 | 1.92 - 7.62 | 5.6 | 1.24 - 25.09 |

Similarly, as a result of the study of the second polymorphic variant - rs1801133 in the MTHFR gene, the distribution of alleles and genotypes was as shown in Table 2 (Table 6).

Table 6 Distribution frequency of alleles and genotypes of MTHFR 1298 A>C (rs1801131) gene polymorphism

| G groups | Alleles are variable | | | | Distribution of genotypes is average | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | | C | | A / A | | A / C | | C / C | |
| | n | % | n | % | n | % | n | % | n | % |
| Main group (n = 8 0) | 125 | 78,13 | 35 | 21.88 | 48 | 60 | 29 | 36.25 | 3 | 3.75 |
| First group , (n = 20) | 39 | 97.5 | 1 | 2.5 | 19 | 95 | 1 | 5 | 0 | 0 |
| Second group , (n = 26) | 39 | 75 | 13 | 25 | 13 | 50 | 13 | 50 | 0 | 0 |
| Third group, (n = 34) | 47 | 69.12 | 21 | 30.88 | 16 | 47.06 | 15 | 44.12 | 3 | 8.82 |
| Control group (n=20) | 39 | 97.5 | 1 | 2.5 | 19 | 95 | 1 | 5 | 0 | 0 |

When the indicators obtained on the distribution of genotypes in the MTHFR gene rs1801131 polymorphism were checked based on the Hardy-Weinberg law, it became clear that the results obtained from the main and control groups corresponded to the Hardy-Weinberg law ( $\chi$ 2 <3.84; R>0.05) (5.1. Table 10).

Table 7 Comparison of empirical-observed results with theoretical-expected results calculated by the Hardy-Weinberg law of MTHFR gene rs1801131 polymorphism

| Main group | | | | | |
|---|---|---|---|---|---|
| Alleles | Distribution of alleles | | | | |
| A | 0.781 | | | | |
| C | 0.219 | | | | |
| Genotypes | Distribution of genotypes | | $\chi^2$ | p | df |
| | Observed | Expected | | | |
| A / A | 0.6 | 0.6 1 | | | |
| A / C | 0.3625 | 0.342 | | | |
| C / C | 0, 0 375 | 0.048 | | | |
| General | 1 | 1 | 0.29 | 0.86 | 1 |

| Control group | | | | | |
|---|---|---|---|---|---|
| Alleles | Distribution of alleles | | | | |
| A | 0.975 | | | | |
| C | 0.025 | | | | |
| Genotypes | Distribution of genotypes | | $\chi^2$ | p | df |
| | Observed | Expected | | | |
| A / A | 0.95 | 0.95 | | | |
| A / C | 0.05 | 0.05 | | | |
| C / C | 0.00 | 0.00 | | | |
| General | 1 | 1 | 0.01 | 0.99 | 1 |

A, C alleles and A/A, A/C, C/C genotypes when the significance of polymorphisms presented in mild COVID-19 disease was checked , according to the control group: 97.5%, 2.5% and 95%, 5 %, 0%. When analyzing the differences in the prevalence of alleles and genotypes, there was no correlation between the occurrence of mild COVID-19 and the heterozygous A/C genotype: $\chi 2$ =0.0; R=0.99; OR=1.0, 95% CI 0 (Table 8).

Table 8 Allele and Genotype Prevalence Differences of MTHFR 1298 A>C (rs1801131) Gene Polymorphism in Mild COVID-19

| Alleles and genotypes | Number of alleles and genotypes | | | | $\chi^2$ | P | RR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group I | | Control group | | | | | | | |
| | N | % | N | % | | | | | | |
| A | 39 | 97.5 | 39 | 97.5 | 0.0 | p = 0.99 | 1.0 | 0.06 - 15.65 | 1.0 | 0 - 0 |
| C | 1 | 2.5 | 1 | 2.5 | 0.0 | p = 0.99 | 1.0 | 0.06 - 15.65 | 1.0 | 0 - 0 |
| A / A | 19 | 95.0 | 19 | 95.0 | 0.0 | p = 0.99 | 1.0 | 0.06 - 16.23 | 1.0 | 0 - 0 |
| A / C | 1 | 5.0 | 1 | 5.0 | 0.0 | p = 0.99 | 1.0 | 0.06 - 16.23 | 1.0 | 0 - 0 |

A, S alleles and A/A, A/C, C/C genotypes in moderate COVID-19 was 75%, 25% and 50%, 50%, 0%, respectively, while in the control group these indicators 97.5% showed 2.5% and 95% showed 5% 0%. When analyzing the differences in the distribution frequency of alleles and genotypes of the MTHFR gene 1298 A>C polymorphism, it was found that the minor allele – S and the heterozygous genotype – A/C were significantly more prevalent in patients with a moderate course of COVID-19 compared to the results determined from the control group. Therefore, when its pathogenetic influence was examined, the significance of the heterozygous A/C genotype inducing the moderate form of the COVID-19 disease was found - OR=19.0

(95% CI 3.28-109.9) , and the statistical reliability of this result was confirmed $\chi$ 2 =10 ,8; R=0.01. Similarly, the wild homozygous A/A genotype was found to have a protective effect in the pathogenesis of moderately severe COVID-19 (OR=0.1; 95%CI: 0.01-0.3; $\chi$ 2 =10.8; R=0.01 ) ( Table 9 ).

Table 9 MTHFR 1298 A>C (rs1801131) Gene Polymorphism Allele and Genotype Frequency Differences in Moderate-Severe COVID-19

| Alleles and genotypes | Number of alleles and genotypes | | | | $\chi^2$ | p | RR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group II | | Control group | | | | | | | |
| | N | % | n | % | | | | | | |
| A | 39 | 75.0 | 39 | 97.5 | 8.9 | p = 0.01 | 0.8 | 0.46 - 1.29 | 0.1 | 0.01 - 0.42 |
| C | 13 | 25.0 | 1 | 2.5 | 8.9 | p = 0.01 | 1.3 | 0.03- 54.01 | 13.0 | 2.4 - 70.3 |
| A / A | 13 | 50.0 | 19 | 95.0 | 10.8 | p = 0.01 | 0.5 | 0.22 - 1.25 | 0.1 | 0.01 - 0.3 |
| A / C | 13 | 50.0 | 1 | 5.0 | 10.8 | p = 0.01 | 10.0 | 4.19- 23.84 | 19.0 | 3.28 - 109.9 |

In the group of patients with severe COVID-19 disease , the frequency of A, C alleles and A/A, A/C, C/C genotypes was as follows: 69.12%, 30.88% and 47.06%, 44.12 %, 8.82%. When analyzing the pathogenetic significance of alleles and genotypes of the 1298 A>C polymorphism in the MTHFR gene, a statistically reliable correlation was found between the severe course of COVID-19 and the unpleasant A/C genotype: $\chi$ 2 =9.2; R=0.01; OR=15.0, 95% CI 2.62-85.97 . MTHFR gene rs1801131 A/A genotype has been confirmed to have a strong protective effect on the development of a severe form of the disease (OR=0.05; 95% CI: 0.01-0.25) ( Table 10 ).

Table 10 Allele and Genotype Prevalence Differences of MTHFR Gene 1298 A>C (rs1801131) Polymorphism in Severe COVID-19

| Alleles and genotypes | Number of alleles and genotypes | | | | $\chi^2$ | P | RR | 95% CI | OR | 95% CI |
|---|---|---|---|---|---|---|---|---|---|---|
| | Group III | | Control group | | | | | | | |
| | N | % | n | % | | | | | | |
| A | 47 | 69.1 | 39 | 97.5 | 12.5 | p = 0.01 | 0.7 | 0.47 - 1.08 | 0.1 | 0.01 - 0.28 |
| C | 21 | 30.9 | 1 | 2.5 | 12.5 | p = 0.01 | 1.4 | 0.03 - 61.82 | 17.4 | 3.58 - 84.93 |
| A / A | 16 | 47.1 | 19 | 95.0 | 12.7 | p = 0.01 | 0.5 | 0.24 - 1.04 | 0.0 5 | 0.01 - 0.25 |
| A / C | 15 | 44.1 | 1 | 5.0 | 9.2 | p = 0.01 | 8.8 | 4.51 - 17.25 | 15.0 | 2.62 - 85.97 |

Thus, during the research, we determined the homocysteine values of patients of different genotypes observed in the investigated polymorphisms of the MTHFR gene (Table 11). According to him, the results obtained in the first group did not differ statistically significantly (p>0.05) from the results observed in the control group (due to the insufficient identification of heterozygous genotype patients in the first group, statistical comparison was not performed). Similarly, there was no statistically significant difference (p>0.05) between the results of patients with the wild-homozygous genotype in the second (patients with moderate form of COVID-19) and the third (patients with severe form of COVID-19) group and the results of the control group. On the other hand, the results of S/T (MTHFR gene 677 C>T polymorphism) and A/C (MTHFR gene 1298 A>C polymorphism) heterozygous genotype results in the second group were approximately 3.3 times and 3 times higher than the results obtained in the control group, respectively. was higher (p<0.05), the third group's scores were about 3.44 and 3.3 times higher than those of the control group, respectively (p<0.05). Interestingly, patients with C/T heterozygous genotype in the second and third groups were found to be approximately 2.84 and 2.9 times higher than patients with C/C genotype in the same group (p<0.05). Similarly, the results of homocysteine concentration in the second and third groups of A/C genotype patients were confirmed to be approximately 2.2 and 2.4 times higher than the results observed in A/A - wild genotype patients (p<0.05) (Table 11). .

Table 11 Results of homocysteine (μmol/l) in different genotypes of MTHFR gene 677 C>T and 1298 A>C polymorphisms

| Groups | MTHFR 677 C>T | | MTHFR 1298 A>C | |
|---|---|---|---|---|
| | C/C | C/T | A/A | A/C |
| First Group | 5.2 ± 0.4 | - | 5.2 ± 0.4 | - |
| Second Group | 5.4 ± 0.5 | 15.9 ± 2.1 [ab] | 5.8 ± 0.7 [a] _ | 14.7 ± 1.9 [ab] |
| Third Group | 6, 8 ± 0.5 | 16.6 ± 1.5 [ab] | 5.5 ± 0.7 _ | 15.8 ± 1.3 [ab] |
| Control group | 4.8 ± 0.7 | - | 4.8 ± 0.7 | - |

Instruction : a - statistical reliability compared to the control group - $p < 0.05$; b - statistical reliability in this polymorphism and in this group, compared to the wild homozygous group - $p < 0.05$.

## Discussion.

MTHFR gene 1(1r36.3) is located in the short shoulder of the chromosome and consists of 11 exons. The length of the coding gene is about 1980 pairs of nucleotides. As a result of MTHFR gene rs1801133 (C677T) polymorphism, cytosine nucleotide 677 is changed to thymine, alanine is changed to valine in the protein-enzyme catalytic domain (p.Ala222Val) . As a result , enzyme activity decreases by 70% in the mutant homozygous variant, and by 35% in the heterozygous genotype. As a result of a sharp decrease in the activity of the enzyme in the homozygous genotype for the C677 allele, the concentration of homocysteine in the blood is sharply above the norm, and in the heterozygous genotype it is significantly higher than the norm. This situation is especially strong when the amount of folates in the blood is low [18].

According to the results of our study, as shown in table 11, MTHFR gene 677 C>T and 1298 A>C polymorphisms were compared to patients with homozygous wild type (C/C and A/A) in patients with heterozygous genotype (C/T and A/C) and hyperhomocysteine levels. Homocysteine was found to be statistically significantly higher. Interestingly, no reliable difference in homocysteine concentrations was found between patients with the wild-type homozygous genotype and controls.

A result similar to ours, that is, a positive association between MTHFR gene 677 C>T and 1298 A>C polymorphisms and hyperhomocysteinemia, has been confirmed by several studies, in particular Yukub M. and colleagues [21], Kakkamo D and colleagues [22] and several other studies.

It is known that homocysteine damages the endothelial layer of blood vessels and activates the coagulation process. Endothelium is a barrier between the vascular wall and circulating blood and produces vasoactive substances, mediators and their inhibitors. Through these biologically active substances, the endothelium is of primary importance in controlling vascular tone . Nitrous oxide is one of these substances. It is constantly produced by the endothelium and has several protective functions, including vasodilation, inhibition of smooth muscle proliferation , and reduction of platelet and other blood cell aggregation. At the same time, nitric oxide reacts with homocysteine and "neutralizes" it. However, hyperhomocysteinemia or neutralization does not occur .

Similarly, homocysteine induces the formation of oxygen radicals. The mechanism for this is that hyperhomocysteine inhibits the activity of some antioxidants, particularly glutathione peroxidase, superoxide dismutase, and heme oxygenase-1. In addition, hyperhomocysteine increases the activity of NADF-oxidases, as a result of which many free oxidative radicals are generated. This leads to a decrease in the activity of endothelial lipid synthetase. As a result, the synthesis of nitric oxide, which is an important factor of vasodilatation and endothelial protection, decreases in hyperhomocysteinemia [19, 20].

result of increased oxidant stress and endothelial dysfunction due to increased homocysteine, rostacyclin, which is considered a vasoactive substance, reduces the amount of PGI 2 and increases the amount of thrombocyte A2 . Both are synthesized from arachidonic acid under the action of cyclooxygenase. Thromboxane enhances thrombus formation by increasing platelet aggregation, causing strong narrowing of blood vessels. Prostacyclin is mainly synthesized in the endothelium of blood vessels, unlike thrombox, it relaxes vascular muscles, reduces platelet aggregation, and stimulates fibrinolysis [20].

Studies have shown that homocysteine activates elastase, and as a result of elastin degradation, the endothelium becomes fragile, which leads to the deposition of calcium, cholesterol, and lipids that deform the vascular wall. At the same time, homocysteine reduces the production of endothelin-1. Endothelin-1 is a protein composed of 21 amino acids synthesized by vascular endothelium. It increases the proliferation of smooth muscle cells by binding to special transmembrane receptors and has a strong vasoconstrictor effect. Normally, endothelial cells resist the adhesion of blood cells to the vessel surface, have antithrombotic and fibrinolytic properties. Endothelial injury as a result of hyperhomocysteinemia increases platelet aggregation [21].

to the literature, homocysteine disrupts the activity of tissue plasminogen activator, causes the aggregation of lipoprotein and fibrin, as a result of which fibrinolysis decreases. At the same time, homocysteine in high concentration disrupts the activity of antithrombin III and protein, which are natural anticoagulants, alters the normal antithrombotic properties of the endothelium, which activates blood clotting factors V, X and XII [21].

In addition, homocysteine not only alters endothelial cells, but also impairs their regeneration by causing hypomethylation in these cells. Similarly, as a result of folate cycle deficiency, the production of HDL (high-density lipoprotein) from hepatocytes decreases, which increases the risk of atherosclerosis [22].

Due to hyperhomocysteinemia-induced endothelial dysfunction, and the cobormid role of cytokine storm in the pathogenesis of COVID-19, patients may develop systemic inflammation. In patients carrying the mutant allele, methyltetrahydrofolate reductase enzyme activity is reduced, which may predispose patients to moderate or severe disease with COVID-19. Because hyperhomocysteinemia induces endothelial dysfunction and creates a propensity for inflammation and hypercoagulation by disrupting the ratio of pro-inflammatory/anti-inflammatory and pro-coagulant/anti-coagulant substances in endotheliocytes. In this case, it may increase the likelihood that the disease will rapidly progress to a severe form in those infected with COVID-19. The comorbid pathological effects of SARS-CoV-2 and hyperhomocysteinemia, especially on the endothelial system, significantly increase the probability of the development of systemic inflammation, ischemic and thromboses.

## Conclusion.

According to the conclusion of the experiment conducted in the Uzbek population, the amount of homocyseine in the blood was significantly higher in patients with the non-wild allele of the MTHFR gene 677 C>T and 1298 A>C polymorphisms, compared to the control group, as well as to patients with the wild homozygous genotype. Similarly, it was found that almost all of the patients with T and C mutant alleles of the polymorphisms presented in the patients had moderate and severe disease. In addition to hyperergic inflammation and hypercoagulopathy induced by COVID-19, hyperhomocysteine cobormide exposure significantly increases the likelihood of severe disease compared to homozygous wild genotype patients.

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