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COMPARATIVE ANALYSIS OF FOLATE CYCLE MARKERS IN PATIENTS WITH TYPE 2 DIABETES COMPLICATED BY DIABETIC NEPHROPATHY

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ABSTRACT

One of the most studied genetic factors in the context of DN is the methylenetetrahydrofolate reductase (MTHFR) gene, specifically the C677T polymorphism. This single nucleotide polymorphism (SNP) is associated with elevated plasma homocysteine levels, which are known to contribute to vascular damage and the progression of nephropathy in diabetic patients. Research consistently shows that the 677T allele, particularly in individuals with the homozygous form (TT genotype), significantly increases the risk of developing DN across various populations.

Key words: mitral valve genetic polymorphisms, diabetic nephropathy, markers.

INTRODUCTION

Diabetic nephropathy (DN) is one of the microvascular complications associated with type 2 diabetes mellitus (T2DM) [10]. It remains one of the leading causes of chronic kidney disease and end-stage renal failure globally [13]. The pathogenesis of DN is multifactorial, involving hyperglycemia, arterial hypertension, and genetic predisposition. Recent studies have highlighted the significance of certain genetic polymorphisms in increasing the risk of developing this condition [1, 3, 4].

One of the most studied genetic factors in the context of DN is the methylenetetrahydrofolate reductase (MTHFR) gene, specifically the C677T polymorphism. This single nucleotide polymorphism (SNP) is associated with

elevated plasma homocysteine levels, which are known to contribute to vascular damage and the progression of nephropathy in diabetic patients [9]. Research consistently shows that the 677T allele, particularly in individuals with the homozygous form (TT genotype), significantly increases the risk of developing DN across various populations [5].

In addition to C677T, the A1298C polymorphism in the MTHFR gene is also being investigated in the context of DN. Although A1298C does not always demonstrate a strong association with DN on its own, its presence in combination with C677T may have a synergistic effect, exacerbating the risk of nephropathy in T2DM patients [11]. The combined impact of these polymorphisms is particularly evident in populations with low folate levels, where they may contribute to the progression from microalbuminuria to more advanced stages of nephropathy.

Another gene of interest is MTRR (methionine synthase reductase), where the A66G polymorphism has been linked to an increased susceptibility to DN, especially when combined with the C677T polymorphism in the MTHFR gene. This interaction suggests a complex genetic interplay that heightens the risk of nephropathy, particularly in individuals with additional risk factors such as obesity [12].

Finally, the role of the MTR (methionine synthase) gene, especially the A2756G polymorphism, should be noted. Although the evidence base for this gene is less extensive compared to polymorphisms associated with MTHFR, its potential contribution to DN development, particularly in specific ethnic groups, warrants further investigation [6].

Thus, understanding the genetic predisposition conferred by polymorphisms in the MTHFR, MTRR, and MTR genes is crucial for identifying individuals at increased risk of developing diabetic nephropathy. This knowledge not only deepens our understanding of DN pathophysiology but also opens avenues for the development of personalized therapeutic strategies aimed at mitigating this severe complication of T2DM.

Objective of the Study

The aim of this study is to assess the impact of genetic polymorphisms (MTHFR C677T, MTHFR A1298C, MTRR A66G, MTR A2756G), which regulate the folate cycle, on the progression of diabetic nephropathy.

Materials and Methods

The study included 122 participants, of which 79 were patients with type 2 diabetes mellitus (T2DM) complicated by diabetic nephropathy (DN), and 43 individuals were in the control group, matched by age and gender. The patients with T2DM were aged between 35 and 70 years with a confirmed diagnosis of

T2DM. Inclusion criteria for the study included a confirmed diagnosis of T2DM, the presence of diabetic nephropathy, and the absence of exacerbations of comorbid conditions. Exclusion criteria included a history of thromboembolism, hereditary coagulopathies, pregnancy, recent surgeries, low hemoglobin levels, the presence of type 1 diabetes, acute complications of diabetes, liver or end-stage renal failure, thyroid dysfunction, and severe somatic or infectious diseases in the decompensated stage.

The T2DM patient group was further divided into subgroups based on the stage of DN: 50 patients without DN, 8 patients with chronic kidney disease (CKD) stages 1-2, and 21 patients with CKD stages 3-4. The control group consisted of individuals without diabetes and without signs of diabetic nephropathy.

Genetic analysis was performed to identify the polymorphisms C677T and A1298C in the MTHFR gene, as well as A66G in the MTRR gene and A2756G in the MTR gene. The identification of polymorphisms was conducted using polymerase chain reaction (PCR) with specific primers, followed by the analysis of amplification products on an automated sequencer.

Statistical analysis was carried out using SPSS Statistics 25.0 software. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Fisher's exact test, Student's t-test, and Mann-Whitney U test were employed to compare the frequency of various genotypes and alleles across subgroups, depending on the normality of the data distribution. Qualitative indicators were analyzed using Pearson's chi-squared test. Differences were considered statistically significant at a p-value of < 0.05.

Results and Discussion:

Table 1. Comparative Analysis of MTHFR C677T in Relation to Diabetic Nephropathy

	Control Group	**No DN**	**DN 1-2**	**DN 3-4**
MTHFR 677 C/C	24 (43.6%)	25 (45.2%)	2 (38.7%)	4 (3.2%)
MTHFR 677 C/T	14 (35%)	16 (11.5%)	3 (73.1%)	7 (3.8%)
MTHFR 677 T/T	5 (18.5%)	9 (22.7%)	3 (40.9%)	10 (31.8%)

Note: There is no statistically significant difference between the groups, Chi2 = 9.092, P = 0.059.

Table 1 presents the distribution of the MTHFR C677T polymorphism among different groups of patients with and without diabetic nephropathy (DN). The most notable findings indicate that the MTHFR 677 C/T genotype is present in 35% of the control group, 11.5% of patients without DN, 73.1% of patients with diabetic nephropathy stages 1-2, and 3.8% of those with stages 3-4. Additionally, the MTHFR 677 T/T genotype is observed in 18.5% of the control group, 22.7% of

patients without DN, 40.9% of those with diabetic nephropathy stages 1-2, and 31.8% of those with stages 3-4. Despite these variations, there are no statistically significant differences between the groups (Chi2 = 9.092, P = 0.059).

An important aspect of the discussion is the comparison of the obtained data with the results of other studies. In a study conducted by Mochulsky and colleagues, it was shown that the MTHFR 677 CT and TT genotypes were statistically more frequent in patients with diabetic nephropathy than in healthy individuals. Their study found that the frequency of the CT and TT genotypes increased with the progression of nephropathy, reaching 68% in patients with chronic renal failure, which is comparable to our data showing an increase in the frequency of CT and TT genotypes in patients with more advanced stages of diabetic nephropathy [5].

On the other hand, the study by Mtiraoui and colleagues demonstrated that the MTHFR 677 TT genotype is associated with elevated homocysteine levels and an increased risk of developing diabetic nephropathy in type 2 diabetes patients, which also supports our findings of a higher frequency of the TT genotype in patients with diabetic nephropathy stages 3-4 [7].

Thus, the data obtained in our study, as reflected in Table 1, align with the findings of other research, confirming the association between the MTHFR C677T polymorphism and the risk of developing diabetic nephropathy.

Table 2. Comparative Analysis of MTHFR A1298C in Relation to Diabetic Nephropathy

	Control Group	**No DN**	**DN 1-2**	**DN 3-4**
MTHFR 1298 A/A	27 (47.4%)	22 (73.3%)	3 (10.0%)	5 (16.7%)
MTHFR 1298 A/C	13 (33.3%)	20 (76.9%)	1 (3.8%)	5 (19.2%)
MTHFR 1298 C/C	3 (11.5%)	8 (34.8%)	4 (17.4%)	11 (47.8%)

Note: Statistically significant differences between the groups were observed, Chi2 = 11.950, P < 0.05.

Table 2 presents the distribution of the MTHFR A1298C polymorphism among different groups of patients depending on the presence of diabetic nephropathy (DN). The most striking findings indicate that the MTHFR 1298 C/C genotype is present in 11.5% of the control group, 34.8% of patients without DN, 17.4% of patients with diabetic nephropathy stages 1-2, and 47.8% of those with stages 3-4. Notably, the differences between the groups were statistically significant (Chi2 = 11.950, P < 0.05).

In discussing these findings, it is essential to reference the study by Rahimi and colleagues, who found that the presence of both MTHFR 1298C and 677T

alleles significantly increases the risk of diabetic nephropathy progression, particularly during the transition from microalbuminuria to macroalbuminuria. Their study showed that the combined presence of these alleles increases the risk of developing macroalbuminuria by 7.8 times, which aligns with our data showing a higher frequency of the MTHFR 1298 C/C genotype in patients with diabetic nephropathy stages 3-4 [9].

Moreover, a study by El-Baz and colleagues established that the MTHFR 1298C polymorphism in a dominant model is a significant risk factor for the development of macroalbuminuria in type 2 diabetes patients, confirming our data on the high frequency of this genotype in patients with progressive diabetic nephropathy [2].

Therefore, the results presented in Table 2 demonstrate a significant association between the MTHFR A1298C polymorphism and the development of diabetic nephropathy, particularly in the later stages of chronic kidney disease.

Table 3. Comparative Analysis of MTR A2756G in Relation to Diabetic Nephropathy

	Control Group	**No DN**	**DN 1-2**	**DN 3-4**
MTR 2756 A/A	30 (36.1%)	34 (64.2%)	5 (9.4%)	14 (26.4%)
MTR 2756 A/G	10 (32.3%)	13 (61.9%)	2 (9.5%)	6 (28.6%)
MTR 2756 G/G	3 (37.5%)	3 (60.0%)	1 (20.0%)	1 (20.0%)

Note: There is no statistically significant difference between the groups, Chi2 = 0.648, P = 0.958.

Table 3 provides data on the distribution of the MTR A2756G polymorphism among different groups of patients depending on the presence of diabetic nephropathy (DN). The primary findings indicate that the MTR 2756 A/A genotype is present in 36.1% of the control group, 64.2% of patients without DN, 9.4% of patients with diabetic nephropathy stages 1-2, and 26.4% of those with stages 3-4. The MTR 2756 G/G genotype is observed much less frequently, accounting for 20% in patients with diabetic nephropathy stages 3-4, and there are no statistically significant differences between the groups (Chi2 = 0.648, P = 0.958).

These findings can be compared with the results of a study conducted by Nithya and colleagues, who also investigated the MTR A2756G polymorphism in the context of type 2 diabetes and its complications among a South Indian population. Their study showed that the MTR A2756G polymorphism was not significantly associated with either diabetes or its complications, including diabetic

nephropathy, which corresponds to our data showing no significant differences between the groups of patients with different stages of diabetic nephropathy [8].

Thus, the results presented in Table 3 do not reveal a significant association between the MTR A2756G polymorphism and the development or progression of diabetic nephropathy, as confirmed by other studies.

Table 4. Comparative Analysis of MTRR A66G in Relation to Diabetic Nephropathy

	Control Group	No DN	DN 1-2	DN 3-4
MTRR 66 A/A	18 (37.5%)	17 (56.7%)	5 (16.7%)	8 (26.7%)
MTRR 66 A/G	17 (33.3%)	24 (70.6%)	2 (5.9%)	8 (23.5%)
MTRR 66 G/G	8 (34.8%)	9 (60.0%)	1 (6.7%)	5 (33.3%)

Note: There is no statistically significant difference between the groups, Chi2 = 2.945, P = 0.567.

Table 4 presents data on the distribution of the MTRR A66G polymorphism among various groups of patients with and without diabetic nephropathy (DN). The most significant results indicate that the MTRR 66 A/A genotype is found in 37.5% of the control group, 56.7% of patients without DN, 16.7% of patients with diabetic nephropathy stages 1-2, and 26.7% of those with stages 3-4. The MTRR 66 G/G genotype is less common, accounting for 6.7% in patients with diabetic nephropathy stages 1-2, and there are no statistically significant differences between the groups (Chi2 = 2.945, P = 0.567).

These findings align with the results of a meta-analysis conducted by Zi and colleagues, which demonstrated that the MTRR A66G polymorphism was not associated with the risk of metabolic syndrome or type 2 diabetes. However, this polymorphism may enhance the influence of other genetic factors, such as the MTHFR C677T polymorphism, on the development of these conditions [12].

Therefore, the results presented in **Table 4** confirm the lack of a significant association between the MTRR A66G polymorphism and the development of diabetic nephropathy. However, its interaction with other genetic factors may influence the development of other diabetes-related complications.

Conclusion

The conducted study on folate cycle markers in patients with type 2 diabetes mellitus complicated by diabetic nephropathy, considering genetic polymorphisms in the MTHFR, MTR, and MTRR genes, demonstrated that the MTHFR C677T and A1298C polymorphisms have a significant impact on the risk of developing and progressing diabetic nephropathy, particularly in the later stages of chronic kidney disease. In contrast, the MTR A2756G and MTRR A66G polymorphisms

did not show a significant association with the progression of nephropathy, highlighting the complexity and multifaceted nature of the pathogenesis of this complication, which requires further study. Thus, identifying genetic predisposition to diabetic nephropathy is a crucial step toward improving the prediction and prevention of this condition in modern medicine.

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