



Investigation of the Relationship Between MTHFR C677T Gene Variation and Serum Copper Levels in Patients Diagnosed with Parkinson's

Parkinson Tanısı Almış Hastalarda MTHFR C677T Gen Varyasyonu ve Serum Bakır Düzeyleri Arasındaki İlişkinin Araştırılması

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Abstract

Objective: In this study, we aimed to investigation of the relationship between MTHFR C677T gene variation and serum copper levels in patients diagnosed with Parkinson's.

Materials and Methods: For our study, patient and control groups were formed including 63 Parkinson's patients and 32 healthy controls. Genotype distributions for MTHFR gene variation were determined and serum copper levels were measured. In these processes PCR, RFLP and atomic absorption spectrophotometer methods were applied.

Results: Serum Cu levels of Parkinson's patients were found to be significantly higher than healthy controls. Although the significant difference was not found between the patient and control groups in terms of genotype distributions of the MTHFR C677T gene variation, CC homozygote genotype of this gene variation was observed significantly more than other genotypes in the patient group. In addition, the C allele frequency of this gene variation was determined significantly different from the Hardy-Weinberg distribution in Parkinson's patients. Serum copper levels of Parkinson's patients carrying CT and TT genotypes were detected significantly higher than the serum copper levels of controls carrying the same genotypes.

Conclusion: In our study, in Thrace population, it was determined that the relationship between MTHFR C677T gene variation and serum copper levels may be an important factor for Parkinson's disease. In conclusion, the evaluation of MTHFR C677T gene variation genotype distributions and serum copper levels together is extremely important for prognosis of Parkinson's disease.

Keywords: Parkinson disease; methylenetetrahydrofolate reductase (NADPH2); polymorphism; genetic; copper; polymerase chain reaction.

Özet

Amaç: Bu çalışmada Parkinson tanısı almış hastalarda MTHFR C677T gen varyasyonu ile serum bakır düzeyleri arasındaki ilişkiyi araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmamız için 63 Parkinson hastası ve 32 sağlıklı bireyden oluşan hasta ve kontrol grupları oluşturulmuştur. MTHFR gen varyasyonu için genotip dağılımları belirlendi ve serum bakır seviyeleri ölçüldü. Bu işlemlerde PZR, RFLP ve atomik absorpsiyon spektrofotometre yöntemleri uygulandı.

Bulgular: Parkinson hastalarının serum Cu düzeyleri sağlıklı kontrollere göre anlamlı olarak yüksek bulundu. MTHFR C677T gen varyasyonunun genotip dağılımları açısından hasta ve kontrol grupları arasında anlamlı farklılık bulunmamasıyla birlikte, bu gen varyasyonunun CC homozigot genotipi hasta grubunda diğer genotiplere göre anlamlı olarak daha fazla gözlemlendi. Ayrıca Parkinson hastalarında bu gen varyasyonunun C allel frekansı Hardy-Weinberg dağılımından anlamlı derecede farklı belirlendi. CT ve TT genotiplerini taşıyan Parkinson hastalarının serum bakır düzeyleri, aynı genotipleri taşıyan kontrollerin serum bakır düzeylerinden anlamlı derecede yüksek saptandı.

Sonuç: Trakya popülasyonunda yaptığımız çalışmada, MTHFR C677T gen varyasyonu ile serum bakır düzeyleri arasındaki ilişkinin Parkinson hastalığı için önemli bir faktör olabileceği belirlenmiştir. Sonuç olarak, MTHFR C677T gen varyasyon genotip dağılımlarının ve serum bakır düzeylerinin birlikte değerlendirilmesi Parkinson hastalığının prognozu için son derece önemlidir.

Anahtar Kelimeler: Parkinson hastalığı; metilentetrahidrofolat redüktaz (NADPH2); polimorfizm, genetik; bakır; polimeraz zincir reaksiyonu.

Introduction

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease (1). Parkinson's disease develops as a result of progressive degeneration and loss of

dopaminergic neurons in the substantia nigra in the midbrain and is characterized by tremors, bradykinesia, impaired postural reflexes, alpha-synuclein protein aggregation. Although the

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etiology of Parkinson's disease has not been fully explained, genetic and environmental factors are effective in the pathogenesis of the disease (2-4). Folate plays an important role in the nervous system and brain development. Abnormal DNA methylation develops as a result of chronic folate / methyl deficiency (5-9). Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme that is effective in folate metabolism pathways, so MTHFR gene has been associated with Parkinson's disease susceptibility. MTHFR gene is localised on chromosome 1p36.3 (10). The MTHFR C677T gene variation is characterized by cytosine / thymine base substitution and alanine / valine amino acid change in the N-terminal catalytic region of the MTHFR gene (4,11,12). MTHFR enzyme activity decreases as a result of this genetic variation (13,14). Imbalances in trace element homeostasis, which is known as an important environmental factor in Parkinson's pathogenesis, play an important role in neurodegeneration associated with Parkinson's disease. Abnormal levels of some trace elements may adversely affect the nervous system through induction of the production of reactive oxygen species (15). Cu is an essential cofactor for the antioxidant function of ceruloplasmin and the enzymatic activity of superoxide dismutase (16). It has been suggested that oxidative stress may be an important cause of neuronal death and the development of oxidative stress may be associated with an imbalance in Cu homeostasis. Decreased ceruloplasmin levels in Parkinson's patients demonstrate this (1). Cu plays a dual role in Parkinson's disease. Cu has been associated with increase in oxidative stress, the formation of Lewy bodies through alpha-synuclein protein oligomerization (17). Therefore, in our study, we aimed to investigate the role of correlation between MTHFR C677T gene variation and serum Cu levels in Parkinson's patients.

Materials and Methods

Patients and control subjects: Trakya University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee was applied. The approval of ethics committee was obtained from the komite for our study (Protocol code: TÜTF-BAEK 2017/77). Signed informed consent forms were obtained from these groups. Peripheral venous blood was obtained from Trakya University Faculty of Medicine Neurology Department for our study. Experimental parts of

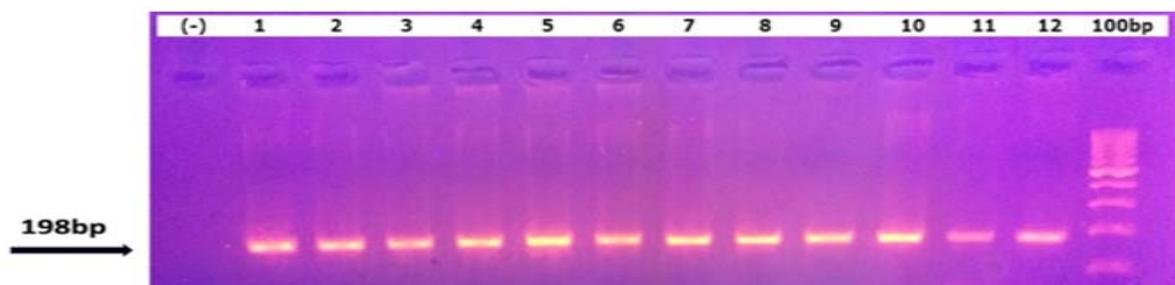
the study were carried out in Trakya University Faculty of Medicine Department of Biophysics. This study consisted of 63 Parkinson's patients and 32 controls. The patient group in our study includes patients diagnosed Parkinson's disease. The patients applied to Trakya University Faculty of Medicine Neurology Outpatient Clinics were diagnosed with Parkinson's disease due to the presence of clinical symptoms such as rigidity, asymmetric tremor, and postural instability as a result of a comprehensive neurological and physical examination. Patients and controls that are younger than 19 years and those have a history of malignancy were excluded from our study. Pregnant and breastfeeding women, individuals with any other neurodegenerative diseases associated with central nervous system were excluded from this study. In addition, people who took trace element supplements were also excluded from our study. Peripheral venous blood containing ethylenediaminetetraacetic acid (EDTA) was used to determine MTHFR C677T gene variation genotype distributions, while peripheral venous blood without EDTA was used to determine serum Cu levels.

DNA Isolation and PCR-RFLP methods: DNA isolation was performed from peripheral blood samples of patients and controls. These blood samples contained EDTA. DNA blood isolation kits (Invitrogen / Thermo Fisher Scientific, USA) were used for DNA isolation. Polymerase chain reaction (PCR) (Techne TC-3000 G, USA) and restriction fragment length polymorphism (RFLP) methods were used to determine the MTHFR C677T gene variation genotype distributions (12) (Table 1). For the PCR method, 25 µl of PCR mixture was prepared and these PCR reaction products were used for the RFLP method. PCR product lengths (198bp) for the MTHFR C677T gene variation were observed in 2% agarose gel electrophoresis (Clever Scientific for electrophoresis tank and EC-105, Clever Scientific MP300 V for power source, United Kingdom) (Figure 1). The genotype distributions of MTHFR C677T gene variation were observed using 2.5% agarose gel electrophoresis (Clever Scientific for electrophoresis tank and EC-105, Clever Scientific MP300 V for power source, United Kingdom). CC homozygous genotype (198bp), CT heterozygous genotype (198bp, 175bp, 23bp) and TT homozygous genotype (175bp, 23bp) were observed for the MTHFR C677T gene variation (Figure 2).

Table 1: Primer series, PCR condition, restriction enzyme and product lengths for MTHFR C677T gene variation

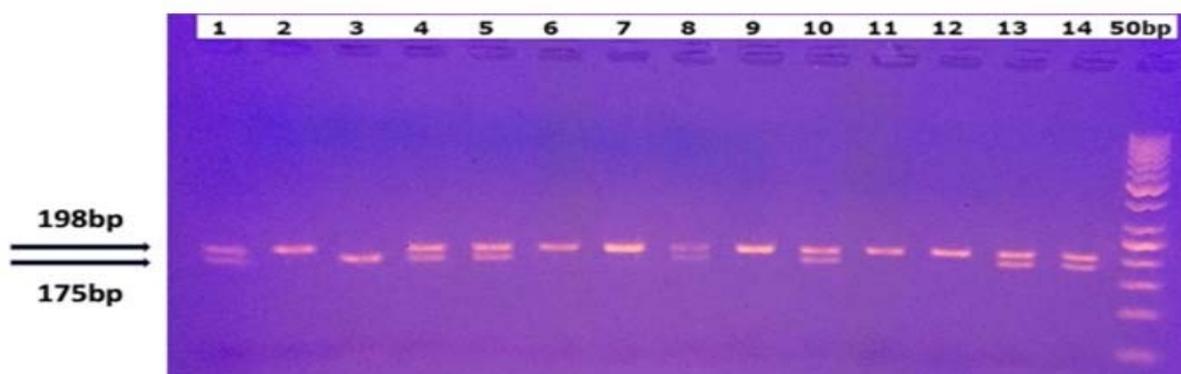
Gene Variations	Primer series	PCR Conditions	RE
MTHFR (C677T)	FP:5'-TGAAGGAGAAGGTGTCTGCGGGA-3' RP:5'-AGGACGGTGCGGTGAGAGTG-3'	5 minutes at 94°C 30 seconds at 94°C 30 seconds at 62°C 30 seconds at 72°C 5 minutes at 72°C	Hinfi
Gene Variation	PCR Product Length	Genotype Distributions	Product Lengths
MTHFR C677T	198bp	CC → 198bp CT → 198bp, 175bp, 23bp TT → 175bp, 23bp (23bp is not observed; 50bp marker)	

FP: Forward primer; **RP:** Reverse primer; **PCR:** Polymerase chain reaction; **RE:** Restriction enzyme



PCR samples of patient and control groups for MTHFR C677T gene variation (198bp, 100bp marker)

Figure 1. PCR samples of patient and control groups for MTHFR C677T gene variation.



RFLP samples of patient and control groups for MTHFR C677T gene variation
CC genotype in MTHFR C677T gene polymorphism (198 bp; Lanes 2, 6, 7, 9, 11 and 12), **TT genotype** (175 bp, 23 bp; Lane 3 numbered band) and **CT genotype** (198 bp, 175 bp, 23 bp; Lanes 1, 4, 5, 8, 10, 13 and 14)
 Note: 23 bp not observed; 50 bp marker.

Figure 2. RFLP samples of patient and control groups for MTHFR C677T gene variation.

Determination of serum Cu levels: EDTA-free peripheral venous blood samples of Parkinson's patients and healthy controls were centrifuged at 5000 rpm for 5 minutes. Serum samples were separated from centrifuged blood samples. Distilled water was added to these serum samples and the total volume was prepared as 5 milliliters. This mixture was homogenized. Standard solutions (0.5, 1.0, 1.5, 2.0, 2.5 ppm) were prepared for serum Cu measurement. Serum Cu levels were measured by atomic absorption spectrophotometer method. Concentration-calibration curve was drawn for element Cu using standard solutions (18).

Statistical analyses: For MTHFR C677T gene variation, the relationships between genotypes and groups were determined by Chi-square test. Odds ratio and 95% confidence interval values are calculated as a result of logistic regression analysis for MTHFR C677T gene variation genotype distributions between patient and control groups. In addition, the conformity of allele frequencies of the MTHFR C677T gene variation to the Hardy-Weinberg distribution was analyzed. Serum Cu levels were compared between Parkinson's patients and controls by Mann-Whitney U test. Independent-Samples Kruskal-Wallis test was used to determine serum Cu levels according to MTHFR C677T gene variation genotypes distribution between Parkinson's patient group and control group. In our study, the results were expressed as mean \pm standard deviation. Statistical significance level was considered as 5% and SPSS (Statistics Package of Social Science, V: 20) for windows was used for all statistical analyses.

Results

In our study CC, CT, TT genotypes of MTHFR gene variation were observed more in Parkinson's patients than in healthy controls. MTHFR C677T gene variation genotype distributions did not show a significant difference between the patient and control groups ($p > 0.05$). However CC homozygote genotype of this gene variation was observed significantly more than other genotypes in the patient group ($p < 0.05$). In addition, C allele frequency of the MTHFR C677T gene variation was found to be significantly different from the Hardy-Weinberg distribution in Parkinson's patients ($p < 0.05$) (Table 2). Serum Cu levels were detected significantly higher in Parkinson patients than controls (Table 3). The significant difference was determined between the patient and control groups in terms of serum Cu levels ($p < 0.05$). In Parkinson's patients carrying TT genotype, serum

Cu levels were detected higher than those carrying CT and CC genotypes. Serum Cu levels of Parkinson's patients carrying CT and TT genotypes were detected significantly higher than controls carrying the same genotypes ($p < 0.05$) (Table 4).

Discussion

Parkinson's disease is a neurodegenerative disease and may eventually affect the quality of life of patients. Parkinson's disease is known as a multifactorial disease stem from a combination of genetic factors and environmental factors (19). Many genetic variations have been identified in the MTHFR gene and especially two of these genetic variations may affect the MTHFR enzymatic activity. In a meta-analysis study, in Caucasian population, TT and CT genotypes have been associated with higher risk of Parkinson's disease compared to CC homozygous genotype (3). In another meta-analysis study, in Asian population, T allele of the MTHFR C677T gene variation was detected as genetic risk factor in Parkinson's disease (20). In another study, MTHFR C677T gene variation has been associated with a reduced risk of Parkinson's disease (21). In a study, MTHFR C677T gene variation has been associated with increased susceptibility for Parkinson's disease (22). In our study, CC, CT and TT genotypes of MTHFR C677T gene variation were observed more in Parkinson's patient group than in healthy control group. MTHFR C677T gene variation genotype distributions did not show a significant difference between the patient and control groups ($p > 0.05$). However CC homozygote genotype of this gene variation was observed significantly more than other genotypes in the patient group ($p < 0.05$). In addition, the C allele frequency of the MTHFR C677T gene variation was determined significantly different from the Hardy-Weinberg distribution in Parkinson's patients ($p < 0.05$) Imbalance in some trace element levels may adversely affect the central nervous system. Oxidative stress can develop as a result of abnormalities in these trace element levels and oxidative stress may be an important environmental risk factor in the pathogenesis of Parkinson's disease (17). Trace elements have important roles in the pathogenesis of Parkinson's disease and neurotoxic effects of trace elements such as Cu have been reported. In Parkinson's disease, Cu ions bind less to ceruloplasmin and free Cu ions can coordinate with low molecular weight ligands. Free or active

Table 2. Comparison of genotype distributions between patient group and healthy control group

Genotype distributions		Patient group=63 (%)		Control group=32 (%)		p	
MTHFR	CC	30 (71.4%)		12 (28.6%)		0.572 ^a	
C677T	CT	21 (60.0%)		14 (40.0%)			
	TT	12 (66.7%)		6 (33.3%)			
Genotype distributions		Patient group (n=63) and Control group (n=32)				p	
MTHFR	CC	OR: 1.515 (0.635-3.616)				0.349	
C677T	CT	OR: 0.643 (0.269-1.539)				0.321	
	TT	Reference OR: 1.00				-	
Genotype distributions		Patient group (n=63)				p	
MTHFR	CC	30 (71.4%)				0.029 ^{a*}	
C677T	CT	21 (60.0%)					
	TT	12 (66.7%)					
Genotype distributions		Control group (n=32)				p	
MTHFR	CC	12 (28.6%)				0.598 ^a	
C677T	CT	14 (40.0%)					
	TT	6 (33.3%)					
Gene Variations		Patient group (n=63)			Control group (n=32)		
	<i>Allele</i>	<i>Case</i>	<i>Frequency</i>	<i>Allele</i>	<i>Case</i>	<i>Frequency</i>	
	C	81	0.64286	C	38	0.59375	
	T	45	0.35714	T	26	0.40625	
MTHFR	Total	196	1.00000	Total	162	1.00000	
C677T	Hardy-Weinberg Equilibrium Test: Pearson chi2 = 4.73235 Pr= 0.0296 ^{b*}			Hardy-Weinberg Equilibrium Test: Pearson chi2 = 0.27747 Pr= 0.5984 ^b			

^aChi-square test; ^bHardy-Weinberg Equilibrium test; *: Significance (p<0,05) CC: Cytosine–Cytosine; CT: Cytosine–Thymine; TT: Thymine–Thymine

Table 3. Comparison of copper levels between patient and control groups

Trace element	Groups		P
	Patient (Mean ± SD) (n=63)	Control (Mean ± SD) (n=32)	
Cu (µg/dl)	180.30 ± 6.467	143.69 ± 4.862	0.001 ^{a*}

^aMann Whitney U test, *: Significance (p<0,05) Cu: Copper

Table 4. Cu levels according to C677T gene variation genotype distributions

MTHFR C677T Gene Variation	Genotypes	Trace elements	Parkinson group n (Mean ± SD)	Control group n (Mean ± SD)	P
		CC	Cu (µg/dl)	30 (150.33 ± 19.149)	12 (147.42 ± 26.983)
	CT	Cu (µg/dl)	21 (198.71 ± 63.728)	14 (141.71 ± 31.267)	0.001 ^{a*}
	TT	Cu (µg/dl)	12 (222.50 ± 38.668)	6 (140.83 ± 22.203)	0.001 ^{a*}

^aIndependent-Samples Kruskal-Wallis test, *: Significance (p<0,05) CC: Cytosine-Cytosine; CT: Cytosine-Thymine; TT: Thymine-Thymine; Cu: Copper

copper ions contribute to neurodegeneration by showing redox activities and thus play a pathogenic role. Cu-induced oxidative stress has been associated with the activation of transcription factors that regulate neuroinflammatory reactions. Progression of free

Cu accumulation tendency may be associated with significantly increased Cu neurotoxicity in Parkinson's patients (19). The main component of Lewy bodies is alpha-synuclein, and alpha-synuclein binds to the Cu ion in the oxidation states of Cu. Aggregate formation and oxidative

stress may occur as a result of this binding. Imbalance in Cu homeostasis has been associated with protein aggregation and development of oxidative stress (1). In some studies, significantly increased Cu levels were detected in the substantia nigra and cerebrospinal fluids of Parkinson's patients (23,24). In another study, serum Cu levels were found to be significantly higher in patients with severe Parkinson's than in healthy controls (25). In a study performed with Spanish population, increased serum Cu concentrations were determined in the cerebrospinal fluids of Parkinson's patients (16). In a study performed with the Russian population, decreased serum Cu concentrations were detected in Parkinson's patients (1). The differences in these results may be due to the variability in the clinical parameters of Parkinson's disease, different demographic parameters, different lifestyle and environmental conditions, and different selection criteria for the patient and control groups (19). In our study, serum Cu levels were determined significantly higher in Parkinson's patients compared to controls. The significant difference was detected between these groups in terms of serum copper levels ($p < 0.05$). In our study, in Parkinson's patients carrying TT genotype, serum copper levels were observed higher than carrying CC and CT genotypes. Also, in Parkinson's patients carrying CT and TT genotypes, serum copper levels were determined significantly higher than controls carrying the same genotypes ($p < 0.05$). Consequently, in our study, serum copper levels according to MTHFR C677T gene variation genotype distributions may be evaluated as risk factor for Parkinson's disease.

Conclusion

In our study, MTHFR C677T gene variation and serum Cu levels were evaluated together in the Parkinson's patients. It is thought that the obtaining of some biomarkers in our study may be important in the early diagnosis, prognosis and progression of Parkinson's disease. Serum Cu homeostasis may be impaired and Cu trace element activity and metabolism may be affected as a result of MTHFR C677T gene variation. As a result, it is thought that it may contribute to neurodegeneration due to oxidative stress. It is thought that the different results obtained in studies performed with different populations may be due to different selection criteria for patient and control groups, lifestyle differences, genetic variability, and sample size. In our study, in Thrace population, it was determined that the relationship between MTHFR C677T gene

variation and serum copper levels may be an important factor for Parkinson's disease. The evaluation of genetic factors such as genetic variations that may be effective in the pathogenesis of Parkinson's disease and environmental factors such as trace elements together and obtaining biomarkers are very important in terms of developing new therapeutic strategies and drugs related to Parkinson's disease.

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Compliance with Ethical Standards

Ethical approval: For our study, ethics committee approval was obtained with TÜTF-BAEK 2017/77 protocol code from Trakya University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee.

Author Contribution: 1. Concept: NA, AA; 2. Design: NA, AA; 3. Control: NA, AA; 4. Materials; 5. AA, SG; 6. Data Collection and Processing: NA, AA; 7. Analysis and Interpretation: NA, AA; 8. Literature Review: NA, AA; 9. Writing-Original Draft: NA, AA; 10. Writing Review and Revision: NA, AA; 11. Critical Review: NA, AA; 12. Software and Visualization: NA, AA.

Consent to Participate: A signed informed consent form was obtained from each of the individuals with Parkinson's disease and the control group.

Conflict of Interest: The authors declare that they have no conflict of interest.

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