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The effects of MDR-1 gene polymorphisms on the clinical course of chronic hepatitis B infection

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ABSTRACT

Aims: Chronic HBV infection is associated with a high morbidity and mortality rate due to the increased risk of hepatic cirrhosis and hepatocellular cancer. Treatment modalities and resistance are currently being investigated. Several mechanisms underlie drug resistance. P-glycoprotein (P-gp), the product of the multidrug resistance gene (MDR-1), is a well-known mechanism of the MDR phenotype. MDR gene C1236T polymorphism is associated with decreased p-gp function. The mutation of the MDR gene can affect the clinical course of the disease and response rate to treatment. The aim of our study was to investigate the relationship between MDR gene polymorphism and clinical course and treatment responses in chronic HBV infection.

Methods: A total of 90 (male/female: 69/21) patients with chronic HBV infection under Lamivudine treatment were enrolled in this study. Mean ages were 49.8±12.6 (range: 22-75) years. The patients were categorized as: Treatment-respondent (group 1: HBV-DNA is negative at the 24th week) and treatment-refractory (group 2: HBV-DNA is still positive after the 24th week). Group 1 consisted of 51 (M/F: 38/13) and group 2 consisted of 39 (M/F: 31/9) patients. There was no significant difference between the ages and genders of the two groups. Histologic activity indexes (HAI), total bilirubin, AST and ALT levels, and HBV-DNA titers were significantly higher in the patients in group 2 than in group 1 ($p<0.05$).

Results: Genotype distributions; homozygous CC genotype was in 8 (15.7 %), heterozygous CT genotype was in 37 (%72.5), and homozygous TT genotype was in 6 (11.8%) in patients in group 1. The homozygous CC genotype was in 13 (33.3%), the heterozygous CT genotype was in 21 (53.8%), homozygous TT genotype was in 5 (12.8%) in patients in group 2. CC genotype was more common in group 2 than in group 1 ($p=0.044$). C and T alleles' frequencies in groups 1 and 2 were 51.96% and 60.26%, 48.04%, and 39.74%, respectively ($p>0.05$). In group 2 (n:11) patients with a YMDD mutation, 5 (45%) had the CC genotype, 5 (45%) had the CT genotype, and 1 (9%) had the TT genotype. Three (37%) of the patients with a negative YMDD mutation in group 2 (n: 8) had the CC genotype, while five (63%) had the CT genotype. CC genotype was more common in the patients with a positive YMDD mutation than in group 1 ($p=0.043$). Furthermore, the CC genotype was more common in patients with HBV-DNA positivity than in group 1 at the 12th month of Lamivudine treatment ($p=0.042$).

Conclusion: Consequently, MDR-1 and p-gp polymorphisms are important factors in the clinical course of chronic HBV infection and may influence treatment responses. In the current study, it was found that the CC genotype of the MDR-1 gene C1236T was more common in patients with lamivudine-resistant HBV infection.

Keywords: Hepatitis B, Lamivudine resistance, MDR-1 gene polymorphism

INTRODUCTION

Chronic hepatitis B is a major health problem and one of the most common infectious diseases. Liver cirrhosis and cancer, which are fatal liver diseases, develop in 25% of chronic hepatitis B patients.[1,2]

Lamivudine is the first nucleoside analogue approved for the treatment of chronic hepatitis B. However, the relapse of the disease and the development of drug resistance after an average of 3-6 months following the discontinuation of the treatment are the limitations of lamivudine treatment.[1]

MDR1 gene (Multidrug Resistance) encodes a transmembrane transporter protein named P-glycoprotein (P-gp). P-gp controls the intracellular entry and exit routes of many drugs and chemicals. Response to drugs and drug-related adverse events vary across individuals in the same population due to genetic changes in drug-metabolizing enzymes. This difference may be explained by the increased expression of MDR genes that lead to drug resistance.[3,4]

The increased expression of the MDR1 gene product, P-gp, is the most well-studied among the mechanisms that create the MDR phenotype. It has been reported in various studies that single nucleotide polymorphisms manifested in the MDR1 gene lead to alterations in P-gp expression and/or function.[4] It is considered that P-gp expression is high in some alleles, and this causes resistance to drugs and some substances.[5] P-gp

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is expressed in various organs and is associated with drug distribution in intestinal erythrocytes, endothelial cells of brain capillaries, proximal tubule and hepatic canalicular cells.[6]

The three commonly observed SNPs in the MDR1 gene and the most emphasized in the literature are C3435T, G2677T and C1236T, and these SNPs are common haplotype components.

In this study, the association between treatment response rates and C1236T polymorphism in the MDR1 gene in chronic hepatitis B patients was investigated.

METHODS

Ninety patients treated for Chronic Hepatitis B that applied to Gaziosmanpaşa University Faculty of Medicine Gastroenterology Clinic between 2009 and 2012 and received lamivudine, were enrolled in the study. In this study, which was approved by the Ethics Committee of Gaziosmanpaşa University Faculty of Medicine by the decision dated 30.03.2012 and numbered 2012-30, all genetic and laboratory analyzes were performed in Gaziosmanpaşa University Faculty of Medicine Genetics Laboratory.

Selection of Cases

Patients with positive HBS Ag results detected by the ELISA method and significant HBV-DNA levels detected by PCR method were included in the study. YMDD mutation analysis was done in lamivudine resistant cases via HBV Quantitative & YMDD Mutation Real Time PCR Kit (Shanghai ZJ Bio-Tech Co.,Ltd, China, HD-0003-04).

Patients enrolled in the study were divided into two groups.

Group 1: Patients on lamivudine therapy for chronic hepatitis B and responding to treatment with negative HBV-DNA titer.

Group 2: Patients non-responsive to treatment with an increase or no significant decrease in HBV-DNA titer during monitoring of the patients with chronic hepatitis B on lamivudine therapy. YMDD mutation was studied in patients in group 2 and YMDD mutation was examined in two subgroups as positive/negative.

A total of 90 patients diagnosed with chronic hepatitis B were included in the study. Patients with negative HBV-DNA titer at week 24 during lamivudine therapy were considered to be responsive to treatment (Group 1, n:51). Patients applied to the hospital at the 24th and later weeks on lamivudine therapy and still showed positive HBV-DNA titers were considered treatment-resistant (Group 2, n:39). There were 51 (Male/Female:38/13) patients in group 1 and 39 (Male/Female:31/8) patients in group 2. There were 11 patients with YMDD mutation who were non-responsive to lamivudine therapy in group 2, 11 patients with positive YMDD mutation, and 8 patients with negative YMDD mutation and 20 patients whose YMDD mutation could not be detected as positive/negative due to technical issues.

Genetic Analysis

Genomic DNA isolation was performed from the blood sample collected from the patients in a 5-cc EDTA tube via the Invitrogen Genomic DNA Isolation Mini Kit (K1820-02, Invitrogen Life Technologies, Carlsbad, CA, USA). Afterwards, PCR was performed for the MDR1 gene C1236T locus using the appropriate primers as previously defined. The fragmented PCR products were resolved in 3% Nusieve 3:1 agarose gels containing 0.5 mg/ml of ethidium bromide and they were visualized by using Vilber–Lourmat Gel Quantification and Documentation System QUANTUM-ST4 (Vilber Lourmat BP 66, Torcy, France). Genotyping was performed based on the restriction lengths obtained (Allele T: 269+97 bp and Allele C: 269+62+35 bp).

Statistical Analysis

Statistical analyses were performed using SPSS 15.0. Visual and analytical methods (Kolmogorov-Smirnow/Shapiro-Wilk tests) were used to confirm that the data was normally distributed. Values are given as mean±standard deviation. The T-test was used to compare numerical variables between independent groups and the chi-square test was used to compare categorical variables. The Mann-Whitney U test was used to compare the parameters that did not fit the normal distribution.

The allelic/genotypic frequencies for SNP in patient groups and haplotype frequencies were determined by the Arlequin 3.11 software program. Fischer's exact chi-square test was used to detect genotype frequencies. SPSS 15.0 was used to compare the data from the patient and control groups and to calculate the OR (Odds Ratio). $p<0.05$ was considered as statistically significant.

RESULTS

A total of 90 (M/F:69/21) patients diagnosed with chronic hepatitis B were included in the study. The mean age of the patients was 49.8±12.6 (range; 22-75) years. The patients were divided into 2 main groups:

The mean age of the patients in the first group was 50.78±12.66 years, and 74.5% of the patients were male while 25.5% were female. The mean age of the second group was 48.62±12.69 years, and 79.5% of the patients were male while 20.5% were female. In the second group, the mean age of the patients with positive YMDD mutation was 50.64±12.20 years, 81.8% of the patients were male while 18.2% were female, and the mean age of the patients with negative YMDD mutation was 45.38±11.04, 82.5% of the patients were male while 17.5% were female. No statistically significant difference was found between the mean age and sex of both groups ($p>0.05$). The clinical and laboratory features of the patients are given in **Table 1**.

Table 1: Clinical and laboratory features of patient groups prior to treatment

Feature	Responsive to Lamivudine N:51	Resistant to Lamivudine N:39	P
Age, year	50.78±12.66	48.62±12.69	0.324
Sex			0.624
Male	38 (74.5%)	31 (79.5%)	
Female	13 (25.5%)	8 (20.5%)	
HBV-DNA, IU/ml	1483696.02 ±7298719.11	5842435.95 ±17252984.918	0.001*
HAI	9.20±3.40	10.62±3.70	0.036*
Fibrotic stage			0,117
Stage 1	12 (23.5%)	10 (25.6%)	
Stage 2	21 (41.2%)	9 (23.1%)	
Stage 3	9 (17.6%)	15 (38.5%)	
Stage 4	2 (3.9%)	3 (7.7%)	
Stage 5	1 (2%)	1 (2.6%)	
Stage 6	6 (11.8%)	1 (2.6%)	
Lamivudine duration, months	33.05±22.1	16.97±12.26	0.001*
AST, U/L	36.21±17.41	60.07±43.99	0.002*
ALT, U/L	46.07±32.24	94.41±75.71	0.001*
Albumin, gr/dl	4.43±0.36	4.40±0.39	0.416
T. Bilirubin, g/dl	0.69±0.48	0.83±0.44	0.042*

The CC, CT and TT gene mutations of MDR1 C1236T were compared genotypically between the treatment-respondent group and the treatment-resistant group. In group 1, CC gene mutations were found in 8 (15.7%), CT gene mutations in 37 (72.5%), and TT gene mutations in 6 (11.8%) patients. In group 2, CC gene mutations were found in 13 (33.3%), CT gene mutations in 21 (53.8%), and TT gene mutations in 5 (12.8%) patients. In the treatment-resistant group (group 2), CC gene mutations were found in 5 (45%) patients with positive YMDD mutation, CT gene mutations in 5 (45%) patients, TT gene mutation was found in 1 (9%) patient while CC gene mutations were detected in 3 (37%) patients with negative YMDD mutation and CT gene mutations were detected in 5 (63%) patients. YMDD mutation rate in patients with treatment resistance was found 12.2% (11/90) (**Table 2**).

The CC, CT, and TT genotype distributions were investigated among the patient groups (**Figure 1**). While CT and TT genotypes were detected at similar rates Group 1 and 2, CC genotype was present by 15.7% in Group 1 and 33.3% in Group 2. The difference was statistically significant (p=0.044) (OR 2.69%; 95% CI: 0.99-7.27).

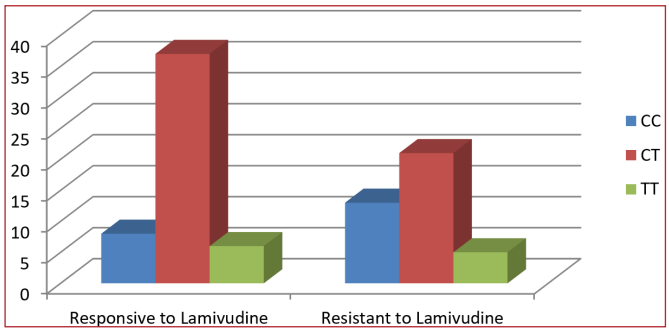

Figure 1: C1236T genotype distribution among patient groups

Allele frequencies between groups were compared. Allele C frequency was 51.96% in Group 1 and 60.26% in Group 2. Allele T frequency was 48.04% in Group 1 and 39.74% in Group 2. When the groups were compared in terms of the frequency of both alleles, no statistically significant difference was found (p>0.05).

CC, CT, TT mutations of 11 patients in group 2 with positive YMDD mutation and 8 patients with negative mutation were found to be genotypically similar (p>0.05). 11 lamivudine-resistant patients with positive YMDD mutation and group 1 which consists of 51 patients sensitive to lamivudine, were compared in terms of genotypic CC mutation, and the difference between the two groups was found to be statistically significant (p=0.043) (OR=4.48, 95% CI: 1.15 – 17.38).

10 of 11 YMDD-positive patients in group 2 carry the allele C. In terms of CC mutation, when 8 patients with positive YMDD mutation with a detected lamivudine resistance in group 2 and whose total duration of lamivudine use did not exceed 12 months, and 51 lamivudine sensitive patients were compared, a statistically significant difference was found (p=0.046 OR=5.38 95% CI 1.20-24) (**Table 2**).

CC mutation was detected in 8 (15.69%) patients in group 1 and in 8 (38.1%) patients in group 2 with a 12-month duration of Lamivudine use, and the difference regarding CC mutation was found statistically significant (p=0.042) (OR 3.31, 95%). CI: 1.06 – 10.33) (**Table 2**).

DISCUSSION

Lamivudine is a nucleoside analogue approved by the FDA in 1998 for the treatment of chronic hepatitis B. The only limitation, when compared to other anti-viral agents used in the treatment of chronic hepatitis B, is drug resistance, which may arise during lamivudine therapy.[7-15]

Factors that may be associated with the development of lamivudine resistance in the literature have been reported as the patient's age, sex, body-mass index, HBeAg positivity, HBV genotype, pathological condition of the liver prior to treatment, pre-treatment serum ALT and HBV-DNA levels.[7,8,12,16]

In this study, apart from the factors triggering the development of resistance mentioned in the literature, it has been investigated whether MDR1 gene polymorphism is associated with the development of lamivudine resistance.

Yuen et al.[8] reported that YMDD motif mutation occurrence was associated with pre-treatment serum ALT and HBV-DNA levels. In a similar vein, in our study, we have found a significantly higher HBV-DNA level in the treatment-resistant group when compared with the sensitive group (p=0.001).

The study conducted by Suzuki et al.[17] presented that the development of a YMDD motif mutation was directly associated with the duration of treatment. Accordingly, they reported that the YMDD motif mutation frequency, which was detected as 12.5% at the end of the 1st year, reached 43% at the end of the 3rd year and 63% at the end of the 5th year. In our study, although a higher rate of lamivudine resistance was found when compared with the previous findings in the literature, the YMDD mutation rate in patients with treatment resistance was found to be consistent with the literature findings as 12.2%.

The pharmacokinetic and pharmacodynamic efficacy of drugs is affected by enzymes responsible for the metabolism of drugs, drug transporters, and genetic variations on receptors

Table 2: Genotypic and allelic distribution of MDR1 gene C1236T polymorphism in patients with HBV based on Lamivudine response, YMDD mutation, and duration of Lamivudine therapy

Genotype	The response to Lamivudine		YMDD Mutation			Lamivudine Resistant Duration of Use	
	Responsive	Resistant	Positive (n:11)		Negative		
			Lamivudine Use 6-12 months	Lamivudine Use > 12 months		<12 months	>12 months
CC	8 (15.7%)	13 (33.3%)*	4 (36%)	1 (%9)	3 (%37)	8 (38.1%)**	5 (27.7%)
CT	37 (72.5%)	21 (53.8%)	4 (36%)	1 (9%)	5 (63%)	11 (52.4%)	10 (55%)
TT	6 (11.8%)	5 (12.8)	0	1 (9%)	0	2 (9%)	3 (16.6%)
Total	51	39	8	3	8	21	18
Allele							
C	53(51.96%)	47(60.26%)	12	3	11	27	20
T	49(48.04%)	31(39.74%)	4	3	5	15	16

*- Among lamivudine responsive and resistant patients, p=0.044, **- Among drug-resistant and responsive patients on Lamivudine <12 months, p=0.042 (OR, 3.31; 95% CI, 1.06-10.33)

or cofactors.[18] The P-glycoprotein encoded by the MDR1 gene is a factor that has an influence on the drug metabolism.[19,20]

For the first time, in a study conducted by Tsurua et al.[21] it was shown that vincristine accumulated in the cell due to the use of trifluoperazine and verapamil in P-gp positive mouse leukemic cells with MDR phenotype, and it was reported that P-gp-associated multi-drug resistance could be reversed. Studies were carried out with the aim of reducing the expression and function of P-gp with anti-MDR-1 oligonucleotides or decreasing the expression of MDR-1 with protein kinase C inhibitors such as staurosporine as well.[22] It was reported that verapamil and trifluoperazine eliminated the resistance to adriamycin in P-gp positive cells.[23]

Schwab et al.[20] emphasized that drug kinetics and the response to drugs vary between societies and individuals based on genetic structure, and they brought forward the development of a "patient-tailored treatment" approach by using genetic databases to be obtained from different populations.[27] In our study, we investigated the presence of other genetic factors affecting lamivudine resistance, apart from YMDD mutation, and the impact of MDR1 gene polymorphism on resistance. The CC genotype was found to be significantly higher in both the lamivudine-resistant group and the resistant patients with YMDD mutation.

More than 50 SNPs (Single Nucleotide Polymorphism) have been identified in the MDR1 gene up to the present, and are increasing in number day by day.[18] Kimichi – Sarfaty et al.[28] reported that the three most observed and emphasized SNPs in the MDR1 gene were C3435T, G2677T/A and C1236T.

Schwab et al.[20] concluded that C3435T and C1236T polymorphisms were silent (synonymous) polymorphisms that did not lead to amino acid replacement, and although C3435T and C1236T were silent polymorphisms.

C3435T polymorphism is the most associated with diseases or drug resistance.[23,24,26,29] It has been reported that C3435T, one of the silent polymorphisms, may be associated with ribavirin resistance in chronic hepatitis C infection.[30] In our study, we investigated whether another silent polymorphism, C1236 T, was associated with lamivudine resistance in chronic hepatitis B patients.

The CT genotype (72.5%) was higher in the lamivudine-responsive group in comparison to the non-responsive group (53.8%), however, no statistical difference was noted. The CC genotype was higher in the lamivudine-resistant group (33.3%) in comparison to the responsive group (15.7%) (p=0.044). When the groups were compared in terms of alleles, allele C was higher in the resistant group (60.26% vs 51.96%), while allele T was higher in the sensitive group (48.04% vs 39.4%). Allele frequencies were determined as similar between the groups. Similarly, CC genotype and allele C were found to be higher in the lamivudine non-responsive group and in the patient group with positive YMDD. The CC genotype was significantly higher in patients who developed resistance in the first year of treatment when compared with the group that responded to lamivudine. In light of these findings, it was concluded that the CC genotype played a role in the development of lamivudine resistance. The elevated CC genotype and allele C frequency in patients with YMDD mutation also support this hypothesis.

The MDR1 genotype is of great importance in terms of disease risk and treatment outcome in AIDS because HIV protease inhibitors used in the treatment of this disease are the substrates of P-gp. Fellay et al.[29] detected a significant increase in CD4+ cells in patients with the C3435TT genotype following 6 months of antiretroviral treatment. As a result, it was concluded that TT genotype was associated with a better response rate and virus resistance to a lesser extent in HIV treatment or allele C was linked to a failure in viral immune response. The data on the C1236T polymorphism in our study also supports this study to a large extent. It was found that Allele C frequency and CC genotype were associated with lamivudine resistance in the treatment of chronic hepatitis B.

When compared with other nucleoside analogues, the rate of development of resistance to lamivudine is higher. The findings of our study have put forth that MDR1 gene mutation may affect response rates to treatment in patients with hepatitis B, especially for lamivudine.

CONCLUSION

Polymorphisms in the MDR-1 gene and its product, P-glycoprotein, are important factors that affect the treatment response during chronic hepatitis B. In our study, MDR1 C1236T CC genotype was more commonly found in the lamivudine-resistant group. We are of the opinion that MDR gene polymorphisms will guide clinicians in the selection and duration of treatment in chronic hepatitis B treatment in the upcoming years. Further and more comprehensive research is needed on this subject.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gaziosmanpaşa University Faculty of Medicine Research and Application Hospital Clinic Ethics Committee (Date: 30/03/2012, Decision No: 30).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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