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Research paper

Association between hepatic steatosis and *MTP* gene –493G/T polymorphism in the patients with HCV genotype 1 infection

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ABSTRACT

Aim: Hepatitis C virus (HCV) affects approximately 250 million people worldwide. If patients are untreated, 80% of patients with chronic HCV develop liver failure, liver cirrhosis (LC), and hepatocellular carcinoma (HCC). HCV genotype 1 is the most prevalent among the infected individuals with HCV. Hepatic steatosis is known as accumulation of lipid molecules in hepatocytes, and its prevalence is approximately 55% in CHC infection. The reason of HCV-related hepatic steatosis in CHC infection is mainly HCV core protein. HCV core protein inhibits activities of microsomal triglyceride transfer protein (MTP) which is a lipid transfer protein expressed in the liver. The –493G/T polymorphism in the promoter region of *MTP* gene has been associated with HCV-related hepatic steatosis. This polymorphism in *MTP* gene influences MTP mRNA expression, therefore which might also affect lipid transfer. We evaluated the association between *MTP* gene polymorphism and the risk of HCV genotype 1-related hepatic steatosis.

Methods: In the current study, *MTP* gene polymorphism was explored in 144 biopsy-proven chronic HCV genotype 1 patients by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results: The results showed that there were no any difference between the steatosis and the non-steatosis groups for the allele and genotype frequencies of the -493G/T polymorphism ($P > .05$). Moreover, *MTP* genotypes (GG vs. TG + TT) were not associated with BMI, fibrosis stages and the levels of biochemical parameters. Additionally, there were statistically significant differences in the biochemical parameters including triglyceride, total cholesterol, LDL, VLDL levels between the two groups ($P < .05$).

Conclusions: In conclusion, the current study demonstrates for the first time that *MTP* gene -493G/T polymorphism has not a major effect on the risk of HCV genotype 1-related hepatic steatosis in Turkish population. Further studies are imperative to clarify the association of this polymorphism with HCV genotype 1 infection in HCV-related hepatic steatosis.

1. Introduction

Hepatitis C virus (HCV) is a ribonucleic acid (RNA) virus which may lead to liver inflammation, liver cirrhosis and hepatocellular carcinoma (HCC) (Hwang and Lee, 2011). HCV infection affects about a quarter billion people worldwide, and of those approximately 80% develops chronic hepatitis C infection (CHC). Further, in 20–30% of the persons with CHC infection may be occur cirrhosis and HCC (Hwang, 2001). HCV genotype 1 is more prevalent among the infected individuals with HCV, and its response to treatment is very hardly (Magri et al., 2017). Hepatic steatosis is known as accumulation of lipid molecules in hepatocytes, and which is a histological characteristic of CHC infection (Zampino et al., 2008), and its prevalence is approximately 55% in CHC infection (Lonardo et al., 2006). Hepatic steatosis in CHC infection is depending on metabolic and viral factors; obesity, insulin resistance (IR), diabetes mellitus (DM), hyperlipidemia and HCV genotypes, viral load, HCV core protein, respectively (Moriya et al., 1997; Barba et al., 1997).

A few animal and cell culture studies have been reported that the reason of HCV-related hepatic steatosis in CHC infection is mainly HCV

Abbreviations: HCV, Hepatitis C virus; SNPs, Single nucleotide polymorphisms; PCR-RFLP, Polymerase Chain Reaction–Restriction Fragment Length Polymorphism; A/G, Guanine/Thymine; bp, Base pair; SD, Standard deviation; CI, Confidence interval; HCC, Hepatocellular carcinoma; LC, Liver cirrhosis; *MTP*, *Microsomal triglyceride transfer protein*; HIV, Human immunodeficiency virus; HBV, Hepatitis B virus; HDV, Hepatitis D virus; LD, Linkage disequilibrium; VLDL, Very low-density lipoprotein

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core protein (Chang et al., 2007; Perlemuter et al., 2002; Ferré, 2004). HCV core protein inhibits activities of microsomal triglyceride transfer protein (MTP) and peroxisome proliferator activating receptor alpha (PPAR-α), which are a crucial players in lipid metabolism (Perlemuter et al., 2002; Dharancy et al., 2005). MTP is a lipid transfer protein expressed in the liver, intestine and heart, and that is consist of a 97-kDa and a 58-kDa subunits, and its pivotal role is to transfer of triglycerides from hepatocytes to apolipoprotein B (ApoB). Moreover, MTP provides correctly assembly and secretion of very low-density lipoprotein (VLDL) together with ApoB (Namikawa et al., 2004; García-García et al., 2005). Karpe et al. (1998) have identified –493G/T (rs1800591) polymorphism in the promoter region of *MTP* gene, and this polymorphism is associated with HCV-related hepatic steatosis and different diseases in a few studies (Mirandola et al., 2006; Zampino et al., 2008; Mirandola et al., 2009; Bernard et al., 2000; García-García et al., 2005; Namikawa et al., 2004). The expression studies reported that while the -493T allele is related to an increased MTP expression in healthy individuals, the -493G allele leads to decreased MTP transcription (Karpe et al., 1998; Namikawa et al., 2004). However, this polymorphism's influence on HCV-related hepatic steatosis is controversial (Mirandola et al., 2008; Petit et al., 2006; Magri et al., 2017; Mirandola et al., 2010). Further, only one study investigated the association of *MTP* gene –493G/T polymorphism with the 93 patients infected with HCV genotype-1 (Siqueira et al., 2012). Therefore, the –493G/T variant was selected as a candidate polymorphism in patients with HCV genotype 1 for the elucidation of its role in hepatic steatosis.

The aim of this study, for the first time, was to analyze the effect of *MTP* gene –493G/T polymorphism on HCV genotype 1-related hepatic steatosis in the Turkish population. *MTP* gene –493G/T polymorphism was investigated in 144 patients with HCV genotype 1 using real time polymerase chain reaction (RT-PCR) assay in Turkish population.

2. Methods

2.1. Patients and methods

The current study was approved by the Ethics Committee of Çukurova University in Turkey. Chronic HCV group was recruited at Çukurova University Balcalı Hospital from March 2013 to May 2017. The current study was performed according to the Helsinki declaration approved on the World Medical Association meeting in Edinburgh. 144 patients with chronic HCV infection, who were positive for both hepatitis C antibody (anti-HCV) and the presence of HCV-RNA, and continued elevation of alanine aminotransferase (ALT) for at least 6 months, were enrolled as the patients group. All patients had only HCV genotype 1 infection. 144 patients underwent biopsy for histological evaluation. Inclusion of the patients to the research study was conditioned by an obtained written informed consent form regarding the use of their blood and biopsy samples. Serum samples were tested for HCV RNA levels using the COBAS AmpliPrep/COBAS TaqMan HCV Test (sensitivity, 43 IU/ml; Roche Molecular Systems, Inc., Branchburg, NJ, USA) and for HCV genotype using the Linear Array Hepatitis C Virus Genotyping Test (Roche Molecular Systems, Inc. Branchburg, NJ, USA) at the Central Laboratory of Çukurova University Balcalı Hospital. The patients who also had human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis D virus (HDV), and had other liver diseases (autoimmune hepatitis, Wilson's disease, haemochromatosis) and being obese, were excluded from the present study. In addition, the patients with alcohol consuming and decompensated cirrhosis were excluded from the study, too. Quantitative HCV RNA, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), hemoglobin (HGB), hematocrit (HCT), triglyceride, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein HDL, very low-density lipoprotein (VLDL) levels were determined for statistical analysis. Additionally, body mass index (BMI) values were calculated as (kg/m^2). Peripheral blood samples of patients were taken from March 2013 to May 2017 in department of gastroenterology. Blood specimens were stored at +4 °C and serum specimens were frozen at −20 °C until analysis.

2.2. Histological evaluation

144 patients underwent a liver needle biopsy for histological evaluation. An experienced pathologist did histological evaluation without knowing the clinical information and HCV genotype of the patients. Fibrosis stages of patients with HCV were scored using the METAVIR scoring system (F0, F1, F2, F3 and F4). Moreover, fibrosis stages were categorized as two groups (F0–2; F3–4) for statistical analysis. Steatosis grades were scored according to the percentages of hepatocytes having lipid droplets (Grade 0: none; Grade 1: 1–33%; Grade 2: 34–66%; Grade 3: > 66%). Steatosis grades were categorized as two groups (Grade 0: the group of non-steatosis; Grade 1–3: the group with steatosis) for statistical analysis.

2.3. MTP gene −493G/T genotype detection

Genomic DNA was extracted from peripheral whole blood using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer protocol. As previously reported (Karpe et al., 1998), the genotyping for *MTP* gene −493G/T polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A 109 base pair (bp) fragment encompassing the G to T polymorphic site in *MTP* gene region was amplified with the forward primer 5′-AGTTTC ACA CATAAGGACAAT CAT CTA-3′ and the reverse primer 5′-GGATTT AAATTTAAA CTG TTA ATT CAT ATC AC-3′. The primers were verified using the NCBI Primer-Blast Tool web site. Amplification was performed in GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Singapore). The 20 µl PCR mixture contained approximately 250 ng DNA, with 0.25 µM of both primers, 0.1 mM of each dNTP, 1 × PCR buffer, 1.5 mM MgCI2 and 1 U Taq polymerase (Promega, Madison, WI, USA). The optimized PCR cycling conditions were 95 °C for 5 min, followed by 40 cycles of 94 °C for 60 s, 57 °C for 60 s and 72 °C for 60 s, with a final extension at 72 °C for 10 min. After validation of accomplished PCR amplification by 3% agarose gel electrophoresis, each PCR product was digested overnight with 5 units *Hph*I (recognizing the sequence 5′..GGTGA(N)$_8$↓..3′) restriction endonuclease enzyme at 37 °C (New England Biolabs Inc., Beverly, MA), and digested products were electrophoresed on 3% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under UV illumination. The digest fragments were 89 and 20 bp bands for the G allele. The T allele had 109 bp band since the recognizing site of HphI enzyme didn't occur when the T allele exist.

2.4. Statistical analysis

To obtain adequate sample size and 80% statistical power in the present study was calculated by Quanto 1.1 ver. software (http://hydra.usc.edu/gxe) using minor allele frequency data from HapMap (http://hapmap.ncbi.nlm.nih.gov/). Data analysis was performed using the computer software IBM Statistical Package for Social Sciences (SPSS; SPSS, Inc., Chicago, IL, USA) for Windows (version 20). Continuous variables are presented as the mean (standard deviation, SD) for normal distributions or median (min-max) for non-normal distributions, and categorical variables are presented as frequencies (%). Comparisons in the distributions of demographical characteristics of the patients with chronic HCV infection were evaluated using the Student's *t*-test or Mann-Whitney *U* test for continuous variables depending on their Gaussian distribution, and chi-square test for categorical variables. The observed genotype frequencies were compared with expected values calculated from Hardy–Weinberg equilibrium theory. Logistic regression analysis was used to analyses the association of genotypes in

Table 1
Distribution of selected characteristics in patients.

Variables	Total patients n = 144, (%)	Steatosis group n = 105, (%)	Non-Steatosis group n = 39, (%)	*P* value
Age[e]	57.52 ± 12.55	57.10 ± 11.30	58.68 ± 15.91	0.51[c]
Male (%)	51 (35.4)	35 (33.3)	16 (41.0)	0.39[b]
BMI (kg/m^2)[e]	26.71 ± 2.68	27.32 ± 2.04	24.88 ± 3.66	0.03[c]
Fibrosis F0–2/ F3–4	41/103	29/76	11/28	0.97[b]
ALT (U/L)[d]	35 (8–192)	40 (8–192)	28 (9–102)	0.07[a]
AST(U/L)[d]	34 (12–275)	35 (14–275)	33 (12–127)	0.68[a]
GGT(U/L)[d]	26 (7–426)	26(7–426)	26.5 (10–250)	0.66[a]
HGB (g/dl)[e]	12.89 ± 1.92	12.95 ± 1.89	12.72 ± 1.99	0.53[c]
HCT (%)[e]	38.39 ± 5.56	38.83 ± 5.52	37.29 ± 5.58	0.14[c]
Triglyceride (mg/dl)[d]	97 (27–357)	112.5 (30–357)	78.5 (27–180)	0.009[a]
T. cholesterol (mg/dL)[d]	160 (70–352)	168.5 (80–352)	143 (70–327)	0.021[a]
LDL (mg/dl)[d]	94 (32–248)	98 (40–245)	80 (32–248)	0.015[a]
HDL (mg/dl)[d]	42 (8–93)	42.5 (8–87)	41 (14–93)	0.67[a]
VLDL (mg/dl)[d]	19.8 (6–70)	22.5 (6–70)	16.3 (6–36)	0.021[a]
HCV RNA, log (IU/ml)[d]	6.24 (2.61–7.71)	6.23 (4.83–7.71)	6.30 (2.61–7.08)	0.67[a]

[a] P values were calculated by Mann-Whitney test.
[b] P values were calculated by chi-square test.
[c] P values were calculated by student *t*-test.
[d] Data were shown as median (min-max).
[e] Data were shown as mean ± SD.

inheritance models (co-dominant, dominant, recessive). Moreover, statistical analyses of genotypes were analyzed using the website for SNP Statistics: https://www.snpstats.net/start.htm?q=snpstats/start.htm. Results are expressed as odds ratios with 95% confidence interval (CI). All tests were two-sided and *P* value < .05 was considered significant.

3. Results

The clinical and demographic characteristics of the patients were shown in Table 1. All patients were Turkish and Caucasian in the present study. The most of the patients were female (64.6%). The patients' steatosis grades were as follows: grade 0, n = 39 (27.1%); grade 1, n = 66 (45.8%); grade 2, n = 27 (18.8%); grade 3, n = 12 (8.3%). 39 patients with grade 0 (27.1%) were classified as the group of non-steatosis, and 105 patients with grade 1–3 (72.9%) were classified as the group with steatosis. Fibrosis stages of the patients were as follows: F0, n = 2 (1.4%); F1, n = 20 (13.9%); F2, n = 18 (12.5%); F3, n = 75 (52.1%); F4, n = 29 (20.1%). Fibrosis stages were categorized into two groups (F0–2, n = 40 (27.8%); F3–4, n = 104 (72.2%)) to provide adequate statistical power. No significant correlation was found between the stages of fibrosis and grades of steatosis. Additionally, there was no significant difference between the non-steatosis group and the group with steatosis in terms of gender. There were statistically significant differences in the biochemical parameters including triglyceride, total cholesterol, LDL, VLDL levels between the two groups (Table 1). The patients with steatosis had higher triglyceride, total cholesterol, LDL and VLDL levels than the patients with non-steatosis (median values: 112.5 vs 78.5; 168.5 vs 143; 98 vs 80; 22.5 vs 16.3, respectively). Moreover, there was a significant association between these two groups for BMI. The patients with steatosis had higher BMI than the patients with non-steatosis (Table 1). We, also, evaluated the clinical and demographic characteristics of the patients according to *MTP* genotypes. *MTP* genotypes were categorized into two groups (GG wild type genotype versus combined TG + TT genotypes as mutant allele carrying genotypes) to provide adequate statistical power. *MTP* genotypes (GG vs. TG + TT) were not associated with BMI, fibrosis stages and the levels of biochemical parameters (Table 4).

3.1. The allele and genotype frequencies of MTP gene −493G/T polymorphism

The overall allelic frequencies of *MTP* gene −493G/T polymorphism were 74% and 26% for G and T, respectively. There was no significant association between the steatosis and the non-steatosis groups for allele frequencies (P = .317) (Table 2). In addition, the genotype frequencies of −493G/T polymorphism were not significantly different between these two groups (P = .49) (Table 2). Furthermore, when the patients were categorized with respect to gender, we didn't find any difference between the steatosis and the non-steatosis groups for the allele and genotype frequencies of the −493G/T polymorphism (Table 3). Additionally, the patients with and without steatosis frequencies were also in Hardy–Weinberg equilibrium and had no selection bias (P = .34 and P = .63, respectively).

3.2. The association between MTP genotypes and the risk of hepatic steatosis

To identify whether there was a statistically significant increased risk of steatosis in terms of the *MTP* gene −493G/T genotypes, we

Table 2
Allele and genotype frequencies of *MTP* polymorphism in steatosis and non-steatosis groups as well as the association with the risk of steatosis.

	Steatosis group n = 105, (%)	Non-Steatosis group n = 39, (%)	*P*-value	OR (95% CI)
Allele frequency				
G	152 (72.4)	61 (78.2)		1.00 (Reference)
T	58 (27.6)	17 (21.8)	0.32[a]	1.37 (0.74–2.54)
Co-dominant model				
GG	56 (53.3)	25 (64.1)		1.00 (Reference)
GT	40 (38.1)	11 (28.2)	0.22[b]	1.68 (0.73–3.85)
TT	9 (8.6)	3 (7.7)	0.64[b]	1.40 (0.35–5.64)
Dominant model				
GG	56 (53.3)	25 (64.1)		1.00 (Reference)
GT + TT	49 (46.7)	14 (35.9)	0.23[b]	1.61 (0.75–3.48)
Recessive model				
GG + GT	96 (91.4)	36 (92.3)		1.00 (Reference)
TT	9 (8.6)	3 (7.7)	0.82[b]	1.17 (0.30–4.61)
Overdominant model				
GG + TT	65 (61.9)	28 (71.8)		1.00 (Reference)
GT	40 (38.1)	11 (28.2)	0.24[b]	1.61 (0.71–3.65)

P values were adjusted for age and sex.
[a] P values were calculated by chi-square test.
[b] Data were calculated by logistic regression analysis.

Table 3
Allele and genotype frequencies of *MTP* polymorphism according to gender, as well as the association with the risk of steatosis.

Gender	Allele genotype	Steatosis group n, (%)	Non-Steatosis group n, (%)	*P* value	OR (95% CI)
Male		35 (68.4)	16 (31.4)		
	G	47 (67.1)	25 (78.1)		1.00 (Reference)
	T	23 (32.9)	7 (21.9)	0.26[a]	1.75 (0.66–4.64)
	GG	16 (45.7)	9 (56.2)		1.00 (Reference)
	GT + TT	19 (54.3)	7 (43.8)	0.49[b]	1.53 (0.46–5.02)
Female		70 (75.3)	23 (24.7)		
	G	105 (75)	36 (78.3)		1.00 (Reference)
	T	35 (25)	10 (21.7)	0.65[a]	1.20 (0.54–2.67)
	GG	40 (57.1)	16 (69.6)		1.00 (Reference)
	GT + TT	30 (42.9)	7 (30.4)	0.29[b]	1.71 (0.63–4.69)

MTP genotypes were categorized into two groups (GG wild type genotype versus combined TG + TT genotypes as mutant allele carrying genotypes) to provide adequate statistical power. P values were adjusted for age.
[a] P values were calculated by chi-square test.
[b] Data were calculated by logistic regression analysis.

Table 4
Association between *MTP* genotypes and selected characteristics of patients.

Variables	GG (wild type genotype)	GT + TT (carrying mutant T allele)	*P* value
BMI (kg/m^2)[e]	26.15 ± 3.12	27.46 ± 1.83	0.21[c]
Fibrosis F0–2/F3–4	19/62	21/42	0.19[b]
ALT (U/L)[d]	29.5 (8–164)	42.5 (11–192)	0.11[a]
AST (U/L)[d]	32 (12–275)	43 (14–122)	0.16[a]
GGT (U/L)[d]	26 (9–281)	27 (7–426)	0.87[a]
HGB (g/dl)[e]	12.87 ± 2.04	12.90 ± 1.76	0.94[c]
HCT (%)[e]	38.11 ± 5.66	38.78 ± 5.45	0.49[c]
Triglyceride (mg/dl)[d]	96 (27–357)	104 (30–272)	0.87[a]
Total cholesterol (mg/dL)[d]	172 (76–352)	150 (70–264)	0.08[a]
LDL (mg/dl)[d]	102 (32–248)	86 (36–195)	0.12[a]
HDL (mg/dl)[d]	43 (14–93)	41 (8–87)	0.42[a]
VLDL (mg/dl)[d]	19.60 (6–70)	20.80 (6–54)	0.95[a]
HCV RNA, log (IU/ml)[d]	6.25 (4.21–7.15)	6.24 (2.61–7.71)	0.72[a]

MTP genotypes were categorized into two groups (GG wild type genotype versus combined TG + TT genotypes as mutant allele carrying genotypes) to provide adequate statistical power.
[a] P values were calculated by Mann-Whitney test.
[b] P value were calculated by chi-square test.
[c] P values were calculated by student *t*-test.
[d] Data were shown as median (min-max).
[e] Data were shown as mean ± SD.

performed logistic regression analysis between the steatosis group and the non-steatosis group (Table 2). The patients carrying T allele or TT genotype had a higher risk of steatosis when compared with those carrying G allele or GG genotype, but it was not statistically significant (OR = 1.37; 95% 0.74–2.54, P = .32; OR = 1.61; 95% 0.75–3.48, P = .22, respectively) (Table 2). We also performed logistic regression analysis in the male and female groups for steatosis risk, although the T allele and combined GT + TT genotype increased the risk of steatosis, they were not statistically significant (OR = 1.75, P = .26 for T allele and OR = 1.53, P = .49 for GT + TT genotype in male; OR = 1.20, P = .65 for T allele and OR = 1.71, P = .29 for GT + TT genotype in female respectively) (Table 3).

4. Discussion

The present study explored that the effect of the *MTP* gene −493G/T polymorphism on the risk of hepatic steatosis in Turkish patients with chronic HCV genotype 1 infection. Some researchers revealed that the *MTP* gene −493G/T polymorphism was associated with hepatic steatosis in CHC infection which is especially genotype-3 HCV infection (Mirandola et al., 2009; Magri et al., 2017; Zampino et al., 2008). Moreover, these researchers also investigated the relation between the *MTP* -493G/T polymorphism and HCV genotype non-3 which was composed of genotype 1, 2, 4 and 5. However, they didn't investigate the association of *MTP* –493G/T polymorphism with just HCV genotype-1 (Mirandola et al., 2009; Magri et al., 2017; Zampino et al., 2008). Only one study investigated the association of *MTP* gene −493G/T polymorphism with the 93 patients infected with HCV genotype 1-related hepatic steatosis (Siqueira et al., 2012). That's why, in the current study, the MTP -493G/T polymorphism was selected as the candidate polymorphism due to its crucial role in HCV-related hepatic steatosis, and this study was performed to clarify the association of this polymorphism with HCV genotype 1.

In this study, the overall allele frequencies of *MTP* –493G/T polymorphism were found 74% and 26% for G and T alleles, respectively. According to the previous results, the frequencies of G and T alleles among the populations were distributed as 67.8% and 32.2% in Brazilian, 75% and 25% in Swedish, 76.3% and 23.7% in Japanese, 70.6% and 29.4% in Italians, 74.5% and 25.5% in French, 75% and 25% in Spanish, respectively (Magri et al., 2017; Karpe et al., 1998; Namikawa et al., 2004; Zampino et al., 2008; Bernard et al., 2000; García-García et al., 2005). Additionally, European HapMap Cohort study reported that G and T allele frequencies were in 76% and 24% for European population which has sample size 18,446 persons (https://www.ncbi.nlm.nih.gov/snp/rs1800591). The allele distributions reported in the populations are in agreement with results of the current study. In the present study, although the allele and genotypes of this polymorphism increased the risk of HCV-related hepatic steatosis, there were no statistically significant correlations in all genetic models. It was reported that the GG and GT genotypes of *MTP* polymorphism were associated with severe steatosis in type II diabetes and NASH (Bernard et al., 2000; Namikawa et al., 2004), but in the HCV infection, especially HCV genotype 3, patients carrying T allele had an increased risk of hepatic steatosis (Mirandola et al., 2009; Magri et al., 2017; Siqueira et al., 2012; Zampino et al., 2008). Moreover, the some of these researchers reported that T allele of this polymorphism was associated with severe steatosis in the total HCV cohort or in HCV genotype non-3 which was composed of genotype 1, 2, 4 and 5 (Mirandola et al., 2009; Siqueira et al., 2012). Conversely, the some studies reported that no association was found between hepatic steatosis and this polymorphism in the total HCV cohort or in HCV genotype non-3 which was composed of genotype 1, 2, 4 and 5 (Zampino et al., 2008; Magri et al., 2017; Petit et al., 2006). The current study was performed in the patients infected only with HCV genotype 1. There is only one study to compare with the results of current study. In that study, Siqueira et al. (2012) reported that there wasn't an association of MTP gene −493G/T polymorphism with the 93 patients infected with HCV genotype-1, but in total HCV cohort (93 HCV genotype 1 and 45 HCV genotype non-1) this

polymorphism was associated with hepatic steatosis. This finding on HCV genotype 1 is consistent with the result of the current study. It should be noted that the group of HCV genotype non-3 used in other studies, except for Siqueira et al. (2012), consisted of mixed HCV genotypes. Therefore, it is not an accurate to compare with result of the current study.

The present study analyzed whether there is a difference between the two groups in terms of biochemistry parameters. The patients with steatosis had higher triglyceride, total cholesterol, LDL, VLDL levels than the patients with non-steatosis. Contrary to the results of the present study, Siqueira et al. (2012) reported that total cholesterol and LDL levels had lower in steatosis group than those in non-steatosis group in the patients with HCV genotype 1. Moreover, Magri et al. (2017) showed that in the total HCV cohort, total cholesterol and LDL levels had lower in steatosis group. Furthermore, Mirandola et al. (2006) revealed that while the steatosis group with HCV genotype 3 had lower total cholesterol, HDL and LDL levels than those in non-steatosis group with HCV genotype 3, whereas in the patients with HCV genotype non-3, lipid parameters didn't differ.

The current research also analyzed the association of this polymorphism with HCV genotype 1-related hepatic steatosis by gender. Even though the allele and genotypes of this polymorphism an increased the risk of HCV-related hepatic steatosis, neither allele nor genotype frequency of this polymorphism was related to gender in HCV genotype 1-related hepatic steatosis (P = .26 OR = 1.75; P = .49 OR = 1.53 in males, P = .65 OR = 1.20; P = .29 OR = 1.71 in females). There is no any study which evaluating association between gender and this polymorphism, to compare with the current study in the HCV-related steatosis. However, in studies which are different from HCV studies, the authors reported that there was no significant difference between gender and MTP genotypes (García-García et al., 2005; Bernard et al., 2000). These results are similar to result presented in the current study. The present study also investigated relation to *MTP* polymorphism with lipid parameters, but not significant associations were found. Moreover, Zampino et al. (2008) reported that neither alleles nor genotypes of this polymorphism were associated with lipid parameters in the HCV genotype 3 and HCV genotype non-3. The results of this study were consistent with the results presented in current study. Contrarily, Mirandola et al. (2009) reported that the patients carrying TT genotype had higher HDL cholesterol levels than the G allele carriers, but others of lipid parameters were not significant by *MTP* polymorphism. Additionally, in studies which are different from HCV studies, while the T allele carriers had lower levels of triglyceride and VLDL cholesterol (García-García et al., 2005), whereas total cholesterol, LDL cholesterol, HDL cholesterol were not association with this polymorphism (García-García et al., 2005; Bernard et al., 2000; Namikawa et al., 2004). Moreover, Ledmyr et al. (2002) reported that individuals with TT genotype had lower the levels of LDL and total cholesterols in the healthy men. The results of all these studies are controversial.

Recently, HCV-related hepatic steatosis has been associated with the *MTP* gene −493G/T polymorphism for HCV genotype-3, but not related to HCV genotype 1. While the T allele of −493G/T polymorphism was determined to have increased the MTP transcription in vitro in healthy persons (Karpe et al., 1998), but hepatic MTP mRNA expression and MTP activity didn't differ according to genotypes of this polymorphism in CHC infection (Mirandola et al., 2009). On the contrary, Nakamuta et al. (2009) reported that the MTP expression level was higher in the total HCV cohort who was composed of genotype 1 and 2 compared to controls. In all these studies, the reasons for having different results which regarding steatosis and lipid levels relation to this polymorphism are not clear. Different populations, different diseases, gene-gene interactions and gene-environment interactions can cause changes in lipid metabolism.

The limitations of the current research: a) this study does not cover all HCV genotype 1 patients in the Turkish population, because this work was implemented around the Adana and neighboring provinces. b) It should be noted that the very rare occurrence of the TT genotype frequency in this study limited the statistical analysis by gender. That's why; more extensive studies with a larger sample size of gender are required in the patients with HCV genotype 1. c) This work has been limited with Turkish population owing to differences in allele distribution among different ethnic groups.

In conclusion, the results of current study demonstrate for the first time that *MTP* gene −493G/T polymorphism has not a major effect on the risk of HCV-related hepatic steatosis in the HCV genotype 1 infection in Turkish population. Considering only one study in HCV genotype 1 infection, further studies are imperative to clarify the association of this polymorphism with HCV genotype 1 infection in HCV-related hepatic steatosis.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Each author's contribution to the paper is equal. All authors are in agreement with the content of the manuscript.

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