



# Evaluation of Paraoxonase 1 Activities in Patients Infected with Hepatitis C Virus Genotype 1b and Genotype 4 in the Kayseri Province of Turkey

Türkiye'nin Kayseri Bölgesindeki HCV Genotip 1b ve Genotip 4 ile Enfekte Hastalarda Paraoksonaz 1 Aktivitesinin Değerlendirilmesi

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## ABSTRACT

**Objective:** Hepatitis C virus (HCV) infection is an important cause of chronic liver disease and is associated with oxidative stress. The type of treatment given for chronic HCV infection depends on the genotype of the virus. Genotype 1b is known to be associated with poor prognosis, although knowledge about genotype 4 is not sufficient. In this study, we aimed to compare serum paraoxonase (PON1) activities in HCV genotype 1b-infected patients with those in HCV genotype 4-infected ones, and the relationship of PON1 activity with hepatic damage demonstrated as liver fibrosis.

**Materials and Methods:** In this study, we included 49 patients with HCV genotype 1b and 42 with genotype 4. Serum PON1 activity was analyzed by spectrophotometric method. Serum triglycerides, total cholesterol, LDL, HDL, and transaminases levels and liver histological activity index (HAL) and fibrosis scores were evaluated retrospectively.

**Results:** Serum PON1 activity was significantly lower in genotype 1b compared to that in genotype 4 (147.6±73.9 U/L and 192.6±99.0 U/L, respectively; p=0.015). There was no significant correlation between PON1 activity and HCV RNA viral load and fibrosis scores (p>0.05). However, there was no difference in the other serum parameters between the two genotypes (p>0.05).

**Conclusion:** Antioxidant enzyme PON1 activity is lower in genotype 1b compared to that in genotype 4. This may affect response to therapy and prognosis. Further studies with larger samples are needed to understand the association between PON1 activity and HCV genotypes to elucidate if PON1 activity could be a prognostic factor.

**Key Words:** PON1, HCV, genotype 1b, genotype 4

**Conflict of interest:** The authors reported no conflict of interest related to this article.

## ÖZET

**Amaç:** Hepatit C virüs (HCV) kronik karaciğer hastalıklarının önemli bir nedenidir ve oksidatif stres ile ilişkilidir. Kronik hepatit C enfeksiyonunda uygulanacak tedavi kişinin enfekte olduğu HCV genotipine bağlıdır. Genotip 1b'de prognozun kötü olduğu bilinmekle birlikte genotip 4 için yeterli veri bulunmamaktadır. Bu çalışmada, genotip 1b ve genotip 4 HCV'li hastalarda serum paraoksonaz 1 (PON1) aktivitesinin karşılaştırılması ve karaciğer fibrozisi ile gösterilen hepatik hasar ile ilişkisinin araştırılması amaçlanmıştır.

**Gereç ve Yöntemler:** Bu çalışmaya, 49 HCV genotip 1b ve 42 HCV genotip 4 ile enfekte hasta dahil edilmiştir. Serum PON1 aktivitesinin ölçümü spektrofotometrik olarak yapılmıştır. Serum trigliserid, total kolesterol, LDL, HDL, serum transaminaz ve karaciğer histolojik aktivite indeksi (HAL) ve fibrosis skorları retrospektif olarak değerlendirilmiştir.

**Bulgular:** Serum PON1 aktivitesi, genotip 1b ile enfekte hastalarda (147,6±73,9U/L) genotip 4 ile enfekte hastalara (192,6±99,0 U/L) göre daha anlamlı olarak düşük bulunmuştur (p=0,015). Serum PON1 aktivitesi ile HCV RNA viral yük ve fibrosis skorları arasında anlamlı korelasyon bulunmamıştır (p>0,05). Her iki genotipin diğer serum parametreleri arasında istatistiksel olarak anlamlı bir fark olmadığı gösterilmiştir (p>0,05).

**Sonuç:** Antioksidan enzim olan PON1 aktivitesi, HCV genotip 1b ile enfekte hastalarda HCV genotip 4 ile enfekte hastalara göre daha düşük olarak saptanmıştır. Bu durum prognozu ve tedaviye cevabı etkileyebilir. PON1 prognostik bir faktör olarak kullanılacaksa HCV genotipleri ile arasındaki ilişkiyi incelemek için daha geniş bir hasta popülasyonunda yapılmış ileri çalışmalara ihtiyaç vardır.

**Anahtar Kelimeler:** PON1, HCV, genotip 1b, genotip 4

**Çıkar çatışması:** Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemişlerdir.

## Introduction

Hepatitis C virus (HCV) infection is a serious infectious condition that may cause chronic liver disease, cirrhosis, and hepatocellular carcinoma in Turkey and in the world, besides, it is associated with oxidative stress (1). Viral and host factors are responsible for the progression of hepatic damage in HCV infection. The various genotypes and subtypes of HCV have been associated with different geographical spread patterns. HCV genotyping is of crucial importance in the determination of the treatment protocols and the follow-up of the clinical course since treatment success rate is low and the duration of treatment is longer in HCV genotype 1-infected patients (2). HCV genotype 1b is the most common type in the Central Anatolian Region in Turkey. Indeed, the prevalence of genotype 4 in Kayseri, a city in the Central Anatolia, is higher than the national HCV genotype distribution (3).

In recent years, an imbalance between oxidant production and antioxidant capacity was determined to be implicated in cell destruction (1). HCV is capable of producing high oxidative stress within hepatocytes and also, reactive oxygen species (ROS)-mediated liver injury may be triggered by lipid peroxidation (4,5). Paraoxonase-1 (PON1) which is synthesized mainly by the liver is found in the circulation associated with high-density lipoproteins (HDLs) (6). PON1 protects low-density lipoprotein against oxidative modification and prevent LDL oxidation. Due to hydrolyzing lipid function of peroxides in oxidised lipoproteins, human PON1 is recognized as an anti-oxidant enzyme. This function of PON1 can be useful for monitoring of chronic hepatitis disease (1,7,8). In recent studies, it has been reported that serum PON1 may be significantly reduced in patients with chronic liver disease (7, 9,10,11,12).

In this study, we aimed to compare serum PON1 activities in HCV patients with genotype 1b with those in patients with genotype 4, and the relationship of PON1 activity with hepatic damage demonstrated as liver fibrosis and histological activity index (HAI) scores in fine needle biopsy materials.

## Materials and Methods

### Patients

Hepatitis C virus genotypes were retrospectively evaluated in a total of 91 patients (57 female, 34 male) with chronic hepatitis C admitted to our hospital. Gender, age, serum triglycerides, total cholesterol, LDL and HDL cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels, HCV RNA viral load, HAI score and stage of liver fibrosis in all patients were determined. Patients who were admitted our laboratory for analysis of HCV genotypes for determining the treatment modality and those who did not receive any treatment were selected for this study. Biochemical parameters and HCV RNA titers were studied on samples drawn on the same day with the liver biopsy. The serum was then separated from blood cells by centrifugation and stored at -80 °C until PON1 analysis. Forty controls were also analyzed for serum PON1 activity.

Forty healthy subjects were included as control group. All control subjects were HCV RNA-negative and healthy and were evaluated for any symptoms of clinical evidence of chronic diseases (diabetes mellitus, renal disease, cardiovascular disease). None of the control subjects smoked or used alcohol.

### Quantification of HCV RNA

HCV RNA levels were determined by a commercial real time polymerase chain reaction system, the COBAS® AmpliPrep/COBAS® TaqMan® 48 HCV test (ROCHE).

### HCV Genotyping

All HCV RNA-positive samples were subjected to HCV genotyping by pyrosequencing methods. HCV RNA-positive samples were sequenced by PyroMarkQ24 assay (Qiagen, Germany) following the manufacturer's instructions.

### Histopathological Evaluation

The stage of fibrosis was assessed according to the METAVIR scoring system. Liver fat scores (LFSs) were defined as follows: score for fibrosis (F): F0-no fibrosis; F1-portal fibrosis without septa; F2-portal fibrosis with rare septa; F3-numerous septa without cirrhosis; and F4-cirrhosis.

### Measurement of Paraoxonase

PON1 assays were performed without additional NaCl. PON1 was measured in serum samples collected from the patients. PON1 activity was measured by a fully automated paraoxonase activity measurement kit (Rel Assay Diagnostics® Kit, Turkey). Linear increase of the absorbance of p-nitrophenol, produced from paraoxon, was followed at kinetic measurement mode. Nonenzymatic hydrolysis of paraoxon was subtracted from the total rate of hydrolysis. The molar absorptivity of p-nitrophenol is 18.290 M<sup>-1</sup>cm<sup>-1</sup> and one unit of paraoxonase activity is equal to 1 μmol of paraoxon hydrolyzed per liter per minute at 37 °C. The reaction was initiated at 37 °C by the addition of the substrate solution, and using a spectrophotometer (Readwell, India), absorbance was continuously monitored at 405 nm.

### Statistical Analysis

Statistical analyses were performed using SPSS 21.0 (SPSS Inc., Chicago, IL, USA) with p<0.05 as the probability level. The Kolmogorov-Smirnov test was used evaluating the histograms to assess the normality of the data. Values were expressed as frequencies, mean±standard deviation according to the type variance and normality. Comparisons of patients and control groups were computed by one-way ANOVA. To compare the differences between genotype 1b and genotype 4 for HAI, fibrosis scores and HCV RNA, independent two-sample t-test was performed. In addition, Pearson's correlation analysis was performed for any correlation between PON1 activity and HCV RNA viral load and fibrosis scores.

## Results

The mean PON1 activity in control group (n=40) was 194.5±36.9 and in HCV patients (n=91) was 168.8±89.5 (p=0.028). PON1 activities and demographic data of patients and control group are shown in Table 1. There was no significant difference in total cholesterol, LDL and HDL cholesterol levels between the two

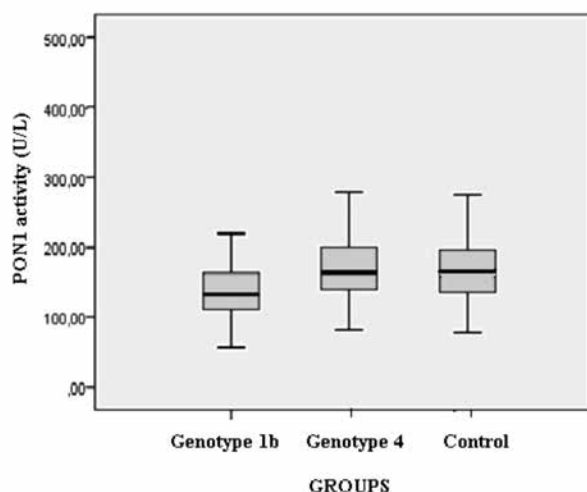
genotypes and control group ( $p>0.05$ ). Besides, no significant difference was observed between two genotypes in HAI, fibrosis scores and HCV RNA viral load. Serum transaminase levels in patient groups were significantly higher than in control group ( $p<0.05$ ). However, serum PON1 activity was lower in genotype 1b compared to that in genotype 4 and control groups ( $147.6\pm 73.9$

U/L,  $192.6\pm 99.0$  U/L and  $194.5\pm 36.9$ , respectively;  $p=0.015$ ). There was no correlation between PON1 activity and liver enzymes, HCV RNA viral load and fibrosis scores ( $p>0.05$ ).

## Discussion

HCV is capable of producing high oxidative stress in hepatocytes. The defense against oxidative injury is made through antioxidant pathways. Since the antioxidant activity of PON1, which is originated from the liver, was found to be reduced in chronic liver damage, it may be particularly meaningful as an index of liver function status (7,9). It has been previously reported that oxidative stress is increased in patients with chronic liver disease (7,10,11,12).

The most prevalent two HCV genotypes in Kayseri Province in Turkey are 1b and 4 (64.7% and 28.3%, respectively) (3). Genotype 1b is known to have poor prognosis, although knowledge about genotype 4 is not sufficient. Since genotype 4 is the second most common HCV genotype and knowledge about its prognosis is not enough, we compared the antioxidant enzyme levels, serum lipids, liver function tests and fibrosis scores in patients with genotype 4 with those in patients with genotype 1b, the most common HCV genotypes in our region. Therapy in patients infected with HCV depends on the genotype of the virus. Prognosis and life expectancy in patients with chronic HCV infection depends on how much the liver is damaged and how well a person responds to treatment. Hepatic damage may be due to a direct viral cytopathic effect or an immune-mediated process in patients infected with



**Figure 1.** Comparison of serum PON1 activities of genotype 1b, 4 and control group. Genotype 1b is different from genotype 4 and control group ( $p=0.015$ )

<b>Table 1.</b> PON1 activities and general characteristics of patients with genotype 1b and genotype 4 and control group			
	<b>Genotype 1b (n=49)</b> (mean $\pm$ SD)	<b>Genotype 4 (n=42)</b> (mean $\pm$ SD)	<b>Control (n=40)</b> (mean $\pm$ SD)
Age (year)	60.3 $\pm$ 7.9	63.1 $\pm$ 8.2	59.7 $\pm$ 8.3
Gender (Female/Male)	32/17	25/17	21/19
PON1 activity (U/L)	147.6 $\pm$ 73.9 a	192.6 $\pm$ 99.0	194.5 $\pm$ 36.9
*HAI	7.42 $\pm$ 1.8	7.17 $\pm$ 1.8	-
*Fibrosis	3.3 $\pm$ 1.5	2.9 $\pm$ 1.4	-
Total cholesterol (mg/dL)	156.5 $\pm$ 27.1	158.4 $\pm$ 26.8	158.5 $\pm$ 24.1
LDL cholesterol (mg/dL)	81.7 $\pm$ 20.8	85.5 $\pm$ 26.4	86.8 $\pm$ 18.1
HDL cholesterol (mg/dL)	44.9 $\pm$ 12.7	52.4 $\pm$ 16.3	50.5 $\pm$ 15.1
Triglyceride (mg/dL)	129.8 $\pm$ 62.3	102.5 $\pm$ 31.6	105.2 $\pm$ 28.1
AST (IU/mL)	55.0 $\pm$ 27.0	47.2 $\pm$ 27.6	23.5 $\pm$ 6.6 b
ALT (IU/mL)	59.7 $\pm$ 36.4	45.5 $\pm$ 31.2	25.9 $\pm$ 7.1 c
*HCV RNA (IU/mL)	2.99 $\pm$ 86.2 $\times$ 10 <sup>6</sup>	4.41 $\pm$ 95.5 $\times$ 10 <sup>6</sup>	-

HAI: Liver histological activity index, LDL: Low density lipoprotein, HDL: High density lipoprotein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, p: Differences of three groups were computed by one way ANOVA test.

\*: Differences between Genotype 1b and Genotype 4 for HAI, Fibrosis and HCV RNA were performed by independent two-sample t test and no significant difference was observed between groups ( $p>0.05$ )

a: Genotype 1b is different from Genotype 4 and control ( $p<0.05$ ), b: Control is different from Genotype 1b and Genotype 4 ( $p<0.05$ ), c: Control is different from Genotype 1b and Genotype 4 ( $p<0.05$ )

chronic hepatitis C. Since patients with low levels of fibrosis respond better to treatment, liver biopsy is of crucial importance in the determination of the treatment modality and response to treatment in patients with chronic hepatitis C. Besides, HCV genotype is considered to be an independent factor affecting the response to interferon therapy (13).

Previously, several studies have reported decreased PON1 activity in infected patients with chronic hepatitis C (7,10,11,12). Ferre et al. (14) investigated PON1 activity and its correlation with oxidative stress, fibrosis and soluble fatty acid synthase in patients with chronic liver diseases. They have suggested an active role of PON1 in the regulation of oxidative stress, fibrosis and hepatic cell apoptosis in chronic liver diseases. There are two theories explaining this situation: Firstly, the decrement in PON1 activity may be the result of hepatic dysfunction. In the present study, we have found that antioxidant enzyme PON1 activity was lower in genotype 1b compared to that in genotype 4. However, HAI, fibrosis scores, liver function tests in the two genotypes were similar. Therefore, we can conclude that PON1 activity is lower in genotype 1b independently from hepatic damage. The other theory was; PON1 could be decreased as a consequence of changed synthesis and/or secretion of HDL (7). PON1 is a structural enzyme of HDL. In a study, Dirican et al. (15) reported that a reduction in HDL may lower PON1 activity. In this study, although HDL levels were mildly lower in genotype 1b than in genotype 4, there was no statistically significant difference between the two genotypes. Thus, the difference between PON1 activities cannot be explained with the degree of fibrosis or HDL levels.

The standard liver function tests (serum ALT, AST levels) are recognized as markers reflecting the degree of the histological damage and they serve as parameters for starting therapy or judging response to antiviral treatment in chronic hepatitis C. However, most studies show conflicting results about the relationship of the degree of histological damage, and serum ALT and AST levels with HCV RNA viral load in chronic hepatitis C (15,16,17,18). In genotype 1b and 4, we also could not find any correlation between liver enzyme levels and viral load. In this study, there was no difference in serum AST and ALT levels between the genotypes.

There are some limitations in our study. It was a retrospective study and some clinical data could not be obtained.

We found that antioxidant enzyme PON1 activity was lower in genotype 1b compared to that in genotype 4, despite similar lipid profile, serum transaminase levels, and HAI and fibrosis scores. Further studies in larger samples are needed to understand the association of PON1 activity with other antioxidants and HCV genotypes and whether it could be a prognostic factor.

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