Changes in serum levels of cytokeratin-18 fragments in patients with chronic hepatitis C under antiviral therapy

Kronik hepatit C hastalarında antiviral tedavi ile serum sitokeratin-18 düzeylerinin değişimi

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Background and Aims: *Cytokeratin-18 is the known substrate for caspases, which are encountered during hepatic and pancreatic acinar apoptosis. Studies performed in recent years have indicated that the cleavage level of serum cytokeratin-18 (M30 antigen) is correlated with hepatic fibrosis and disease severity in both chronic hepatitis C and non-alcoholic steatohepatitis. It was shown that antiviral therapy in chronic viral hepatitis C patients significantly reduced hepatocellular apoptosis and cytokeratin-18 is accepted as a reliable marker of hepatocyte apoptosis. Our aim was to determine the correlation between the cytokeratin-18 level and treatment response in patients with chronic viral hepatitis C.* ***Materials and Methods:*** *Sixty patients with chronic viral hepatitis C were included in the study. A 48-week course of peginterferon-ribavirin therapy was given to appropriate patients. Hepatitis C virus RNA was measured at 0, 12, and 24 weeks at the end of therapy and 72 weeks. In addition, cytokeratin-18 levels were measured at 0, 24, and 72 weeks.* ***Results:*** *The mean age of 60 patients was 52±10.9 years. While 31 (51.6%) of patients were in the sustained viral response group, 29 (8.4%) of patients were in the non-sustained viral response group. It was determined that while the cytokeratin-18 level at week 0 in the sustained viral response group was 243±21, the cytokeratin-18 level at week 24 was 115±12 U/L and the difference between the level of cytokeratin-18 at weeks 0 and 24 were 127±209 U/L (p: .014). While the cytokeratin-18 level at week 0 in the non- sustained viral response group was 270±14; at week 24, the cytokeratin-18 level was 136±19 U/L and the difference between cytokeratin-18 levels at weeks 0 and 24 was 136±156 U/L (p > .5). At week 72, the cytokeratin-18 level in the sustained viral response group was 109±38 and the difference between weeks 0 and 72 was 134±215 (p < .002).* ***Conclusion:*** *In chronic viral hepatitis C patients, there was a correlation between sustained viral response and cytokeratin-18, which is a marker of apoptosis. During treatment, it was found that there was a relationship between sustained viral response and the decrease in cytokeratin-18 levels. This finding indicates that cytokeratin-18 level monitoring may be used as a predictive marker of sustained viral response.*

Key words: *Chronic hepatitis C, cytokeratin-18, sustained viral response*

Giriş ve Amaç: *Sitokeratin-18, hepatik ve pankreatit asiner apopitozisi sırasında ortaya çıkan kaspazların bilinen substuratıdır. Son zamanlarda yapılan çalışmalarda; serum sitokeratin-18 (M30 antijen) düzeyi ile non-alkolik steatohepatit ve kronik hepatit C'nin şiddeti ve hepatik fibrozis ile korelasyonu belirtilmiştir. Kronik viral hepatit C hastalarında, başarılı antiviral tedavi ile hepatosellüler apopitozisin anlamlı olarak azaldığı, hepatosit apopitozisini göstermede sitokeratin-18'in güvenilir bir markır olduğu gösterilmiştir. Bizim amacımız kronik viral hepatit C'li hastalarda sitokeratin-18 düzeyi ve tedavi yanıtı arasındaki korelasyonu belirlemektir.* ***Gereç ve Yöntem:*** *Kronik viral hepatit C tanısı alan 60 hasta çalışmaya alındı. Tedavi için uygun hastalara 48 hafta peginterferon-ribavirin tedavisi verildi. Tedavinin 0-12-24. haftalarında, tedavinin sonunda ve 72. haftada hepatitis C virusu RNA miktarı ölçüldü. Ayrıca tedavinin 0-24 ve 72. haftasında sitokeratin-18 düzeyleri ölçüldü.* ***Bulgular:*** *Altmış hastanın ortalama yaşı 52±10.9 yıl idi. Hastaların 31'i (%51,6) kalıcı viral yanıt grubunda iken, 29'u (%48,4) kalıcı viral yanıt elde edilemeyen grupta idi. Kalıcı viral yanıt grubunda 0. haftada sitokeratin-18 düzeyi 243±21 U/L iken, 24. haftada sitokeratin-18 düzeyi 115±12 U/L saptanmış olup 0. hafta ile 24. hafta arasındaki değişim 127±209 U/L bulunmuştur (p: 0.014). Kalıcı viral yanıt elde edilemeyen grupta 0. haftada sitokeratin-18 düzeyi 270±14 U/L iken, 24. haftada sitokeratin-18 düzeyi 133±19 U/L saptanmış olup 0. hafta ile 24. hafta arasındaki değişim 136±156 U/L bulunmuştur (p>0,5). Kalıcı viral yanıt grubunda, 72. haftada sitokeratin-18 düzeyi 109±38 saptanmış olup 0. hafta ile 72. hafta arasındaki değişim 134±215 bulunmuştur (p<0.002).* ***Sonuç:*** *Apopitozisin göstergesi olan sitokeratin-18 ile kronik viral hepatit C hastalarında kalıcı viral yanıt arasında korelasyon tespit edildi ve tedavi seyrinde sitokeratin-18 düzeyinde azalma ile kalıcı viral yanıt arasında ilişki bulunduğu gösterildi. Bu da sitokeratin-18 düzeylerinde azalmanın kalıcı viral yanıtı ön görmede bir parametre olarak kullanılabileceğini göstermektedir.*

Anahtar kelimeler: *Kronik viral hepatit C, sitokeratin-18, kalıcı viral yanıt*

INTRODUCTION

Chronic HCV (CHC) infection is one of the major causes of severe liver diseases like chronic hepatitis, cirrhosis, and hepatocellular carcinoma. HCV infection, which affects approximately 3% of the world's population, is a widespread and serious health problem. Pegylated-interferon (Peg-IFN) α2a and 2b in combination with ribavirin are administered as the standard therapy for CHC. Treatment success rates with this therapy are 50%–60%

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among genotype 1 patients, and 80%–90% among genotypes 2 and 3 patients (1,2). Genotype 1, male gender, high viral load, age over 40 years, obese, immunosuppressive conditions, alcoholism, and advanced stage fibrosis confirmed by liver biopsy may be listed among factors that unfavorably affect treatment response to CHC (2,3).

Studies performed in recent years showed that cellular death, which occurred in chronic viral hepatitis C disease, was a significant determinant of clinical disease progression. There are two types of cell death in hepatocytes: apoptosis and necrosis. Apoptosis is programmed cell death with both morphological and biochemical changes, which is mediated by caspase enzymes following the apoptotic stimulus. In different pathophysiological conditions, the equilibrium between cellular proliferation and cellular death can deteriorate, and apoptotic pathways may be activated. Cytokeratin-18 (CK-18) is a major fibrillary protein and is present in glandular epithelial tissue like the pancreas, liver, biliary duct, and intestines. CK-18 is also the known substrate of caspases (aspartate-specific cysteine proteases), which is revealed during hepatic and pancreatic acinar apoptosis (3, 4). It is released with cytokeratins 8 and 18 by hepatocyte cells (5). CK-18 is the major cytoplasmic intermediate filament protein of hepatocytes and is specifically fragmented during apoptosis. Recently performed studies have revealed that the serum cleavage level of CK-18 (M30 antigen) by apoptotic caspases (caspases 3,6,7) might significantly reflect liver damage in chronic hepatitis C and non-alcoholic steatohepatitis (NASH), and it was correlated with both hepatic fibrosis and disease severity (6). Over the years, it has been shown that there was not only a close relationship between apoptosis level and treatment responses of chronic viral hepatitis B and C but a significant correlation between decreases in CK-18 levels during both treatment and sustained viral response (SVR) (7-9).

Our aim in this present study was to reveal the relationship between CK-18 level, an indicator of apoptosis, and treatment responses of patients with chronic viral hepatitis C; and to show whether CK-18 level will be a predictive parameter for treatment response in the future.

MATERIALS and METHODS

In this present study, 60 patients, who were diagnosed with chronic viral hepatitis C in the Gastroenterology Clinic of Izmir Atatürk Training and Research Hospital between December 2009 and December 2010, and had HCV-RNA positivity longer than 6 months, were enrolled. Patients, who were pregnant or lactating; had known allergy against drugs; had decompensated liver disease, severe coronary artery or cerebrovascular or neuropsychiatric diseases, renal failure, solid organ transplantation history, and alcohol and/or narcotic drug abuse, were excluded from the study. Before initiation of treatment, patients provided peripheral venous blood samples for HCV genotyping, HCV-RNA tests, CBC, CRP, AST, ALT, ALP, GGT, total bilirubin, INR, thyroid function tests, and antibodies. Liver biopsy was performed on patients, and samples were evaluated according to the modified histological activity index (ISHAK).

Eligible patients who fulfilled treatment criteria received the following: genotype 1 patients received 180 µg/week dose of PEG-interferon alpha-2a or 1.5 µg/kg/week dose of PEG-interferon alpha-2b for 48 weeks with 1000 mg/day ribavirin for patients <75 kg or 1200 mg/day for patients >75 kg; genotype 2 patients received 180 µg/week dose of PEG-interferon alpha-2a for 24 weeks or 1.5 µg/kg/week dose of PEG-interferon alpha-2b with 800 mg/day dose of ribavirin.

Laboratory tests were repeated in each month after the treatment initiation and at the end of treatment. HCV RNA was measured in Weeks 4, 12, and 24, at the end of treatment, and 24 months after treatment ended by the enzyme-linked immunosorbent assay (ELISA) meth-

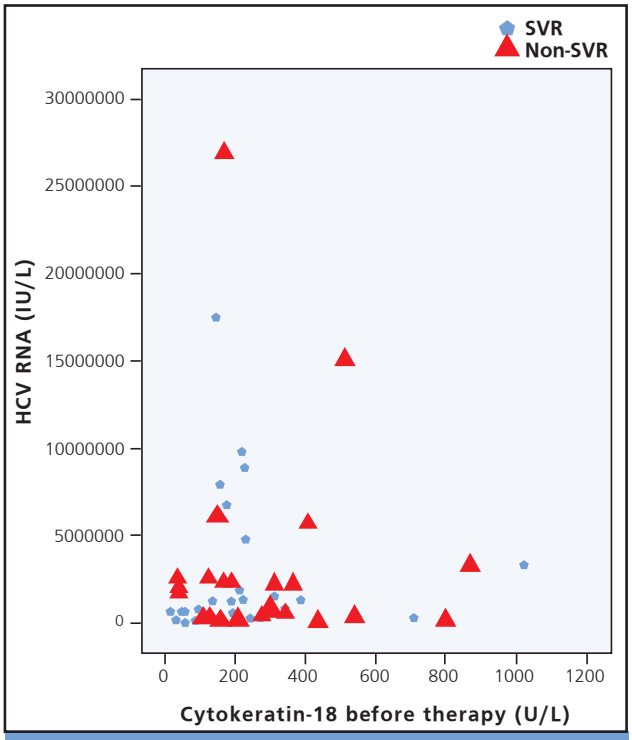

Figure 1. Distributions of CK-18 and HCV-RNA before treatment.

SVR: Sustained viral response. Non-SVR: Non-sustained viral response.

od. Therefore, rapid viral responses, early viral responses, end of treatment responses, and SVRs of patients were defined. Patients, who had no detectable HCV RNA 24 weeks after the end of treatment, were defined as SVR group; who had less than a 2 log decrease in Week 12 of treatment or had detectable HCV RNA levels in Week 24 of treatment, were defined as the nonresponder group; and who had positive HCV RNA 6 to 12 months after the end of treatment were defined as recurrences. Nonresponder and recurrent patients were defined as patients without sustained viral response (non-SVR patients). Additionally, serum CK-18 levels were measured in Weeks 0, 4, 12, 24, and 72 of treatment by M30-ELISA (Peviva) kits, which used multiwell plates with cells reactive for 96 tests and were developed for quantitatively specific and sensitive measurement of caspase-cleaved cytokeratin 18 (CK18Asp396-NE: M30 neo-epitope).

Statistical Analysis

Data were evaluated using SPSS for Windows 15.0 program. For comparisons of two independent groups, an independent t-test and Mann–Whitney U test were used, whereas the ANOVA method was used to compare more than two independent groups. Correlations between continuous variables were analyzed by correlation analysis, whereas correlations between categorical variables were tested by the Chi-square test.

RESULTS

The mean age of the 60 patients was 52 ± 10.9 years; 25 (41.6%) were males, and 35 (58.4%) were females. Of patients, 31 (51.6%) were sustained viral responders (SVR), and 29 (48.4%) were in the group without sustained viral response (non-SVR); and no significant difference was detected in age, gender, body mass index (BMI), HAI, and modified ISHAK scores, ALT values, and CK-18 levels in Week 0 between these two groups. There were statistically significant differences in baseline HCV-RNA and HOMA-IR values of groups (P = .038/.025). Differences in both laboratory and demographic characteristics of the two groups are given in Table 1. There was no correlation between baseline viral loads and CK-18 levels of patients (Figure 1).

Table 1. Laboratory and demographic characteristics of patient population

	SVR	Non-SVR	P
Age (years)	51.4±11.1	52.5±10.7	NS
Gender (male/female)	12/19	13/16	NS
BMI (kg/m²)	26.4±4.2	27.4±5.0	NS
HCVRNA (IU/ml)	2645855±5024572	2703832±4683512	.038
HAI	5.1±1.9	5.2±1.7	NS
Fibrosis-Stage	2.4±1.9	2.8±1.7	NS
ALT	65±38	54±27	NS
HOMA-IR	3.9±3.6	7.7±7.1	.025
Ferritin	94±138	227±327	NS
CK18 in Week 0	243±214	270±143	NS

SVR: Sustained viral response, Non-SVR: Non-sustained viral response, BMI: Body mass index, HAI: Histological activity index, ALT: Alanine aminotransferase, HOMA-IR: Homeostatic model assessment for insulin resistance.

Table 2. CK-18 levels and correlation between CK-18 levels and treatment responses in SVR and non-SVR patients

	SVR	Non-SVR	P
M30-WEEK 0	243±21	270±14	NS
M30-WEEK 24	116±12	134±23	<.001
M30-Difference b/w WEEKS 0&24	127±20	136±15	
P	**.014**	NS	
M30-WEEK 72	109±38	251±43	**.017**
M30- Difference b/w WEEKS 0&72	134±21	115±10	
P	<.002	.049	

SVR: Sustained viral response, Non-SVR: Non-Sustained viral response.

At week 0, the CK level in the SVR group was 243±21 whereas it was 270±143 in the non-SVR group. This difference between week 0 CK levels was not statically significant (p > .5). At week 24, whereas the CK-18 level in SVR group was 116±12.4, the CK-18 level in the non-SVR group was 134 ± 23. The difference between these levels at week 24 was statically significant (p < .001). At week 72, CK-18 level was 109±37.0 in the SVR group. While at week 0, the CK-18 level in SVR group was 243±21, at week 24 it was 116±12, and the difference was 127±20. This difference was statically significant (p: .014).

While in non-SVR patients the CK-18 level was 270±14 at week 0, 134±23 at week 24, and the difference between weeks 0 and 24 was 136±156. This difference was not statically significant (p > .5).

While in SVR patients the CK-18 level was 243±21 at week 0, it was 109±38 at week 72, and the difference between weeks 0 and 72 was 134±21. This difference was statically significant (p < .002). The relationship between the CK-18 level and treatment response in SVR and non-SVR patients is shown in Table 2.

DISCUSSION

Infection with CHC is one of the leading causes of severe liver diseases, like chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC). Hepatocellular apoptosis plays a major role in the pathogenesis of chronic hepatitis C. Compensatory regeneration and inflammation are initiated in hepatocytes, which are progressing to programmed cellular death. During apoptosis, intracellular cysteine caspases are activated, and specific degeneration products are formed. Cytokeratins are secreted from apoptotic or proliferating cells. Cytokeratin 18 (CK-18) is the major intermediate filament protein in hepatocytes. During apoptosis, CK-18 is cleaved at aspartate 238 and aspartate 396 by caspases. The M30 monoclonal antibody specifically recognizes the cleaved fragment of CK-18 at aspartate 396 (M30 antigen). Therefore, CKs can be used as apoptotic indicators (10). CK-18 is cleaved at a special position for apoptosis so that it will form a new epitope formation in cells progressing to apoptosis. The new antigen formed is called the caspase-cleaved CK-18 or M30 antigen. M30 antigens can be secreted from apoptotic cells into serum and can be easily measured by ELISA. It has been observed that the M30 antigen is an important prognostic marker in cancer patients. Because the M30 antigen can be measured in the serum, it seems to be a promising appropriate biomarker especially for follow up of diseases of epithelial origin (epithelial cancers) in routine clinical practice (11). Total CK-18 levels are increased in patients with chronic liver diseases and NASH in correlation with hepatic inflammation and steatosis (6-9,12).

The correlation between hepatic fibrosis and apoptotic CK-18 level in the circulation has been demonstrated. In patients with successful HCV clearance, both disease progression and fibrosis related to a significant decrease of CK-18 in the circulation during hepatocellular apoptosis are decreased (5-7).

Our aim in this present study was to reveal the relationship between CK-18 levels as an apoptosis marker and both SVR and non-SVR in patients with chronic viral hepatitis and to define whether CK-18 levels would be not only a marker but also an indicator for the responsiveness to chronic viral hepatitis C treatment in the future. Levels of CK-18 were defined in blood samples of patients drawn in Weeks 0, 24, and 72 of the treatment. When differences in CK-18 levels were evaluated between treatment weeks of 0, 24, and 72 in SVR patients, and differences in CK-18 levels were evaluated between treatment weeks of 0 and 24 in non-SVR patients; the difference between Weeks 0 and 24 in SVR patients was statistically significant (P = .014). When the difference between Weeks 0 and 72 was evaluated in SVR patients, it was defined as significant (P < .002). In non-SVR patients, the difference between Weeks 0 and 24 was not significant (P > .5). Even though the level of CK-18 in the SVR and the non-SVR group was similar at first, the distribution was not homogeneous, and CK-18 levels in the non-SVR group were much higher initially. This increase was associated with cirrhotic patients who were more typically found in the non-SVR group even though there was no significant difference in HAİ and fibrosis scores between the two groups. Bantel et al. showed that CK-18 level was associated with higher stages of fibrosis and a significant correlation between CK-18 levels and liver damage in 59 patients with chronic viral hepatitis C (5).

Sgier et al. reported significant decreases in CK-18 and ALT levels in SVR patients among 315 patients with chronic viral hepatitis C following treatment. This observed decrease was not significant in patients with relapse or without response. A decrease in hepatocellular apoptosis caused a significant decrease in CK-18 level in the circulation during hepatocellular apoptosis after HCV clearance by successful antiviral treatment. Also in the same study, no significant correlation was detected between HCV RNA and CK-18 level (8). Volkmann et al. reported in their study performed in 23 patients with chronic viral hepatitis C that CK-18 levels were significantly decreased in patients who responded to antiviral

treatment when compared with non-responsive patients or relapsing patients (13). Similarly, a significant decrease in CK-18 level was detected in SVR patients in our study (P < .002). However, no significant correlation was found with CK-18 levels in relapsing and non-responsive patients (P > .5). Sgier et al. showed that CK-18 levels were high in relapsing patients (8). Kronenberg et al. reported that a high basal apoptosis level was correlated with the slow decrease in HCV RNA level in their 19-patient study (6). Parfieniuk-Kowerda et al. reported that elevated serum M30-CK-18, an indicator of enhanced apoptosis of hepatocytes, parallels active hepatic inflammation and fibrosis but also biochemical activity in chronic viral hepatitis C; thus, it may serve as a comprehensive non-invasive marker of disease activity (14). El-Zefzafy et al. showed CK-18 is a sensitive indicator of the severity of liver disease. Patients with CHC infection can be followed up by measuring serum levels of CK-18 which can predict the development of HCC (15).

In our study, no statistically significant difference was detected in CK-18 levels between SVR and non-SVR patients (P > .5).

A statistically significant difference was detected in CK-18 levels between SVR and non-SVR patients measured in treatment week 24 (P < .001). At Week 72, the CK-18 level in the SVR group continued to decrease, whereas the opposite occurred in the non-SVR group since the CK-18 level increased. It was thought that this happened because the nonresponder patient ratio was 69% (20/29) in the non-SVR group and treatment of these patients had to be interrupted before treatment was completed. Sgier et al. defined a significant correlation between decreases in both CK-18 level and ALT level (8). Furthermore, a significant correlation was not detected between baseline HCV RNA levels and CK-18 level in SVR patients. In conclusion, the findings of this study show that hepatocellular apoptosis was significantly decreased in chronic viral hepatitis C patients with successful antiviral therapy; and CK-18 was a reliable marker to indicate hepatocyte apoptosis. However, there are contradictory results reported from studies performed on the availability of basal CK-18 levels in predicting treatment response. Thus, larger scale studies should be performed to reach results about this issue that are more conclusive. When our results are evaluated with the results of previous studies, CK-18 can be used as a follow up to assess treatment efficacy and improvement in apoptosis level as well as a non-invasive marker instead of liver biopsy.

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