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Possible correlations among viremia, genotypes and liver histology in HCV(+) hemodialysis patients

HCV(+) hemodiyaliz hastalarında viremi, genotip ve karaciğer histolojisi arasındaki ilişkiler

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Summary

Aim: The aim of this study was to address whether there were possible correlations between viral load or HCV genotype and clinicopathological features in hemodialysis patients with chronic HCV infection.

Material and Methods: Seventy three HCV antibody (+) hemodialysis patients (Mean age: 47.3 ± 15.6 years, Male/ Female: 40/33) were enrolled in the study. The biochemical, viral and histological data were analyzed retrospectively in these patients on dialysis (mean duration; 10.4±4.4 years).

Results: ALT level was normal in 50 patients (68.5%), intermittently high in 18 patients (24.8%) and persistently high in 5 patients (6.8%). HCV RNA (57, 5 %) was positive in 42 of 73 patients. HCV genotype was determined in 29 patients. HCV genotype 1a was detected in three cases and HCV genotype 1b was detected in 26 patients. There was no difference between HCV RNA positive patients and HCV RNA negative ones with regard to age, gender, duration of dialysis, ALT and albumin levels. A liver biopsy was performed in 21 of 73 patients. It was found that histological features were not different between HCV RNA (+) and HCV RNA (-) patients. Moreover, HAI and fibrosis scores were found to be similar among patients with HCV genotype 1a, HCV genotype 1b and an undetermined genotype.

Conclusion: Our results demonstrate that viral load or HCV genotype do not correlate with clinicopathological features in hemodialysis patients with chronic HCV infection. But further studies should be performed to clarify possible clinicopathologic correlations in HCV Ab (+) hemodialysis patients.

Key words: HCV RNA, HCV genotypes, liver injury, hemodia.

Özet

Amaç :*Kronik hepatit C virus (HCV) infeksiyonu, hemodiyaliz hastalarında sık görülen ancak yavaş ilerleyen bir karaciğer hastalığıdır. HCV nin viral yükünün ve genotipinin hastalığın ilerlemesinde rol oynadığı düşündüren kanıtlar vardır. Bu çalışmada, kronik C hepatiti olan hemodiyaliz hastalarında HCV RNA pozitifliği ve HCV genotipleri ile klinikopatolojik bulgular arasındaki ilişkilerin araştırılması amaçlanmıştır.*

Yöntem ve Gereç: *Çalışmaya HCV antikoru pozitif olan yaş ortalaması 47,3 (22-80) yıl, 40 (%55) ı erkek olan 73 kronik hemodiyaliz hastası alınmıştır. Ortalama 10.4±4.4 yıllık hemodiyaliz süresi olan bu hastaların biyokimyasal, virolojik ve histopatolojik verilerinin retrospektif analizi yapılmıştır.*

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Bulgular: *ALT düzeyi açısından HCV antikoru pozitif hastaların 50 (%68.5) sinde normal, 18 (%24) inde intermittent yükseklik, 5 (%6,8) inde persistan olarak yüksek seyretmiştir. Yetmiş üç hastanın 42 (57.5%) sinde HCV RNA pozitifliği saptanmıştır. Bu 42 hastanın 29 unda HCV genotipi saptanabilmiştir. Bu hastaların 3 ünde genotip 1a, 26 sında genotip 1b saptanmıştır. HCV-RNA pozitif ve negatif hastalar arasında yaş, cinsiyet, diyaliz süresi, ALT yüksekliği ve albumin düzeyleri açısından fark bulunmamıştır. Karaciğer biyopsisi yapılmış 21 hastada HCV RNA (+) ve genotipleri ile hastalığın histolojik evresi ve derecesi arasında anlamlı ilişki dikkati çekmemiştir.*

Sonuç: *Sonuçlarımız HCV antikoru pozitif kronik hemodiyaliz hastalarında vireminin yüksek, genotip olarak da 1b nin çok daha sık olduğunu, bu viral özelliklerle klinikopatolojik bulgular arasında anlamlı ilişki olmadığını düşündürmektedir. Ancak, bu hasta grubunda virolojik ve klinikopatolojik bulgular arasındaki ilişkilerin belirlenmesi için ileri çalışmalara gereksinim vardır.*

Anahtar Kelimeler: *HCV RNA, HCV genotipi, karaciğer hastalığı, hemodiyaliz.*

Introduction

Hepatitis C virus (HCV) infection is a common health problem worldwide. According to World Health Organization (WHO) data, about 3% of the general population is infected by HCV, a single-stranded RNA virus, thus indicating that approximately 200 to 300 million individuals may be affected. One million new cases of infection are reported annually, and HCV is believed to be more prevalent than the hepatitis B virus (HBV) infection. Nearly 4 million people are infected in the United States, while it is estimated that annually 30,000 new acute infections occur. This number is predicted to triple in the next 10 to 20 years if no effective intervention is implemented (1). HCV is transmitted by direct percutaneous inoculation or transfusion of blood and blood products, transplantation of tissue or organs from infected donors, or administration of drugs with a contaminated syringe. Transmission by direct contact with body fluids and sexual transmission occurs less frequently (2). Hemodialysis (HD) treatment is an independent risk factor for transmission of HCV infection. The risk factors for HCV transmission among hemodialysis patients are a breakdown in infection control practices in HD centers, use of contaminated HD equipment, and blood transfusions (3). The prevalence of anti-HCV seropositivity was reported to be 13.2% among HD patients according to the registry in the Turkish Society of Nephrology, Dialysis and Transplantation (4). The mechanisms leading to liver cell injury are still unknown. However, most of the experimental and clinical data suggest an immune-mediated destructive mechanism instead of viral cytopathic effect (5).

It has been known that the natural course of chronic hepatitis C infection has important inter-individual variability. Therefore, the identification of predictors of fibrosis progression is critical. Several risk factors for progression have been identified, such as older age at infection, male gender, alcohol abuse and coinfection with other pathogens, such as the hepatitis B virus or the human immunodeficiency virus (6, 7). One of the factors that may account for interindividual differences in the progression of HCV infection is individual HCV genotypes. According to analysis of the viral 5'-noncoding- region, classifying HCV strains into six major types (HCV-1 to -6) has been introduced. Analysis of the more variable coding regions of the viral genome (core, envelope, nonstructural [NS]-3, and NS-5) indicated that major genotypes are composed of two or more distinct subtypes, termed 1a, 1b, 2a etc (8, 9). The aim of this study was to address whether there were differences in ALT levels and the degree of histological damage with regard to HCV RNA positivity and HCV genotypes in hemodialysis patients with chronic hepatitis C.

Materials ans Methods

Patient Population

We enrolled 73 HCV infected hemodialysis patients who were diagnosed by serologic assays that detect specific antibodies to HCV (anti-HCV) (indirect tests). Patients with active hepatitis B virus or human immunodeficiency virus infection and those with continued alcohol abuse were excluded. The study was approved by the ethical committee of the Akdeniz University. We performed liver biopsy in 21 of 73 patients to evaluate histopathologic features described below.

Virologic diagnosis and monitoring of HCV infection

The serum HCV RNA level and HCV genotype were determined at Düzen laboratory in Ankara. The serum HCV RNA level was determined by The COBAS® AmpliPrep/COBAS® TaqMan® 48 HCV Test (ROCHE DIAGNOSTICS GmbH Mannheim, Germany)and HCV genotyping was performed by RNA sequencer analysis assay.

Histopathological Evaluation

Sections from fixed and paraffin-embedded liver biopsies were stained with hemotoxylin-eosin, Mason trichrome, and Prussian blue reaction. A modified form of the ''histology activity index'' (HAI), originally introduced by Knodell et al (10) served to assess grading and staging of chronic HCV infection. HAI consists of the following four separate categories: 1) periportal/bridging necrosis; 2) intralobular degeneration and focal necrosis; 3) portal inflammation; and 4) fibrosis/cirrhosis. The first three categories comprise necro-inflammatory changes and represent grading, whereas the fourth is a method of staging. Histopathological evaluation was performed without knowing the pathological condition of the patient's biochemical or clinical data, respectively.

Statistical Analysis

Statistical analysis was done by SPSS (Version 16). Numerical variables were given as mean ± standard deviation (SD) or median (min-max). Numerical variables were compared by unpaired Student's t test or ANOVA. The $\div^2$ test was used for nonnumeric data. *P <0.05* was considered statistically significant.

Results

As shown in Table 1, the mean age of patients was 47.3 ± 15.6 years, the male/female ratio was 40/33 and the mean dialysis duration was 10.4±4.4 years. It was found that the ALT level was normal in fifty patients (68.5%), intermittently high in eighteen patients (24.8%) and persistently high in five patients (6.8%). The comparisons of demographic, biochemical and histology by HCV RNA status are shown in Table 2.

Table 1. Demographics, biochemistry and virological features of HCV AB(+) hemodialysis patients.

Characteristic	Data
Gender (M/F)	73 (40/33)
Age (years)	47.5 ± 15.6 (22-80)
Dialysis Duration (years)	10.4±4.4
Biochemistry	
ALT* (U/L)	25 (5-80)
Hgb (g/dl)	11.8 ± 1.5
Ferritin*	436 (11-659)
Albumin(g/L)	4.0 ± 0.2
Virology	
HCV RNA positivity	42 (57.5%)
HCV genotypes	
Type Ia	3 (7,1 %)
Type Ib	26 (61,9 %)
Undetermined	13 (30,9%)

*: median (min–max)
Hgb: hemoglobin, Htc: hematocrit

Table 2. Comparisons of demographics, biochemistry and histology by HCV RNA status.

	HCV RNA (+) (n=42)	HCV RNA (–) (n=31)	*P*
Gender (M/F)	25/17	15/16	*NS*
Age (years), (min-max)	49.8 ± 15.0 (22-80)	44.4 ± 16.0 (23-74)	*NS*
Dialysis Duration (years)	10.8 ± 4.6	9.8 ±3.0	*NS*
HCV duration (years)	6,7±3	6,6±2	
Biochemistry			
ALT (U/L)	32.0 ± 15.6	28.0 ± 18.6	*NS*
Hgb (g/dl)	12.2 ± 1.6	11.3 ± 1.4	*0.02*
Albumin(g/L)	3.9 ± 0.2	4.0 ± 0.3	*NS*
Histopathology			
Liver biopsy	14	7	
HAI*	1,8 (0-15)	1,34(0-13)	*NS*
Fibrosis*	0,25 (0-4)	0,21(0-3)	*NS*
Hemosiderosis*	0,37 (0-3)	0,45(0-3)	*NS*

*: median (min-max)

Hgb: hemoglobin, Htc: hematocrit, HAI: histology activity index,

Table 3. Comparisons of demographics, biochemistry and histology by HCV genotypes.

Parameter/Genotype	Ia (n:3)	Ib (n:26)	Undetermined (n:13)	Significance
Gender (M/F)	3/0	17/9	5/8	ns
Age, years (min-max)	55,6±7,5 (47-60)	52,2±17 (22-80)	37,8±11 (23-53)	0,08
HD duration, years	7,3±0,6	10,3±4,8	12,7±4,3	ns
HCV duration, years	5,2±0,7	6,3±3,2	7,6±3,1	ns
Biochemistry				
ALT (U/L)	28,6±6,0	33,6±1,9	29,7±12	ns
S Alb. (g/dl)	4,1±0,2	3,9±0,3	4,07±0,26	ns
Hb (g/dl)	11,4±0,7	12,1±1,7	12,5±1,5	ns
Ferritin*	285(282-382)	386 (11-893)	437 (189-893)	ns
Histopathology				
Liver biopsy	1	7	6	
HAI*	NA	5,7(4-15)	6(4-8)	ns
Fibrosis*	NA	0,8(0-4)	1,33(0-2)	ns
Hemosiderosis*	NA	0,15(0-3)	2,5(1-3)	ns

*: median (min-max)

There was no difference between HCV RNA positive and HCV RNA negative patients with regard to age, gender, duration of dialysis and ALT and albumin levels (Table 2). In contrast to this finding, we found that the mean Hgb level in HCV RNA positive patients was higher than in HCV RNA negative patients (12.2 ± 1.6 vs 11.3 ± 1.4 g/dl, $p<0.05$).

There was no difference between patients with HCV genotype 1a and genotype 1b in terms of mean age, hemoglobin, albumin and ALT level (Table 3). Moreover, it was also found that the histological features were similar between patients with HCV genotype 1a and HCV genotype 1b.

No difference was found between HCV RNA positive patients and HCV RNA negative ones in terms of HAI, fibrosis and hemosiderosis. Furthermore, HCV genotype 1a was found in three cases and HCV genotype 1b was found in twenty-six.

Discussion

The natural history of chronic hepatitis C infection is characterized by a slowly progressing liver injury with a 20–30% incidence of cirrhosis after 20–30 years of HCV infection (11, 12). What determines this progression is unknown but both viral and host factors may be involved. At present, it is not known whether liver damage is due to a direct viral cytopathic effect and/or an immune-mediated process. The first finding in our study is that the serum ALT level and HAI were not different between HCV RNA (+) patients and HCV (-) ones. In general, chronic hepatitis C patients with elevated ALT levels and high HCV RNA titers in the sera are considered to have active HCV replication in the liver and to be at risk for continued liver injury on a clinical basis. Also, the serum ALT level is recognized as a marker reflecting the degree of the histological damage and has served as a parameter for starting therapy or judging response to antiviral treatment in chronic hepatitis C. However, a number of recent studies show conflicting results in the relationships among the degree of histological damage, serum ALT level, and HCV RNA titers in chronic hepatitis C (13-16). Many factors may account for these discrepancies. First of all, different kinds of tests were used to determine the quantity of HCV RNA. Gretch et al. indicated the limitations of assay for the quantity of HCV RNA, especially when viremia was very low or very high (14). Secondly, because serum HCV load fluctuates

and that is not a stable parameter, it can not reflect the degree of liver damage in a given subject (17). Thirdly, HCV replicates in extra-hepatic sites as well as within the liver (18). Thus, a high amount of circulating HCV does not always imply a more active state of viral replication in neither the liver, nor a more severe degree of liver disease (19). Lastly, the discrepancy may result from the time interval between the testing of ALT and HCV RNA and performing a liver biopsy. Furthermore, the determination of the enzyme level on a single serum sample might not be related to the ALT profile over time (20). However, in this study both the ALT test and HCV RNA titer were studied on samples drawn on the same day with the liver biopsy. Our results indicate that the severity of liver disease is independent of serum markers of hepatitis C virus infection. The precise mechanism by which hepatitis C virus damages the liver remains poorly understood. Until recently, a direct cytopathic effect of the virus was considered as the primary form of liver injury caused by the virus. It has been suggested that the degree of liver damage is the result of a complicated interaction between the virus and immune response of the host. Immune mediated liver damage is believed to be initiated by HCV-specific T cells and is enhanced by HCV-induced HLA-A, B and C and intracellular adhesion molecules.

The second important finding in this study is that there was no difference between patients with HCV genotype 1a and 1b regarding histological changes and HCV RNA status. The sero-prevalences of HCV genotypes differ considerably among different geographic regions. Genotype 1a is predominant in North America and Europe, while genotype 1b is the major type in Japan (21). It was reported that distribution of HCV genotypes among end-stage renal disease in Turkey was as follow: 75% genotype 1b, 19.1% genotype 1a, 3.4% genotype 2, and 2.2% genotype 4 (22). Previous studies conducted both in humans and chimpanzees have clearly demonstrated that different genotypes of HCV are not associated with major biologic differences. All the genotypes and subtypes have been found to be both hepatotropic and pathogenic. Importantly, all HCV genotypes can induce chronic infection. The role of genotypes in the progression of chronic HCV infection remains controversial (23). Genotype 1b was reported to be associated with more severe liver disease compared to infection with other genotypes by some investigators (24-25). In contrast, other investigators failed to show a difference regarding pathogenicity between genotype 1 and 2 (26-27). Although the underlying reasons for these contradictory results are still unknown, several confounding factors may have contributed. Among them, the fact that patients infected with genotype 1b were significantly older than those infected with other genotypes, strongly supports the hypothesis that a cohort effect could explain the association between genotype 1b and the likelihood of cirrhosis or hepatocellular carcinoma (23). As mentioned above, we also did not find any difference in HAI and fibrosis scores by HCV genotype despite the small number of patients enrolled in our study.

In conclusion, our study shows that viral load or HCV genotype does not accurately predict the degree of liver injury in HD patients with chronic HCV infection. Further investigations on large patient populations should be carried out in the hemodialysis environment to explain which other factors may be implicated in the progression of HCV infection.

Kaynaklar

1. Sulowicz W, Radziszewski A, Chowaniec E. Hepatitis C virus infection in dialysis patients. Hemodial Int. 2007;11:286-295.
2. Thomas DL: Hepatitis C. Epidemiologic Quandaries. Clin Liver Dis. 2001;5:955–968.
3. Fabrizi F, Poordad FF, Martin P. Hepatitis C infection and the patient with end-stage renal disease. Hepatology. 2002;36:3–10.
4. Erek E, Suleymanlar G and Serdengecti K. Registry of The Nephrology, Dialysis and Transplantation In Turkey (2007).www.tsn.org
5. Wejstal R. Immune-mediated liver damage in chronic hepatitis C. Scand J Gastroenterol. 1995;30:609-613.
6. Massard J, Ratziu V, Thabut D et al. Natural history and predictors of disease severity in chronic hepatitis C. J Hepatol 2006;44:S19–S24.
7. Asselah T, Rubbia-Brandt L, Marcellin P, Negro F. Steatosis in chronic hepatitis C: why does it really matter? Gut 2006;55:123–130.
8. Chan S-W, McOmish F, Holmes EC, et al. Analysis of a new hepatitis C virus type and its phylogenetic relationship to existing variants. J Gen Virol. 1992;73:1131-1141.

9. Simmonds P, McOmish F, Yap PL, et al. Sequence variability in the 5* non-coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. J Gen Virol 1993;74:66-668
10. Knodell RG, Ishak KG, Black WC et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;5:43-435.
11. Di Bisceglie AM, Goodman ZD, Ishak KG et al: Long-term clinical and histopathological follow-up of chronic posttransfusion hepatitis. Hepatology.1991;14:969–974.
12. Poynard T, Bedossa P, Opolon P: Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet. 1997;349:825–831.
13. Magrin S, Craxi A, Fabiano C et al. Hepatitis C viremia in chronic liver disease: relationship to interferon-alpha or corticosteroid treatment. Hepatology .1994; 19: 273-279.
14. Gretch D, Corey L, Wilson J et al. Assessment of hepatitis C virus RNA levels by quantitative competitive RNA polymerase chain reaction: high-titer viremia correlates with advanced stage of disease. J Infect Dis 1994; 169: 1219-1225.
15. Filipak CL, Gordon SC, Silverman AL. Liver histology and hepatitis C RNA levels in patients with hepatitis C and normal or near normal aminotransferase values. Am J Gastoenterol. 1994;89:1671-1675.
16. Haber MM, West AB, Haber AD, Reuben A. Relationship of aminotransferases to liver histological status in chronic hepatitis C. Am J Gastroenterol 1995;90:1250-1257.
17. Zeuzem S, Schmidt JM, Lee JH, Ruster B, Roth WK. Effect of interferon alfa on the dynamics of hepatitis C virus turnover in vivo. Hepatology 1996;23: 366-371.
18. Muller HM, Pfaff E, Goeser T et al. Peripheral blood leukocytes serve as a possible extrahepatic site for hepatitis C virus replication. J Gen Virol. 1993;74:669-76.
19. Ballardini G, Manzin A, Giostra F et al. Quantitative liver parameters of HCV infection: relation to HCV genotypes, viremia and response to interferon treatment. J Hepatol. 1997;26:779-786.
20. McGuinness PH, Bishop GA, Lien A et al. Detection of serum hepatitis C virus RNA in HCV antibody-seropositive volunteer blood donors. Hepatology. 1993;18:485-90.
21. Takada N, Takase S, Takada A et al. Differences in the hepatitis C virus genotypes in different countries. J Hepatol. 1993;17:277–283.
22. Abacioglu YH, Davidson F, Tuncer S, et al. The distribution of hepatitis C virus genotypes in Turkish patients. J Viral Hepat. 1995;2:297–301.
23. Lee CM, Hung CH, Lu SN, Changchien CS Hepatitis C virus genotypes: clinical relevance and therapeutic implications. Chang Gung Med J. 2008;31:16-25.
24. Chen CH, Sheu JC, Wang JT et al. Genotypes of hepatitis C virus in chronic liver disease in Taiwan. J Med Virol 1994;44:234-236.
25. Ikeda K, Kobayashi M, Someya T, et al. Influence of hepatitis C virus subtype on hepatocellular carcinogenesis: A multivariate analysis of a retrospective cohort of 593 patients with cirrhosis. Intervirology. 2002;45:71-78.
26. Reid AE, Koziel MJ, Aiza I, et al. Hepatitis C virus genotypes and viremia and hepatocellular carcinoma in the United States. Am J Gastroenterol 1999;94:1619-1626.
27. Yotsuyanagi H, Koike K, Yasuda K, et al. Hepatitis C virus genotypes and development of hepatocellular carcinoma. Cancer 1995;76:352-5.