Research Article

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The Relationship Between the Serum RNA Titers of Hepatitis C Virus and Biochemical Parameters in Chronic Hepatitis C Patients

Kronik Hepatit C Hastalarında Serum Hepatit C Virüs RNA Titreleri ile Biyokimyasal Parametreler Arasındaki İlişkinin Değerlendirilmesi

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ABSTRACT

Objective: Liver biopsy, as well as some non-invasive biochemical parameters are also used in monitoring patients with chronic hepatitis C (CHC). The aim of this study was to investigate the relationship between serum biochemical markers and HCV RNA titers in patients with previously untreated CHC.

Materials and Methods: We performed a retrospective study on anti-HCV and HCV-RNA-positive 82 patients with CHC. Eighty two healthy subjects constituted the control group. Complete blood counts, total protein (TP), albumin (ALB), C-reactive protein (CRP), γ-glutamyl transpeptidase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and HCV RNA levels were recorded for each patient and control subject. Neutrophil-lymphocytes ratio (NLR) and the fibrosis index based on the 4 factors (FIB-4 index) were calculated using formulas.

Results: There was a significant difference in ALT, AST, GGT, TP, CRP, red cell distribution width (RDW), lymphocytes (LYM), platelets (PLT), FIB-4, and NLR between CHC patients and controls (p=<0.05). Values of HCV RNA viral load were correlated with ALT (r=0.271; p=0.014), TP (r=-0.256; p=0.02), WBC (r=-0.365; p=0.001), NEU (r=-0.362; p=0.001) and NLR (r=0.282; p=0.01) levels.

Conclusion: We have shown that ALT, AST, GGT, TP, CRP, RDW, LYM, FIB-4, and NLR values are increased in CHC patients but, LYM and PLT levels were decreased. Also, levels of ALT and NLR have correlated with HCV RNA titers in CHC patients. These results have implied that noninvasive biochemical parameters may contribute to monitoring patients with CHC.

Keywords: Biochemical parameters, HCV RNA titers, chronic hepatitis C

ÖZ

Amaç: Karaciğer biyopsisinin yanı sıra noninvaziv bazı biyokimyasal parametreler de kronik hepatit C (KHC) takibinde kullanılmaktadır. Bu çalışmanın amacı, daha önce tedavi edilmemiş kronik HCV hastalarında serum biyokimyasal belirteçler ve HCV RNA titreleri arasındaki ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Anti-HCV ve HCV-RNA pozitif 82 KHC hastası retrospektif olarak incelendi. Hastaneye başvurmuş herhangi bir hastalığı olmayan 82 sağlıklı birey kontrol grubu olarak belirlendi. Hasta ve kontrol grubundaki her bir bireyin, tam kan sayımı, total protein (TP), albumin (ALB), C-reaktif protein (CRP), γ-glutamil transpeptidaz (GGT), aspartat aminotransferaz (AST), alanin aminotransferaz (ALT) ve HCV RNA düzeyleri kaydedildi. Nötrofil-lenfosit oranı (NLR) ve 4 faktöre dayalı fibroz endeksi (FIB-4 endeksi) formüller kullanılarak hesaplandı.

Bulgular: KHC hasta ve kontrol grubu arasında ALT, AST, GGT, TP, CRP, kırmızı hücre dağılım genişliği (RDW), lenfositler (LYM), trombositler (PLT), FIB-4 ve NLR değerlerinde anlamlı bir fark vardı (p<0.05). HCV RNA viral yük değerleri ile ALT (r=0,271; p=0,014), TP (r=-0,256; p=0,02), NEU (r=-0,365; p=0,01), WBC (r=-0,362; p=0,001) ve NLR (r=0,282; p=0,01) seviyeleri korelasyon gösterdi.

Sonuç: ALT, AST, GGT, TP, CRP, RDW, LYM, FIB-4 and NLR değerlerinin CHC hastalarında arttığını, LYM ve PLT değerlerinin ise azaldığını bulduk. Ayrıca, ALT ve NLR seviyeleri KHC hastalarında HCV RNA titreleri ile korelasyon gösterdi. Bu sonuçlar noninvaziv biyokimyasal parametrelerin kronik hepatit C hastalığının takibine katkı sağlayabileceğini göstermektedir.

Anahtar Kelimeler: Biyokimyasal parametreler, HCV RNA titreleri, kronik hepatit C

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Introduction

Hepatitis C virus (HCV), a family member of Flaviviridia, is a single-stranded 9.600 kb RNA virus (1,2,3). HCV RNA genome has genetic heterogeneity with its 6 major genotypes which are divided into more than 80 subtypes. HCV genotype distribution varies according to geographical location or route of transmission (4). HCV is mainly transmitted via parenteral route, by blood transfusion, substance abuse and accidental needle pricks. Dental surgery, acupuncture, hemodialysis and procedures such as tattooing also pose a risk of transmission of HCV (1,5,6).

HCV infection is a significant public health issue. Currently, it is estimated that worldwide there are 175 million chronic hepatitis infection cases and 350.000 patients die every year due to complications of HCV such as cirrhosis and hepatic cancer (7). HCV infection is an insidious disease with slow progression. HCV infection can be manifested as an acute infection and in around 20% of the patients, the disease spontaneously resolves, but becomes chronic in 80% of cases (3,8,9). HCV infection can lead to chronic hepatitis C (CHC), liver cirrhosis and hepatocellular carcinoma (HCC) (10,11). Mechanism of liver injury due to acute or chronic HCV infections has not been fully understood. The high rate of chronicity in HCV infections is explained by escape of virus from immune control as a result of genetic heterogeneity due to tendency to rapid mutation (12). The natural history of HCV infection is affected by a number of host and virus variables (13,14). The duration and route of transmission of the disease, viral genotype, viral load, alcohol abuse and co-infection with human immunodeficiency virus (HIV) and hepatitis B virus (HBV) are among the factors affecting the progression of the disease (5,9). Chronic hepatitis B (CHB) is usually indolent and incidentally recognized during routine serological tests, blood biochemistry tests or histological tests (5,15). The diagnosis of HCV infection is established by detecting antibody formed against the virus (anti-HCV) and by measuring HCV RNA by nucleic acid amplification method (1).

Liver biopsy is considered as the gold standard for grading and staging (9,11,14,16,17). In general, liver biopsy is a reliable method, however, it is invasive, costly and has risk of complication though minimal (9,11,16,17). Because of these limitations, numerous studies have focused on developing simple, inexpensive and, most importantly, non-invasive biochemical markers as an alternative to liver biopsy (11,17).

There are non-invasive methods evaluating hepatic inflammation and fibrosis (9). In HCV-positive patients, complete blood count, routine biochemical blood tests, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP) and measurement of serum HCV RNA levels are carried out (18,19). There are several studies that have investigated the association between liver injury and serum ALT levels, HCV viral load and HCV genotypes but the results were inconsistent (11,14,20,21,22,,23,24,25,26).

The objective of this study was to evaluate and determine the potential correlation between HCV viral load and different biochemical parameters in chronic hepatitis C.

Materials and Methods

The current study is a retrospective analysis of 82 patients, who were known to be HCV antibody (anti-HCV)- and HCV-RNA-positive, admitted to Ahi Evran University Training and Education Hospital between July 2014 and December 2015 and received the diagnosis of CHC. The control group was consisted of 82 volunteers from similar age groups and sex who attended the hospital during the same time period for any reason and had normal biochemistry tests and negative anti-HCV results.

Serum anti-HCV was analyzed by chemiluminescence enzyme immunoassay (Roche Modular Analytics, cobas 6000 analyzer; Roche Diagnostics, Germany). HCV-RNA test was performed by real time polymerase chain reaction (PCR) with an automated system (Roche/Cobas TaqMan System) according to the manufacturer's instructions. The linear range of the HCV RNA assay was 25 to 391.000.000 IU/ml. HCV genotypes were determined using the Linear Array Hepatitis C Virus Genotyping Test (Roche Molecular Systems).

The biochemical assessment included serum total protein (TP), albumin (ALB), C-reactive protein (CRP), γ-glutamyl transpeptidase (GGT), AST, and ALT which were measured on a AU5800 analytical system (Beckman Coulter, FL, USA) using commercially available reagents and an enzyme-based kit. A complete blood count of hemoglobin (HB), hematocrit (HTC), white blood cells (WBC), neutrophils (NEU), lymphocytes (LYM), platelets (PLT), mean platelet volume (MPV) and red cell distribution width (RDW) was determined using a ABX Pentra DX 120 cell counter (Horiba Ltd., Kyoto, Japan). NEU-LYM ratio (NLR) was calculated as the ratio of NEUs and LYM, both obtained from the same automated blood sample at the time of admission to the study. The fibrosis index based on the 4 factors (FIB-4 index) was calculated using the following formula (16):

FIB-4 index=age (years) × AST (IU/L)/PLT count ($\times 10^9$/L) × (ALT [IU/L])$^{1/2}$

Statistical Analysis

All statistical analyses were carried out using the SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA). Categorical variables were presented as frequencies and percentages; continuous variables were expressed as means and standard deviation. Statistical comparison of clinical data between the two groups consisted of unpaired t-tests for parametric data. Correlations were assessed with the Pearson's correlation coefficient, and a chi-square test was used for categorical variables. A p value of less than 0.05 was considered statistically significant.

Results

Eighty-two patients with CHC (37 male, 45 female) and 82 control subjects (43 male, 39 female) were included in the present study. The median age of CHC and control patients was 59.84±16.22 and 58.78±12.76, respectively. There was no statistically signiŞcant difference in sex and age between the groups (p>0.05). The mean HCV-RNA level in the serum was 3.747.400±6.527.860. The distribution of HCV genotypes was as follows genotype 1: 59.3%; genotype 2: 13%; genotype 3: 11% and genotype 4: 16.7%. Dominant genotype in our study group was genotype 1 which is significantly higher than other genotypes. The demographic characteristics and some biochemical features of the participants are shown in Table 1. There was a significant difference in ALT, AST, GGT, TP, CRP, RDW, LYM, PLT, FIB-4 and NLR between the groups. Biochemical values in patients with CHC and controls are also shown in Table 1.

When the correlation between serum HCV RNA levels and various biochemical parameters were evaluated, it was observed that serum HCV RNA titers correlated with ALT (r=0.271; p=0.014), TP (r=-0.256; p=0.02), WBC (r=-0.365; p=0.001), NEU (r=-0.362; p=0.001), and NLR (r=0.282; p=0.01) levels (Table 2).

Discussion

In this study, we have determined that there was a significant difference in ALT, AST, GGT, TP, CRP, FIB-4, RDW, LYM and NLR parameters between HCV-positive patients and control group and we have also found that ALT, TP, WBC, NEU and NLR levels significantly associated with the indicator of viral load, namely HCV RNA level.

As it is known, HCV infection affects nearly 3% of the whole population; and 80% of the cases becomes chronic and its mortality rate is getting higher (1,27). Clinical consequences of HCV infection depend on the balance between replication rate of the virus and specific, rapid and effective response of immune system to the virus (21,28). A number of clinical, biochemical and histological parameters are used in the evaluation of CHC progression (29). Although liver biopsy is considered as the gold standard in determining the effect of HCV on liver, in recent years, studies have focused on developing a non-invasive marker to replace liver biopsy (10,16,17,30,31,32,33,34,35).

Systemic inflammatory response can be evaluated by the increase in NLR. NLR is a simple and inexpensive marker that can be measured by using the results of routine complete blood count. Previous studies have revealed that NLR is related to cardiovascular diseases associated with systemic inflammation and pathologies such as cancer and is a predictor of disease severity and mortality (10,36,37). In their study, Abdel-Razik et al. (38) have found a relationship of NLR with disease severity and hepatic fibrosis in patients with nonalcoholic steatohepatitis (NASH). In their study, Kuo et al. (10) have investigated the association between NLR and response of CHC patients to antiviral therapy and have found a significant correlation between HCV RNA viral load and NLR increase. This finding led to the conclusion that high NLR has a negative impact in evaluation of antiviral therapy (10). In our study, consistent with the literature, CRP and NLR levels were significantly higher in CHC patients compared to controls and also, there was a significant correlation between HCV RNA levels and NLR.

RDW is a measure for variations in circulating red blood cell size. Elevated RDW has been reported to associate with higher mortality risk among the general population (39). Cengiz et al. (40) have also reported a positive correlation between RDW values and fibrotic scores in patients with NASH. Hu et al. (41) have observed that RDW increased in chronic patients with CHB and have suggested that RDW may serve as a potential prognostic index in liver disease. We have also investigated a probable relationship

Table 1. Baseline demographic and biochemical characteristics of study populations

Variables	Chronic hepatitis C patients (n=82)	Control group (n=82)	p value
Age(mean ± SD) (yr)	59.84±16.22	58.78±12.76	0.642
Male sex % (n)	45 (37)	52 (43)	0.435
HCV-RNA (IU/mL)	3.747.400±6.527.860	-	
ALT (IU/L)	41.65±37.68	19.30±7.73	<0.001
AST (IU/L)	39.17±21.24	21.12±5.15	<0.001
GGT (IU/L)	51.45±54.97	22.32±9.14	<0.001
TP (g/dL)	7.30±0.98	6.81±0.42	<0.001
ALB (g/dL)	3.95±0.55	3.95±0.28	0.916
CRP (mg/dL)	0.53±1.07	0.22±0.13	0.012
WBC (10^3/µL)	6.63±3.77	6.21±1.21	0.344
HB (mg/dL)	13.72±2.70	14.34±1.49	0.075
HTC (%)	41.74±5.41	41.78±5.91	0.962
RDW (%)	15.05±1.88	13.76±1.05	<0.001
NEU (10^3/µL)	4.08±3.24	3.38±0.87	0.063
LYM (10^3/µL)	1.75±0.64	2.12±0.66	<0.001
PLT (10^3/mm^3)	207.06±63.62	236.02±49.01	0.001
MPV (fL)	8.87±0.80	8.77±0.81	0.414
FIB-4	2.39±2.40	1.29±0.49	<0.001
NLR	2.43±1.57	1.75±0.73	<0.001

Data are presented as median (range) or mean ± SD, TP: Total protein, ALB: Albumin, CRP: C-reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: γ-glutamyl transpeptidase, HB: Hemoglobin, HTC: Hematocrit, WBC: White blood cells, NEU: Neutrophils, LYM: Lymphocytes, PLT: Platelets, MPV: Mean platelet volume, RDW: Red cell distrubition width, NLR: Neutrophil-lymphocytes ratio, TP: Total protein, FIB-4: Fibrosis-4, SD: Standard deviation

Table 2. Correlation between serum HCV RNA levels and various biochemical parameters in patients with chronic hepatitis C

Variables	r value	p value
ALT (IU/L)	0.271	0.014
AST (IU/L)	0.032	0.774
GGT (IU/L)	0.131	0.242
TP (g/dL)	-0.256	0.02
ALB (g/dL)	-0.177	0.111
CRP (mg/dL)	0.201	0.071
WBC (10^3/µL)	0.365	0.001
HB (mg/dL)	0.045	0.688
HTC (%)	-0.029	0.793
RDW (%)	-0.032	0.778
NEU (10^3/µL)	0.362	0.001
LYM (10^3/µL)	0.086	0.442
PLT (10^3/µL)	0.076	0.496
MPV (fL)	0.082	0.466
FIB-4	-0.064	0.567
NLR	0.282	0.01

TP: Total protein, ALB: Albumin, CRP: C-reactive protein, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: γ-glutamyl transpeptidase, HB: Hemoglobin, HTC: Hematocrit, WBC: White blood cells, NEU: Neutrophils, LYM: Lymphocytes, PLT: Platelets, MPV: Mean platelet volume, RDW: Red cell distrubition width, NLR: Neutrophil- lymphocytes ratio, PLT: Platelets, MPV: Mean platelet volume, FIB-4: Fibrosis-4

between RDW and liver disease associated with HCV and have observed that RDW increased in CHC patients similar to that in patients with hepatitis B.

As it is known, FIB-4 index is a non-invasive test used for evaluation of hepatic fibrosis. A score of <1.45 is considered as absence of fibrosis or presence of moderate fibrosis (F0-F1-F2-F3), and >3.25 is considered as presence of severe fibrosis or cirrhosis. It is accepted that like other non-invasive tests, FIB-4 index may replace biopsy in 70% of cases (16). McCombs et al. (42) have shown in their study that patients with a FIB-4 value of >3.25 had significantly higher risk of mortality and clinical course of hepatic disease was worse in these patients. In our study, we could not find a correlation between HCV RNA viral load and FIB-4, however, consistent with the above mentioned studies, FIB-4 value was significantly higher in the CHC group compared to the control group.

It is a generally accepted predication that in patients with CHC, higher HCV-RNA and serum ALT levels indicate presence of active HCV replication in liver and, thus, liver injury implies a clinical risk. The grade of elevated ALT is accepted as a marker of liver injury and it is used as a criterion in starting antiviral therapy or monitoring response to therapy (14,21,28). It has also been determined that HCV RNA titers are correlated with response to antiviral therapy (18,43). In recent years, various studies investigated the association between the grade of liver injury and serum ALT levels, HCV RNA titers in CHC patients and HCV genotype were performed, but the results were inconsistent. In some studies, no clinically feasible association was found between ALT level and liver injury or liver fibrosis (23,24,25). In a study by Lee et al. (21) there was no association between HCV RNA level and grade of liver injury in chronic HCV carriers but serum ALT level was associated with portal inflammation and periportal necrosis.

Fanning et al. (29) have found that serum HCV RNA viral load and ALT level were significantly correlated with the grade of liver inflammation but no such correlation was found between these parameters and liver fibrosis. Al Swaff (20) have found an association between grade 1 and grade 4 liver fibrosis and higher ALT levels in patients with CHC (genotype 4) infection and have detected higher HCV RNA levels in grade 3 liver fibrosis.

Zechini et al. (14) have found a significant correlation between HCV RNA and ALT. in CHC patients and have also found a correlation between histological activity index (HAI) and HCV RNA levels as well as between HAI and AST and ALT levels. They have reported in their study that particularly AST might be associated with liver injury. Shahid et al. (11) have found that HCV RNA titers, AST, ALP and total bilirubin were correlated with grade of fibrosis in patients with CHC (genotype 3a) infection (11). In our study, serum ALT levels in CHC patients were higher than in control group. There was also a significant correlation between HCV RNA titer and ALT levels (p=0.014).

Conclusion

Some limitations should be considered when evaluating our study. The primary limitation is the relatively small sample size. A larger sample size could provide stronger statistical data. Another limitation is the possible effect of medications that were used by CHC patients was not evaluated. Finally, we did not evaluate our patients in terms of ultrasonography and histopathological investigation which are reference methods of hepatic injury.

We have shown that ALT, AST, GGT, TP, CRP, RDW, LYM, FIB-4, and NLR values are increased in CHC patients, but LYM and PLT were decreased. Also, levels of ALT and NLR have significant correlation with HCV RNA titers in CHC patients. These results have imply that noninvasive biochemical parameters may contribute to monitoring of CHC disease and evaluation of its grade. However, further studies including larger patient population and measuring biochemical parameters and HCV RNA titers simultaneously with histopathological evaluation are needed.

Ethics

Ethics Committee Approval: The study did not need to get Ethics Committee approval.

Peer-review: External and Internal peer-reviewed.

Authorship Contributions

Concept: Rukiye Nar, Design: Rukiye Nar, Fikriye Milletli Sezgin, Data Collection or Processing: Rukiye Nar, Fikriye Milletli Sezgin, Analysis or Interpretation: Rukiye Nar, Fikriye Milletli Sezgin, Literature Search: Rukiye Nar, Fikriye Milletli Sezgin, Writing: Rukiye Nar.

Conflict of Interest: No conflict of interest was declared by the authors.

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