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ORIGINAL PAPER

The Kinetics of Virological and Biochemical Responses in the Treatment of Chronic Hepatitis C by Dual Antiviral Therapy

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Introduction: Infection with hepatitis C is often manifested by a mild clinical course, and in many patients it is revealed incidentally, during routine laboratory tests. Progression of the disease often takes 10-20 years with specified high risk of fibrosis and hepatocellular carcinoma. **Material and methods**: The group of subjects with chronic liver disease of viral C etiology was consisted of 50 patients of both sexes, 38 (75%) were male and 13 (25%) females, aged 20-65 years. Patients were selected according to genotype hepatitis C viral infection and subsequently treated according to two current therapeutic protocols. All patients had prior therapy and after completion of treatment using standard methods of laboratory tests were done the following: functional hepatic tests, serological analysis, nucleic acid detection of hepatitis C virus polymerase chain reaction (PCR), quantitatively and qualitatively with the genotyping of the virus C, which determines the length of therapy. In determining the stage of chronic liver disease, histopathological examination of liver tissue samples obtained by biopsy of the liver was done and we analyzed the fibrosis and architectural changes. **Results:** By analyzing the HCV RNA PCR values at the beginning and end of treatment we tested the effect of treatment on PCR with paired samples t-test logarithm values of the PCR and came to the conclusion that the values after treatment are significantly lower with threshold of significance of 0.01. The results showed that the value of PCR before and after therapy, or achieved a response at the end of therapy, which achieved 77% of patients. The values of ALT in the group of patients with CHC were significantly higher than the values in the group of patients after the therapy. AST values in the patients with CHC were significantly higher than the values in the group of patients after therapy. There was a moderate correlation between ALT values at baseline and ALT values upon completion of treatment (0.5061). There was no correlation between HCV RNA PCR and ALT and AST. **Conclusion**: Upon completion of antiviral treatment response at the end of treatment achieved 77% of patients, regardless of the genotype of the virus. Also, regardless of the genotype of the virus antiviral therapy led to statistically significant reduction of AST and ALT, indicating a direct effect of combination therapy on virological and biochemical response with no significant link between these two studied parameters. **Key words**: chronic viral hepatitis c, antiviral therapy.

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1. INTRODUCTION

Millions of patients worldwide suffering from chronic liver disease, which potentially progress to liver cirrhosis. Only a small number of patients (25-30%) are likely to develop significant fibrosis and cirrhosis of the liver (1). This particularly applies to patients suffering from chronic hepatitis C, which is assumed to have the largest increase in prevalence between 2010 and 2015.

Histological image of liver tissue infection is relatively mild, but in the presence of significant liver fibrosis, suggesting that persistent hepatitis C virus (HCV) infection and a mild histological picture disturbs the structure of organs and leads to the occurrence of liver failure (2). Combined antiviral therapy with pegylated interferon and ribavirin, after the use of conventional interferon which did not achieve a satisfactory therapeutic effect, represents a step forward in achieving sustainable virological response, which is considered a therapeutic success.

Nowadays, the combination of pegylated (PEG) interferon alpha (IFN) and ribavirin is the treatment of choice (3,4) with sustained virological re-

sponse (SVR, defined as HCV RNA negativity at the end of therapy and 6 months after completion of treatment) at 42-52 % of patients with genotype 1 (5,6) and in 80% of patients with genotype 2 or 3 infection (7). An effective antiviral treatment for chronic hepatitis C prevents complications such as liver cirrhosis and hepatocellular carcinoma.

1.1. Pathogenesis of liver fibrosis

Liver fibrosis is the result of liver tissue response to different damage, according to the principle of healing wounds (8).

After acute liver injury (acute viral hepatitis), parenchymal cells regenerate and replace necrotic or apoptotic cells. This process is associated with inflammatory response and the limited disposal of extracellular matrix proteins (ECM). If liver damage persists, regeneration is absent and hepatocytes are replaced with large amounts of ECM, including fibrillary collagen. Distribution of this fibrous material depends on the origin of liver damage. In chronic viral hepatitis and chronic cholestatic disease, fibrous tissue is initially created around the portal space, while in alcoholic liver disease are located primarily in the perisinusoidal and pericentral areas (9).

1.2. Pathogenesis of liver fibrosis in chronic hepatitis C

In chronic hepatitis C fibrosis caused by HCV is insufficiently tested because of lack of animal models with persistent HCV infection (10). HCV infects hepatocytes, causing oxidative stress and inducing activation of inflammatory cells. Both factors lead to activation of HSC and deposition of collagen. Several HCV proteins also directly stimulates the activity of inflammatory and fibrogenic HSC (11).

1.3. Antiviral treatment of chronic hepatitis C

The therapeutic goal for chronic hepatitis C (CHC) is the eradication of hepatitis C virus, prevention of cirrhosis development of and hepatocellular carcinoma (12). In the last decade, antiviral therapy has significantly improved, especially treatment with ribavirin and pegylated interferon.

It is known that continuous inflammation associated with HCV infection gradually leads to the development of hepatic fibrosis and eventually leads to the development of hepatocellular carcinoma (HCC). One of the goals of the clinical treatment of refractory HCV infection is the prevention of liver fibrosis progression (13).

It was also revealed that the prognosis and clinical course of chronic liver disease depend on the stage of hepatic fibrosis, because the complications of the disease usually occur in advanced stages of disease. Besides that, the stage of fibrosis is correlated with the incidence of HCC (14).

Long-term treatment with interferon (IFN) may prolong the positive effects of therapy in patients with refractory CHC. In addition to its antiviral effect, there are indications that suggest that IFN can reduce liver fibrosis. IFN inhibits collagen promoter activity in activated stellate hepatic cells, which play a crucial role in the development of liver fibrosis (15).

2. GOALS

Determine the values of liver function tests (serum aminotransferases, PT, bilirubin, serum albumin), and serological analysis (HCV RNA PCR) in patients before initiation and after completion of antiviral therapy. Identify the possible correlation between the serological tests, PCR and values of individual parameters of liver function tests

3. MATERIAL AND METHODS

3.1. Respondents

Group of patients with chronic liver disease of viral C etiology consisted of 50 patients of sexes, 38 (75%) male and 13 (25%) female. The age of patients, who were hospitalized at the Gastroenterohepatology Clinic of Clinical Center, University of Sarajevo was from 20-65 years. Patients were selected according to genotype of hepatitis C virus infection and therefore treated according to two current therapeutic protocols: Genotype 1 and 4: Pegylated interferon alpha 2a 40 kD, 48 weeks with ribavirin (1000-1200 mg/day depending on body weight). Genotype 2 and 3: Pegylated interferon alpha 2a 40 kD, 24 weeks with Ribavirin (800 mg/day).

3.2. Methods

The study was conducted on the basis of history of illness, objective medical examination, laboratory tests and histopathological analysis of a sample of liver tissue obtained by percutaneous liver biopsy.

3.2.1. Biochemical analyses and other tests

To all patients were done the following laboratory tests: functional liver tests (albumin, total proteinogram, prothrombin time, alanine aminotransferase, aspartate aminotransferase, and bilirubin), serological analysis, nucleic acid detection of hepatitis C virus polymerase chain reaction (PCR), quantitatively and qualitatively with the genotyping of virus C.

To all patients in the study were also made hematologic and biochemical tests: SE, erythrocytes and leukocytes count, hemoglobin, hematocrit, coagulation factors, iron, bilirubin, gamma-GT, alkaline phosphatase, creatinine, urea, K, Na, glucose, cholesterol and triglycerides, blood sugar, uric acid, triiodothyronine (T3), thyroxine (T4), thyroid hormone (TSH), markers of autoimmune liver disease and the concentration of AFP.

3.2.2. Percutaneous liver biopsy

Percutaneous liver biopsy provided analysis of liver tissue in a cylinder of length of at least 20 mm.

Routine preparation of samples for histopathological interpretation begins with the immediate fixation in 10% neutral "buffered" formalin. Following the standard and special staining methods (PAS, PAS-D, Ganor, Van Gieson, Masson trichrome) to determine the degree necroinflammatory activity and stage of fibrosis in the liver using the classification by Ishak et al (16).

3.2.3. Serological analysis and viral load monitoring

Markers of HCV infection included detection of antibodies to HCV and viral RNA.

Identification of HCV antibodies was done by Enzyme immunoassays–EIA) and definitive confirmation of the findings was made by Recombinant immunoblot assay–RIBA.

HCV RNA test was performed by molecular analysis of the AMPLICOR and COBAS AMPLICOR HCV MONITOR test v2.0 which confirmed the infection and monitor the outcome of the therapy.

AMPLICOR HCV MONITOR Test v2.0 is the industry standardized test that is used for the quantification of viral load and monitoring patient response to therapy. In the 12th week of therapy was determined the EVR (Early virological Respond) on the basis of whose values was decided on the continuation or discontinuation of therapy. Negative values of HCV RNA PCR or decrease in the value of 2 log compared to the initial values were the confirmation of an early virological response and enable the further continuation of the previous treatment protocol.

The qualitative AMPLICOR and COBAS AMPLICOR HCV test, with the lowest level of detection of 50 IU/ml was used for determining sustainable virological response SVR (17).

Virus genotyping was performed by direct sequencing.

4. RESULTS

The results of genotype distribution according to the total number of subjects are presented in Figure 1,

The results show that the indeterminate genotype (1n) was found in 5 patients (10%); genotype 1a had 17 patients (33%), genotype 1b had 15 patients (29%), genotype 2 was not present, genotype 3 had 10 patients (20%), and x genotype had 4 patients (8%).

Logarithm PCR values before and after treatment are presented in Figure 2.

The results showed the value of PCR before and after therapy, or achieved response at the end of therapy (End of Treatment Respond), which achieved 77% of patients.

ALT values before and after the therapy are presented in Figure 3

Presented are ALT mean values (mean + SEM) in the group of patients before and after therapy.

The results presented in Figure 3 show that the values of ALT in the group of patients with CHC are significantly higher than the values in the group of patients after the therapy.

AST values before and after treatment are presented in Figure 4.

The results presented in Figure 4 show that the values of AST in patients with CHC group are significantly higher than the values in the group of patients after the therapy.

5. DISCUSSION

The main cause of liver fibrosis in developed countries is chronic hepatitis C, excessive alcohol consumption and alcoholic steatohepatitis.

Fibrosis of liver tissue is the result of chronic liver damage, connective tissue proliferation and accumulation of extracellular matrix (ECM) proteins. These changes are characteristic of most chronic liver diseases. The progressive accumulation of fibrillary extracellular matrix (ECM) in the liver is often the result of damage to liver tissue by viral agents (HCV) and the activation process of healing of damaged tissue components (1). Fibrosis of liver parenchyma is considered as a passive irreversible process which has developed as a result of the collapse of the hepatic parenchyma and its substitution with tissue rich in collagen (18,19).

In most patients with chronic hepatitis C there is a long latency period (10-15 years) between HCV infection and detection of minimal fibrosis stage in the presence of obvious degree of necroinflammatory activity. Progressive hepatic fibrosis and cirrhosis develops in 20-30% of patients with chronic hepatitis C (20).

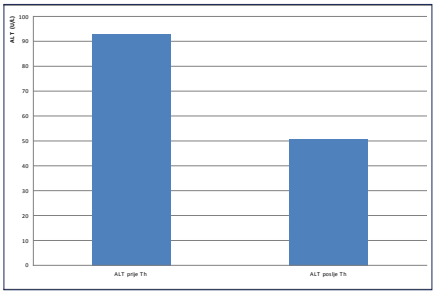

Figure 3. ALT levels in serum of patients before and after antiviral therapy.

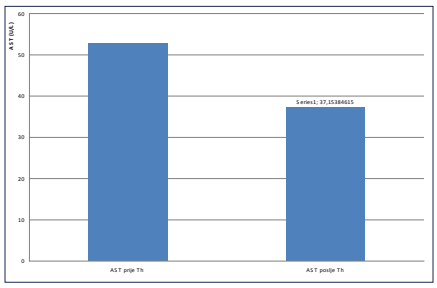

Figure 4. The values of AST in the serum of patients before and after antiviral therapy. Presented are AST mean values (mean + SEM) in the group of patients before and after therapy.

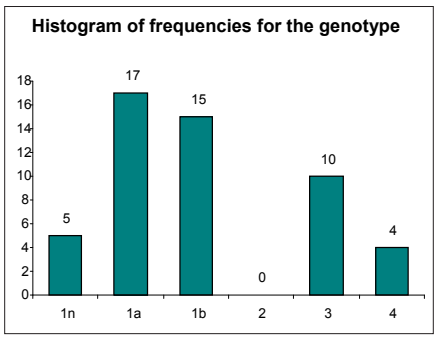

Figure 1. Histogram of frequencies for the genotype. It presents the distribution of Hepatitis C virus genotypes in the tested group.

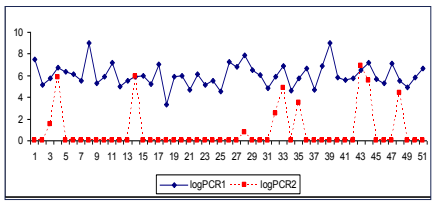

Figure 2. Line diagram of the base 10 logarithm of the PCR values measured before (logPCR1) and after therapy (logPCR2). Negative findings are replaced by zeros.

These results were partially confirmed by the results of our study, which found that the majority of patients had stage 1 fibrosis (60%) and fibrosis stage 2 (29%), whereas stage 3 fibrosis had only 6% of patients and the stage 5 and 6 indicating the presence of incipient and already developed cirrhosis of the liver tissue had a total of 6% of patients.

By comparison with the previously determined ratio of patients who develop significant fibrosis and cirrhosis of the liver tissue, we can conclude that in our group of patients most patients at the time of diagnosis had a lower stage of liver parenchyma fibrosis. Earlier it was shown that continuous inflammation associated with HCV infection gradually leads to the development of hepatic fibrosis and eventually hepatocellular carcinoma (HCC). The aim of antiviral therapy of chronic hepatitis C (CHC) hepatitis C virus is eradication, prevention of development of cirrhosis and possibly hepatocellular carcinoma (12).

In the last decade, HCV antiviral therapy has significantly improved especially combined treatment with ribavirin and pegylated interferon.

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IFN inhibits the collagen promoter activity in activated HSC, which play a crucial role in the development of liver fibrosis (15). In addition to antiviral effects, there are indications that suggest that IFN can reduce liver fibrosis, where it is important to emphasize that the improvement of liver fibrosis was proved only in patients who achieve sustained viral response–SVR.

Chronic hepatitis C is the most intensively studied state and successfully completed combination therapy with interferon and ribavirin, which lead to the disappearance of the virus resulting in regression of fibrosis. It is also important to note that in almost half of the patients with cirrhosis occur significant signs of changes regression (21). It is still not known, however, if this benefit is associated with improvements in long-term clinical outcome. As one of the serum parameters of necroinflammatory activities in the group of patients suffering from chronic hepatitis C, we assessed the activity of alanine aminotransferase (ALT), which confirmed that the values were significantly lower after the treatment, with a significance threshold of 0.01.

The mean ALT before therapy was 94.5 and 53.6 after treatment which is consistent with results of other similar published studies that have noted reduced ALT activity after completion of antiviral therapy. In the same group of patients was analyzed values of other liver function tests before and after the treatment and was found to be significantly different values of AST and so that the values decreased after treatment compared to values before the treatment. Mean AST before treatment was 56.33 and 36.7 after the treatment.

Albumin and INR values before and after therapy did not differ significantly, while the value of bilirubin before and after therapy was significantly different in manner that the values decreased after the treatment compared to values before the treatment onset.

Regardless of the therapeutic protocol and duration of therapy, in the 12th week of treatment was determined early virological response by quantitative analysis of HCV RNA. Achieved early virological response to therapy provides basis for continuation of the previous protocol. In our group of patients early virological response did not reach 17% of patients. In 6% of patients therapy was discontinued either because of inadequate adherence to treatment protocols, or due to adverse events that were the reason for discontinuation of the therapy.

Upon completion of antiviral treatment response at the end of treatment achieved 77% of patients, regardless of the genotype of the virus, expecting that during follow up sustained virological response SVR in genotype 2 and 3 retain the values of ETR and in case of genotype 1 and 4 decrease to values 50% lower which is consistent with the published results of studies by Manns et al. (2001), Fried et al. (2002) and Hadziyannis et al. (2004) (5,7).

By analyzing the HCV RNA PCR at the beginning and end of treatment was tested the effect of treatment on PCR by paired samples t-test for the logarithm values of PCR and came to the conclusion that the values after treatment was significantly lower with a threshold of significance of 0.01.By analyzing the correlation between serological parameters and individual values of liver function tests was found low positive correlation between bilirubin and PCR values after the treatment (0.308). A positive correlation was found between ALT values at the beginning and end of therapy (0.5061), whereas the high correlation was determined between the biochemical parameters of AST and ALT activity (0.714).

6. CONCLUSION

Values of liver function tests (ALT) after antiviral therapy were significantly lower in patients with chronic hepatitis C ($p<0.01$).

Value of serologic parameters (HCV RNA PCR) after treatment was significantly lower in patients with chronic hepatitis C.

According to the results of our research we can determine the positive impact of antiviral therapy on biochemical and serological parameters of the disease. At the same time, no significant correlation between the investigated biochemical and serological parameters of disease activity was found.

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