

ORIGINAL PAPER

Correlation of Pathohistological Changes and Serology Parameters in Chronic Hepatitis C

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Introduction: Viral Hepatitis C, formerly known as non A–non B hepatitis, as a separate clinical entity described in 1975 is most often reported in patients who received blood transfusions, and also called it post transfusion hepatitis. **Aim of the study:** Goal was to quantify the number of HCV RNA copies by PCR method, histologically determine the stage of fibrosis and degree of necroinflammatory activity in biopsies of liver parenchyma, and compare the histopathological changes with the number of the virus copies.

Material and methods: The study was prospective and involved 50 patients suffering from chronic hepatitis C of viral etiology. All patients underwent liver biopsy and the specimens were patohistologically investigated to determine the stage of fibrosis score, and necroinflammatory activities. In every case was determined the concentration of AST, ALT, bilirubin, CBC, DR, and all underwent percutaneous ultrasonography and gastroscopy. We performed genotyping of viruses and virus quantification of HCV RNA-PCR. **Results:** The study showed that women were older than men. The stage of fibrosis and degree of necroinflammatory activities were higher in women than men, meaning that older people carry the virus longer, increasing the number of virus copies the disease lasted longer. According to the etiology of infection the patients who were infected by blood transfusions had a higher stage of fibrosis. Score of necroinflammatory activity was significantly dependent on variables AST with $p=0.02$ and ALT with $p=0.026$. **Conclusion:** Our research has shown that the stage of fibrosis was significantly dependent on alanine aminotransferases, duration of infection, number of virus copies and mode of infection. Patients who received blood transfusions, had the longest duration of infection, higher stage of fibrosis and degree necroinflammatory activity. **Key words:** Chronic hepatitis C, pathohistological changes, serology parameters.

77-80%. For now are identified six genotypes that have a similar effect on the hepatic parenchyma, but react differently to treatment, so it depends on the type of genotype and duration of therapy. Known genotypes can have subtypes, such as type 1 subtypes 1a and 1b, but with no greater significance in clinical treatment.

Biochemical liver function tests, which include level of aminotransferases in serum—AST and ALT, bilirubin, alkaline phosphatase and serum albumin, are hidden predictors of liver parenchymal damage and represent a reliable measure for the assessment of chronic illness (13).

To confirm the degree of histological liver damage, a liver biopsy is performed, which is the best way of assessing the degree of damage to the liver parenchyma.

Today available clinical tests can identify patients with mild or severe degree of illness, but are less reliable in identifying patients with moderately pronounced illness. The most common histopathological features described in chronic viral hepatitis are reflected in the presence of portal aggregation of lymphocytes in the form of follicles and fatty transformation of hepatocytes, bile duct damage and hepatic fatty infiltration. Damage to the bile duct is also very common in patients with chronic hepatitis C of viral etiology. This damage is characterized by inflammatory infiltration that permeates the biliary epithelium, which showed degenerative changes, including cytoplasmic vacuolization and reactive nuclear changes, or pseudo stratification. It is crucial to

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1. INTRODUCTION

Hepatitis C virus is an RNA virus of spherical shape and size of 30-60 nm. It is consisted of the nucleocapsids and envelope. It belongs to the family of Flaviviridae, genus Hepaci virus (1). Viral genome is composed of positively directed RNA consisting of 9400 nucleotides contained in the ORF region (region of open frame) (2), which directly

encodes a viral precursor built of 3010-3033 amino acids. Splitting of the polypeptides are obtained by corresponding structural (C, M and E proteins) and non-structural viral proteins. It is proved that there are 6 genotypes of this virus, numbered 1–6 and more than 80 subtypes designated by letters (a, b, c, d, etc.). The similarity between the sequences ranges 66-99%, and subtypes

note that there is no loss of bile ducts in hepatitis C. Fatty infiltration of the liver is a common histological finding in chronic hepatitis C, particularly in patients infected with genotype 3. The presence, distribution and severity of fatty infiltration are not helpful in making a conclusion about the cause and severity of disease. Typical of chronic viral hepatitis C is usually a mild degree of so-called "interface" hepatitis (piece meal necrosis) and the degree of lobular infiltration is much higher than in chronic hepatitis B. This is one of the reasons why the classification of chronic hepatitis into chronic persistent hepatitis and chronic active hepatitis, which entirely depend on the presence or absence of "interface hepatitis" and "piece meal necrosis" is not used (13). The term "piecemeal necrosis" was coined to describe the progressive tear bar hepatocytes located near the plate boundary. Cell death in the case of apoptosis, not necrosis. Apoptosis is programmed cell death.

2. MATERIAL AND METHODS

Our study was prospective and included 50 patients suffering from chronic hepatitis C of viral etiology, hospitalized at the Clinic for Gastroenterohepatology, Clinical Center of Sarajevo University, from March 2007 to December 2009. Criteria for inclusion in this study were patients referred to the Gastroenterohepatology Clinic to Gastroenterohepatology Counseling in Sarajevo with elevated serum aminotransferases ALT, Patients who have positive anti HCV antibodies, patients who had suspected chronic liver disease of viral C etiology and patients who have signed informed consent. The study included 22 women, or (43.1%) and 28 men or (56.9%). Quantitative determination of HCV-RNA-PCR and copy number and genotyping were done on virology.

Percutaneous biopsy of the liver should provide analysis of liver tissue in a cylinder of length of at least 20 mm. Following the standard and special staining methods (PAS, PAS-D, Ganor, Van Gieson, Masson trichrome) will determine the degree necroinflammatory activity and stage of fibrosis in the liver using the classification by Ishak et al.

(1). We performed a statistical analysis of the groups using appropriate statistical methods for the obtained parameters.

Results are presented in charts and tables. Data were analyzed using: structural analysis, appropriate charts, descriptive statistics, correlation analysis and chi-square tests.

3. RESULTS

The sample consisted of 50 patients, of whom 28 (54.9%) were male and 22 (43.1%) females. Patients were divided into 6 age groups, the first group age from 25-29 years, second group from 30-34 years, a third group from 35-39 years, fourth group from 40-44 years, the fifth group from 45-49 years and the sixth group from 50-55 years. In the group of patients from 25-29 years of age there were 4 patients or 8%, in the group from 30-34 years were 6 patients or 12%, in group from 35-39 years were 13 patients or 26%, in group from 40-44 years were 12 patients or 24%, in a group from 45-49 years were 10 patients or 20%, in the group from 50-55 years there were 5 patients or 10% (Table 1).

Age	No. of patients	%
25 - 29	4	8.00
30 - 34	6	12.00
35 - 39	13	26.00
40 - 44	12	24.00
45 - 49	10	20.00
50 - 55	5	10.00
Total	50	100.00

TABLE 1. The age structure of the respondents

We took the structure of patients by indicator of histopathological changes - grade, where we see that the grade 0.0556 have 2 or 4% of patients, grade 0.1667-1 patient or 2%, grade 0.2222 has 1 patient or 2%, grade 0.2778 have 3 patients or 6%, grade 0.3333 have 2 or 4% of patients, grade 0.3889 has 1 patient or 2%, grade 0.4444 has 1 patient or 2%, grade 0.5000 has 2 patients or 4%, grade 0.5555 has 1 patient or 2%, grade 0.6000 has 1 patient, or 2%, grade 0.6111 has 2 or 4% of patients, grade 0.6667 have 2 patients or 4%, grade 0.9375 has 1 patient or 2%, grade 1.000 has 15 patients, or 30%, grade 2.000 has 8 or 16% of patients, grade 3.000-7 patients.

Thus it follows that the sample is

dominated by patients with a grade in range 0-1 (70%), and within these categories has the most patients with grade 1. Taken was the structure of the sample according to the histopathological changes-stage, where we see that the stage 0.2500 has 9 patients or 18%, with stage 0.5000-3 patients or 6%, the stage 0.6667 has 1 or 2% of patients, with stage 1.000 are 20 patients or 40%, with stage 2.000 are 10 patients or 20%, with stage 3.000 are 6 or 12% of patients, stage 4.000 has 1 or 2% of patients, all of which points to the conclusion that the sample is dominated by patients with stage in interval from 1 and 2 (60%), and in that category are the most patients with stage 1.

Structure of the sample was taken according to the histopathological changes-scores, where we see that the score 0.25 had 1 patient or 2%, with the score 0.50 there were 11 patients or 22%, with a score 1.00 there were 13 patients or 26%, with the score 2.00 there was 13 patients or 26%, with a score 3.00 there were 9 patients or 18%, with a score 4.00 score was 2 or 4% of the patient and with the 6.00 was 1 or 2% of patients, all of the variable scores indicate the heterogeneity of the sample and relatively uniform structural distribution of patients in the sample.

Structure was taken according to the origin of the virus from which the drug addiction was represented by 10 patients or 20%, of unknown origin 7 patients and via blood transfusion 33 patients or 66%, where we see that the sample was heterogeneous, although the largest proportion of patients gets the virus through blood transfusion

Descriptive analysis of variables and testing the assumptions of normality in the complete sample

We conclude that:

- Significantly high values of the parameter range (as the difference between the highest and lowest values of the variables analyzed);
- Significant deviations between the mean, median and modal values;

Significantly high values of the coefficient of variation (especially for HCV-RNA-PCR and copy number), except for the age variable, we can conclude that the distribution of all the variables that

significantly deviate from normal distribution by the coefficient of skewness, we conclude that all have the right (positive asymmetry). The above also points to the heterogeneity of the sample, the presence of outliers and marked variability in the analyzed sample.

The data confirms the conclusions of the previously presented structural analysis:

- According to the variable grade largest number of patient in the sample has a grade 1;
- According to the variable stage most of the patients in the sample has a stage 1;
- According to the variable scores the highest number of patient in sample have a score 1 or 2

We can conclude that most of the patients in the sample according to the values for the number of copies are in the range from 0 to 1.08×10^9 , with a presence that has value for the number of copies greater than the value of that interval. The test result is normal for the value of 3.346 with p value $0.000 < 0.005$, which means that the assumption of normality is not satisfied.

For the variable grade concentration is about values in the range 0-1, however, is represented in the sample and a portion (30%) of patients with grade 2 or 3. The result of Kolmogorov-Smirnov normality test with z value of 2.053 and with p value $0.000 < 0.005$, which means that the assumption of normality is not satisfied.

For the variable stage once again is present the concentration values in the range about 0-2, but the sample is represented by one part (14%) of patients with stage 3 or 4. The test result is with z value of 2.095 with p value $0.000 < 0.005$, which means that the assumption of normality is not satisfied.

According to the scores of variables from a sample of patients are relatively evenly distributed.

Evidently, the presence of (one patient with extremely high scores 6).

The result is normal for the value of 1.595 with p value $0.012 < 0.005$, which

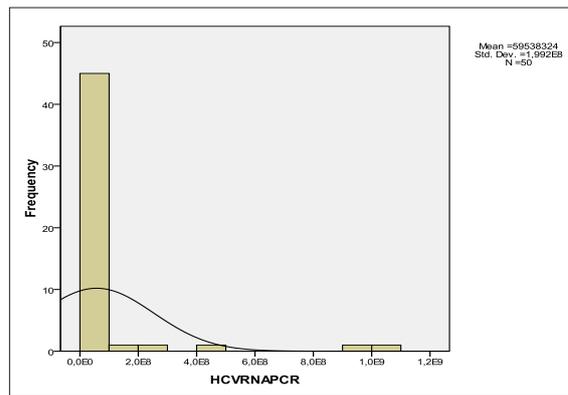


FIGURE 1. Variable—HCV-RNA-PCR in comparison to the normal distribution curve

means that the assumption of normality is not satisfied.

According to the variables age of the patient sample are relatively evenly distributed. The presence of outliers is not evident. The test result is normal for the value of 0.569 with p value of $0.902 > 0.005$, which means that the assumption of normality is satisfied.

4. DISCUSSION

Chronic hepatitis C of viral etiology is a progressive disease that leads to liver cirrhosis and hepatocellular carcinoma. In 50-80% of patients who developed persistent infection, spontaneous release of the virus is rare. There are also cases that the patient is carrier of the virus for decades, and histopathological findings show small changes. However, most patients with chronic active liver disease have hepatitis C progression after 10 years with liver inflammation and fibrosis within the liver cirrhosis. In some patients with hepatitis C virus causes progressive fibrosis weakening, so it take a longer period of time, measured in decades until lead to cirrhosis.

In the observed sample, we had 50 patients suffering from chronic hepatitis C of viral etiology. 28 patients were male 56% and 22 were women 44%. Following investigations by other authors (18, 19, 20, 21), we conclude that other researchers have had more men in their samples to compare with our study there was no deviation. According to the age structure the sample was heterogeneous, so the age ranged from 27-55 years, provided that the women were older age.

Women in the observed sample

were older so that the duration of the infection was longer. In comparison to other researchers (23, 24, 25), we noticed that male patients had an older age. Thus the duration of infection was higher so that the complications were more common in men (22).

Svirlih and associates (16) examined the prevalence of hepatitis C genotypes in Serbia and Montenegro. The conclusions were that genotypes 1b and 3a were predominant and subtype 1b was predominant in older patients. 1b had higher HCV-RNA-PCR and histological activity is stronger which suggests that the infection was long lasting.

Silva Faria et al (15) have also come to the conclusion that the age, duration of infection and HCV-RNA copies correlated with the degree of fibrosis in patients infected by blood transfusions.

When we look at the number of copies of HCV RNA there is a statistically significant difference between groups formed on the basis of age. Since the $p < 0.05$ it means that HCV-RNA has a higher value in the older age group. If we compare the results of studies with results of other researchers (23, 24, 25) we can conclude that the results do not differ from theirs, with the observations that in our group women were older.

The virus infections by origin were divided into three groups: infection through blood transfusion, intravenous drug addict's population and infection of unknown etiology, comparing age groups $p = 0.902$ and stage of fibrosis $p = 0.000$, statistically significant differences in relation to the origin of the virus because $p < 0.05$. In patients who were infected through blood transfusion stage of fibrosis was significantly higher. If we look at the results by Svirlih and colleagues (16) they came to the same conclusion, or the stage of fibrosis is associated with duration of illness.

In older patients who carry the virus for longer time $p = 0.045 < 0.05$, meaning that the variable HCV RNA is associated with patient age and duration of infection.

Our results show that higher concentration of HCV RNA is associated with higher stages of fibrosis. Compared with researches by Mendes and colleagues (24) and Okkaya and colleagues (25), we can conclude that their

results are similar to ours and that the variable grade is not affected by concentrations of ALT, AST, and HCV-RNA-PCR. The results coincide with research of Pradat and associates (13). In female patients, stage of fibrosis was higher in comparison to men, because it is related to age and duration of infection so stage is higher in patients who were infected through blood transfusions.

Variable scores were significantly dependent variables of ALT and AST. Evident is the influence of gender and age. The variable stage is significantly dependent on AST $p=0.017<0.05$, ALT $p=0.035<0.05$ and HCV-RNA copies $p=0.017$. Gender and the origin of the virus infection affecting the stage. Similar results were obtained by other researchers. When we look at the variable grade as the dependent variable based on the correlation matrix, we can conclude that its value does not affect any one of the independent variables: AST, ALT, HCV-RNA-PCR or the number of copies. Not present significant influence of sex, age or origin of the virus on a variable grade.

For the variable stage we notice significant dependence of the variables ALT and AST. There is also a significant effect of gender, age and origin of the virus. In female patients, the stage is higher than in male patients. Older patients (who carry the virus for longer) have a higher stage. In patients who received virus by transfusions the is higher compared to patients who have received the virus as an intravenous drug users.

For the variable score was present significant correlation of the variables ALT and AST. There is also a significant effect of sex and age: female patients have higher score than male patients. Older patients (who carry the virus for longer) have higher scores.

5. CONCLUSIONS

- Both applied methodologies (rank correlation and chi-square test) confirmed that the main hypothesis of the viral load of HCV-RNA and histopathological activity in the liver parenchyma is in significant interdependence in patients with chronic hepatitis C.
- In correlation with the age of

HCV-RNA concentrations and viral load was higher in women.

- The stage of fibrosis and necroinflammatory score was higher in the group of women in relation to a group of men, because they are older and longer carry the virus.
- Patients who were infected by transfusion of infected blood had a higher stage of fibrosis, because the infection lasted longer.
- The highest stage of fibrosis had patients who did not know when they are infected with hepatitis C.
- Patients who were infected through blood transfusions had a higher copy number of HCV-RNA.

Conflict of interest: none declared.

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