Hepatitis and biochemical markers in correlation with alpha-fetoprotein as a diagnostic indicator for the HBV and HCV differentiation

Damir Suljevic[1], Lejla Mehinovic[2], Andi Alijagic[1]

[1]Department of Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina;
[2]Department of Clinical Pathology, Clinical Center, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

Corresponding author: Andi Alijagic
Address: University of Sarajevo, Sarajevo, Bosnia and Herzegovina
E-mail: andialijagic@gmail.com

Abstract

Aim: The number of HBV and HBC patients is growing every day, with the dominance of HBV patients. The aim of this research work was to analyze biochemical and liver tumor parameters in HBV and HCV patients, including AFP correlation with other blood parameters in order to determine the diagnostic parameters important for differentiation of HCV and HBV.

Methods: The research included 48 patients with HBV and HCV during the period 2010-2013 at the University Clinical Center of Sarajevo. The analysis includes biochemical parameters and liver markers, CT, ECHO, MRI, cytology and pH findings.

Results: Most of the affected patients were middle-aged. There were no significant differences between HBV and HCV patients. AST, ALT, γGT, AFP and bilirubin were high in all patients. There was a correlation between the AFP and AST ($r=0.383$, $p<0.05$) and ALT ($r=0.501$, $p<0.01$). Test normality showed significant differences for most parameters ($p<0.05$). In all HBV patients there was anti-HBc (100%) and HBsAg (89.28%), while in HCV patients the anti-HBc (57.14%) dominated. Sex-specific analysis showed almost significant differences for γGT ($P=0.059$).

Conclusion: Increased serum bilirubin, AST, ALT and γGT are present in hepatitis. AFP and γGT are important biochemical markers of hepatitis. Anti-HBc and HbsAg are sufficient for HBV diagnosis, whereas for HCV there should be used biochemical and immune parameters (anti-HBc).

Keywords: alpha-fetoprotein, HbsAg, HBV, HCV, hepatic markers.

Introduction

Cirrhosis is the final stage of several types of chronic liver damage. The main pathological features are irreversible chronic damage of parenchyma accompanied by the formation of fibrosis and regenerative nodules (1). The formation of connective tissue deposits, nodular regeneration of parenchyma and distortion of vascular trough are microscopic features of this disease. In nodules, regenerating hepatocytes are arranged without any order, and various changes can be detected: focal atrophy, necrosis, dysplasia and hyperplasia and malignant alteration (1,2). The most common epidemiological causes of liver cirrhosis are excessive alcohol consumption (in 50-80% of cases) (3), and hepatitis B and C (in 10-30% of cases) (4). Important causes also are primary biliary cirrhosis (5-10%), hemochromatosis (2-5%) and Wilson's disease (about 1%) (5). Hepatitis B virus (HBV) infection causes acute and chronic liver disease and in chronic it carriers 100 times bigger risk of developing HCC (6). HBV contains a small partially double-stranded DNA and belong to the Hepadnaviridae family. Although still under discussion, it is considered that HBV can have direct and indirect effects on hepatocytes (7). The first direct effect is that the viral DNA can integrate into the genome of hepatocytes, causing disorder of chromosomal stability which leads to chromosomal rearrangements or deletions. Integration of HBV DNA can also lead to deregulation of the oncogenes or tumor suppressor genes expression involved in cell survival or cell death (7). Other direct effect of HBV can be attributed to 154 - amino acid (16.5 kDa) virus HBX protein, which transactivates genes involved in the control of cell proliferation, and deregulation of the cell cycle control, DNA repair and apoptosis (8). Hepatitis C virus (HCV) has recently been characterised as the leading cause of cirrhosis and HCC in developed countries (9). HCV belongs to the genus Hepacivirus, Flaviviridae family (6). Unlike HBV, HCV is an RNA virus and does not integrate into the host genome, but HCV infection can cause accumulation of abnormalities in the degeneration-regeneration process. HCV nuclear proteins and non-structural proteins NS3 and NS5A inhibit post-transcriptional expression of the cyclin-dependent kinase inhibitor, which plays an important role in cell-cycle control (10). HCV core protein has a particularly important role by modulating cellular proliferation, apoptosis and the immune response. This protein acts as transcriptional regulator of various cellular genes involved in the regulation of cell growth, including the c-myc proto-oncogene. HCV core protein can also induce carcinogenesis by other mechanisms, such as inhibition of several apoptosis activators, usually Fas and TNF-α (tumor necrosis factor-α) (10,11). Alcohol is an important cofactor in patients with HCV infection. Surprisingly high incidence of this infection (up to 55.5%) was found in alcoholics (12). Patients with HCV infection and simultaneous alcohol abuse develop severe fibrosis and have higher rates of cirrhosis than those who do not consume alcohol (12). In these patients have been reported higher rates of HCV replication and the inhibition of hepatic expression of Bcl-2 resulting in an enhancement of apoptosis and severe hepatic impairment (12). However, the dominant mechanism for the synergy between the alcohol and HCV infection seems to be an increase in oxidative stress. HCV core protein binds to the mitochondrial membrane, changes its permeability and promotes oxidative stress. Ethanol potentiates this mitochondrial injury by further increasing of reactive oxygen species (ROS) production and oxidation of hepatic glutathione. Moreover, the alcohol and the HCV core protein act synergistically in the induction of lipid peroxidation and enhancement of the liver expression of TGF-β and TNF-α (12). Complications of cirrhosis are portal hypertension, bleeding from esophageal varices (in 20-30% of patients with cirrhosis), ascites (most common complication), spontaneous bacterial peritonitis, hepatorenal syndrome, hepatopulmonary

syndrome, hepatic encephalopathy, cardiomyopathy and hepatocellular carcinoma (1,2,13). Treatment of patients with liver cirrhosis involves inhibition of etiological factors (e.g. abstinence from alcohol and treatment of chronic viral hepatitis), avoidance of additional risk factors and prevention of complications (AFP screening every six months, endoscopy, β-blockers, control of diuresis, treatment of infections, control of electrolyte and nutritional support). End stage of cirrhosis is treated by liver transplantation (1,2,5). For assessment of survival Child-Pugh score and MELD-score are the most commonly used (1).

The aim of this study was to analyze the biochemical parameters of liver and tumor markers with special emphasis on AFP in order to determine diagnostic parameters important for HCV and HBV as prognostic parameters.

Methods

Subjects

In this study we analyzed 49 patients with cirrhosis of viral B and C etiology (28 HBV and 21 HCV patients) who are under continuous AFP screening, HCC still unproved. This study was performed in the period January 2010 – March 2013 at the Clinic of Gastroenterohepatology, University Clinical Center, Sarajevo. There was selected a homogeneous sample of respondents in order to reduce multi-causality of the observed parameters. Approval for the study was given by the Ethics Committee of University Clinical Center Sarajevo. All patients were clinical, biochemical and histopathological treated.

Clinical, biochemical and pathohistological analysis

During hospitalization, all patients underwent liver function tests: bilirubin (total, direct and indirect, refferent values 6.8-20.5 mmol/L) and the value of enzymes: aspartate aminotransferase - AST (≤ 38 U/L), alanine aminotransferase - ALT GT (≤ 48 U/L) gamma-glutamyl transferase - gGT (11-55 U/L) and alkaline phosphatase - AP (60-142 U/L). Patients underwent immunological tests for hepatitis markers B and C (HBs Ag, anti-HBs, anti-HBc, Anti-HCV and anti-HBe), abdominal ultrasonography (US) and computed tomography (CT). All the patients were quantified for serum AFP (≤ 7 ng/ml) as a tumor marker. Bilirubin concentration and enzyme activity were determined by analyzer VITROS 5600 Integrated System (Ortho Clinical Diagnostics, USA). For hepatitis markers detection ELISA method was applied, while the concentration of AFP was determined using chemiluminescent microparticle immunoassay ARCHITECT AFP assay - (CMIA, Ireland).

Statistical analysis

Data were analyzed by using SPSS Version 17 software to determine the mean values of different tumor markers and biochemical parameters and to assess the presence of significant difference in the above values with regard to factors supposed to cause variation. The 95% confidence interval and 5% absolute precision were employed for the analysis of variance test (ANOVA). Nonparametric Spearman's test was used for the analysis of correlation, while the normality distribution was determined by Kolmogorov-Smirnov and Shapiro-Wilk tests.

Results

Table 1 shows the percentage ratio of HBV and HCV patients by age. Patients were divided into four groups; young-aged group (age between 18 and 40; n=1), middle-aged group (age between 41 and 64; n=28), old-aged group (age between 65 and 75; n=13), and very old-aged group (≥76, n=7). The largest number of affected patients of both groups belonged to the category of middle-aged, followed by a group of old-aged patients.

In addition to the analysis of biochemical parameters, ultrasonography, CT and MRI of the liver (Imagining Methods), as well as cytology and pH findings were analyzed. The largest number of infected patients had liver cirrhosis.

Statistical testing of biochemical parameters HCV

Table 1. Percentage ratio of HBV and HCV patients

Type of patients	Young-aged (18-40 years)	Middle-aged (41-64 years)	Old-aged (65-74 years)	Very old-aged (≥75 years)
HCV	0	15 (71.42%)	4 (19.04%)	2 (9.52%)
HBV	1 (3.57%)	13 (46.42%)	9 (32.14%)	5 (17.85%)
Total	1	28	13	7

and HBV patients is presented tables 2-5. Table 2 presents the biochemical parameters of 21 patients with hepatitis C virus (males and females) including the mean and standard deviation. In addition, analysis of variance (ANOVA) between men and women was also performed and the resulting p-values are presented. All HCV patients had increased values of bilirubin, except for direct bilirubin in females. We detected very high values of AFP, especially in males. Analysis of enzyme activity showed that the AP value was in the reference interval, a very high value was characteristic for AST and γGT, while ALT was slightly increased, with higher values in males. All the analyzed parameters had higher values in males against females. The largest variations were typical for values of AFP, AST and γGT. Between males and females there was a borderline statistically significant difference (P=0.059) for values of γGT.

Table 2. Biochemical parameters of HCV patients (males and females)

Parameters	Total (n=21)	Males (n=8)	Females (n=13)	P-value
Total bilirubin	37.26±27.79	44.42±30.97	30.90±22.66	0.265
Direct bilirubin	23.88±13.79	27.66±15.07	19.15±8.66	0.182
Indirect bilirubin	37.39±16.67	37.86±14.33	36.80±19.95	0.476
AFP	59.62±135.03	78.85±175.27	42.62±83.35	0.409
AST	91.29±71.69	121.44±85.86	69.85±43.13	0.089
ALT	57.67±38.08	70.89±31.87	48.69±37.91	0.174
γGT	89.05±88.45	128.11±114.57	57.08±35.83	0.059
AP	117.90±43.28	119.89±57.13	113.08±26.11	0.73

Table 3 shows the results of biochemical parameters of 28 patients (including males and females) with hepatitis B virus diagnosis. The analysis included the mean, standard deviation and ANOVA. P values show statistically significant differences between males and females for HBV patients, and P values in the last column represent differences between HCV and HBV patients. For HBV patients we detected higher bilirubin concentration compared to HCV patients, and high variations were found for indirect bilirubin in females. These patients also had increased values of AFP, however they were much less compared to HCV patients. Also, AP values were within referent range. For AST, ALT and γGT, there were observed high values. HBV patients had higher levels of enzymatic activity in comparison to HCV patients. The values of all enzymes in females were higher compared to men, especially AST and γGT. The greatest variations in HBV patients were also present for the value of γGT. However, between HBV and HCV patients, as well as between males and females, there was no statistically significant difference.

Table 3. Biochemical parameters of HBV patients (males and females)

Parameters	Total (n=28)	Males (n=17)	Females (n=11)	P-value	
	Mean ± SD	Mean ± SD	Mean ± SD	Male-female (HBV)	HCV/HBV
Total bilirubin	48.24±37.95	46.06±41.75	51.33±33.35	0.723	0.229
Direct bilirubin	31.95±30.14	32.94±35.54	30.59±22.97	0.872	0.172
Indirect bilirubin	47.81±74.50	27.76±17.08	75.36±111.16	0.176	0.284
AFP	20.33±55.11	26.11±71.53	12.13±12.79	0.511	0.462
AST	122.2±180.8	90.18±63.06	167.67±271.3	0.263	0.452
ALT	88.5±114.95	84.00±105.33	94.92±131.99	0.806	0.236
γGT	126.8±201.6	101.71±122.8	162.50±281.4	0.434	0.389
AP	126.5±109.4	114.41±132	143.67±67.71	0.488	0.695

Correlation between AFP and other biochemical parameters as shown in Table 4 was mostly positive (except ãGT in HBV and total bilirubin and AST in HCV patients). A statistically significant correlation was observed in HBV patients with AST ($p < 0.05$) and ALT ($p < 0.01$).

Table 4. Nonparametric Spearman's correlation test between AFP and biochemical parameters

Parameters		Total bilirubin	Direct bilirubin	Indirect bilirubin	AST	ALT	γGT	AP
HBV	**r**	0.139	0.049	0.351	0.383	0.501	-0.065	0.104
	P	0.480	0.842	0.141	0.04*	0.07**	0.744	0.599
HCV	**R**	-0.42	0.619	0.643	-0.002	0.112	0.107	0.253
	P	0.864	0.102	0.086	0.993	0.628	0.644	0.283

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

Table 5 presents the percentage ratio of patients with positive and negative findings for hepatitis markers, as well as their reference range. All HCV patients did not have a positive test for HBs Ag and anti-HBs. Most important diagnostic immunological liver marker was anti-HBc (57.14%). However, HBV patients usually have a present and the high percentage of almost all markers (except for anti-HCV). Anti-HBc was a marker present in all HBV patients (100%). Kolmogorov-Smirnov normality test (for samples of >50 individuals) and Shaphiro-Wilk normality test (for samples of ≤50 individuals) for HBV and HCV patients including males and females were performed. Most biochemical parameters in HBV patients did not have normal distribution.

Table 5. The link between hepatitis markers for HBV and HCV patients

	Parameters	HBs Ag (0.00-0.99 S/CO)	anti HBs (0.00-9.99 mLU/mL)	anti HBc (0.01-0.99 S/CO)	Anti HCV (0.00-0.99 S/CO)	Anti Hbe -
HBV	**Reactive**	89.28%	68%	100%	45.55%	84.21%
	Nonreactive	10.71%	32%	-	55.55%	15.78%
	Range	0.18-5204.5	0.01->1000	0.14-14.47	0.05-13.65	0.02-1.35
HCV	**Reactive**	30%	38.09%	57.14%		
	Nonreactive	70%	61.90%	42.85%	-	-
	Range	0.25-0.44	0.98-871.84	0.13-12.26		

Discussion

HBV and HCV viral infections are the dominant infectious disease among the world's population. In developed countries, HCV infection is more prevalent compared to HBV infection. Both diseases make important candidates for public health in terms of prevention, early diagnosis and treatment (14). Individuals with HBV and HCV have a high risk for development of cirrhosis and hepatocellular carcinoma in the end. In this study, we analyzed the biochemical and immunological parameters of patients with HBV and HCV viral etiology with the aim of identification of the most important liver markers for differentiation the hepatitis type, the prognosis and course of the disease. Serum bilirubin is one of the earliest known markers of liver. But as its activity increased in other liver diseases too, bilirubin is not sufficient for diagnosis. The concentration of bilirubin in our study is very high as referred in other studies (16). Damage of liver cells cause the release of large amounts of bilirubin which increase levels of serum bilirubin. The ratio of direct and indirect bilirubin depends on the degree of hepatocytes damage. In most patients there were observed necrosis of hepatic tissue, and thus much increased serum bilirubin. However, the bilirubin is not sufficient for diagnosis, but has great diagnostic significance.

In clinical practice, the level of AFP is elevated in a variety of clinical situations, including hepatocellular carcinoma, acute or chronic viral hepatitis, chronic liver disease and gonadal tumors (17). Elevated levels of AFP are used as a marker of liver regeneration after destruction of hepatocytes in viral hepatitis and as a dependent predictor for the patients with HBV and HCV (18). AFP is typically produced during fetal neonatal development of liver, and its concentration decreases after birth when it is present only in trace amounts (less than 10 ng/mL) (19). There is a much more records that AFP levels are significantly raised in anti-HCV-positive patients in comparison with HBsAg-positive patients (17). Serum AFP levels were higer in HCV (59.62 ± 135.03 ng/mL) and HBV (20.33 ± 55.11ng/mL) patients in comparison with referent values ($\leq$7 ng/mL) and there were a non significant difference in HBV patients compared to HCV ($p>0.05$). Increased values of AFP in HBV and HCV patients were reported in the earlier studies (18,21).

We detected a very high value of enzymes AST (91-122 U/L), ALT (57-88 U/L), AP (117-126 U/L) and γGT (89-126 U/L) in HCV and HBV patients. Compared with previous studies (18) our values are significantly higher. Increase in enzyme activity may be a result of the destruction of liver cells and the release of enzymes and increase in their serum concentration. High levels of ALT is the most specific indicator of liver damage (25). It is proved that necrosis of hepatocytes is not necessary the main reason for the release of serum amino-transferases and that the correlation between the levels of liver aminotransferases and degree of hepatocyte damage is weak (26). While some studies detected normal histological findings in patients with normal ALT levels (27), other studies have shown that viral load in patients with normal ALT levels was associated with liver injury (28). On the other hand, increased serum transaminase activity is present especially in patients in which case infection was associated with other diseases. Generally, the AP is usually not changed in HCV infection, but there was a significant increase in γGT in liver cirrhosis, which was also detected in our study (29). Liver ultrasonography showed the prominent and coarser structural changes in HBV compared to HCV patients.

In all patients there were observed the cirrhotic changes in the liver, so the enzyme's activity is in correlation with the degree of the cell's integrity. Therefore, it is evident presence of correlation between the anatomic changes of hepatocytes and the enzyme's activity. Previous studies had shown (22) that bilirubin AST, ALT and ALP correlate with liver damage, while their values correlated with serum HCV RNA were present only in the case of severe stages of fibrosis, but not in

inflammatory processes. Elderly people who are positive for HBV have higher levels of serum bilirubin, transaminases, and a significantly higher prevalence of negativity in HBsAg and anti-HBs (23). Increased levels of bilirubin (particularly indirect) and transaminases (particularly γGT) may suggest to hepatitis, but its type can be identified only by using immunological markers.

The positive correlation between serum AFP and the other biochemical parameters refers to the role of AFP in the pathogenesis of chronic liver disease. Until today many markers were identified and used in the diagnosis and monitoring of disease progression, but serological test cannot clearly diagnose infection. For example, positive HBsAg is a sign of HBV infection but also negative HBsAg can not exclude HBV infection. So far, definitive diagnosis of HBV liver infection relies on a combination of serologic, biochemical and histologic tests. Invention of new hepatic markers will contribute to reduction the number of biopsies. During HBV infection, clearance of hepatitis B surface antigen (HBsAg) is a key event, which implies that the host is not immune tolerant for a long time and enters the low phase of replication (24). Therefore, most of HBV patients (89.28%) have present this antigen, and it could be considered as major marker in HBV positive patients. The presence of HBsAg is a sign of HBV infection and is considered as the first serological marker of acute hepatitis. Its serological presence of more than six months suggests the beginning of chronic HBV infection. The simultaneous presence of this antigen with other clinical and biochemical features usually indicates the occurrence of acute infection in patients with low endemic area, but not in patients with high or medium-endemic regions (20). Chronic HBV infection begins as an acute infection and is characterized by the presence of HBsAg and HBeAg in the serum. The presence of HBeAg indicates continuous viral replication and also indicates a high viral load and infectivity and can last several months or even years. People who are HBeAg positive better respond to antiviral drugs. Our results are comparable with previously presented studies whose results pointed to a large percentage of HBsAg and anti-HBe patients (15). All HBV patients had detected anti-HBc, whereas only 57% of HCV patients had a positive anti-HBc and HBsAg in 30% of patients. Therefore, diagnosis of HCV based on immunological markers is very difficult and unreliable.

Increased levels of serum bilirubin, AST, ALT, gGT can lead to the diagnosis of hepatitis. However, AFP together with γGT have greater diagnostic significance and they can be considered as the most important biochemical markers of hepatitis. For the diagnosis of HBV, anti-HBc and HbsAg are sufficient, while HCV diagnosis requires the combination of biochemical markers and immunological tests, especially anti-HBc.

Conflicts of interest: None declared.

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