Comparative Analysis of Clinical and lab parameters of Patients with Hepatitis C Virus-Related Cirrhosis and Hepatitis C Virus-Related Hepatocellular Carcinoma

Abstract

Objective: We attempted to identify risk factors for the development of HCCby comparing the parameters of patients with HCV related liver cirrhosis without HCC (HCV-LC-without HCC) and those with HCV related liver cirrhosis plus HCC (HCV-LC-HCC).

Material and methods: We retrospectively analysed our database of 40 patients with HCV-LC without HCCand 42 patients with HCV-LC-HCC. A diagnosis of cirrhosis was based on the results of histological examination and/or elastography. HCC was diagnosed histologically or by the detection of consistent findings using at least two imaging techniques from among US, CT, or MRI. Hepatitis C virus infection was detected by enzyme linked immunosorbent assay (ELISA). Other baseline laboratory investigations such as CBC, liver function tests, including serum total bilirubin, ALT, AST, GGT, ALKP, AFP and serum albumin levels, and serum creatinine levels were also measured.

Results: HCC patients had significantly higher serum levels of NLR, PLR, GLR, ALKP and AFP and lower albumin levels, than the non-HCC patients. For the HCC patients, 33.3% had low AFP levels, 35.7% had macroscopic PVT and only 48.1% had a tumor diameter <5cm. Patients with high AFP, PVT and large tumor size had characteristic differences from those with low AFP, absent PVT and smaller tumor size.

Conclusions: Older age, male gender, increased levels of NLR, PLR, GLR, ALKP, AFP and decreased levels of albumin in HCV-related cirrhosis are associated with an increased risk of HCC. In addition, high levels of ALKP, very high levels of AFP (>1000 IU/ml), and presence of large (≥5 cm) tumors increase suspicion of presence of portal vein thrombosis. Consideration of these indicators in routine monitoring may be useful in early diagnosis and treatment of HCC in HCV-related cirrhosis.

Keywords: Carcinoma, Hepatocellular, Liver Cirrhosis, Hepatitis C

Introduction

HCC is the fifth most common malignant disease worldwide [1]. The cause of HCC in most patients is liver cirrhosis, especially HCV-related liver cirrhosis [2]. More than 150 million people are infected with HCV every year and approximately 1/3 of patients die from complications of HCV-related liver cirrhosis [3]. The risk of HCV infection has been significantly reduced as patients achieve a SVR with antiviral drugs[4]. Nevertheless, patients with cirrhosis are at high risk for HCC even after HCV clearance[5,6]. Chronically infected patients may develop liver diseases such as steatosis, fibrosis, cirrhosis, and eventually HCC.In most cases, the disease is diagnosed at advanced stage [7].

Many studies have identified the importance of increasing parameters such as ALP and GGT [8], AFP[9,10], NLR andPLR[11,12,13] in HCC prognosis. In addition, there are other factors that affect the prognosis of this disease: tumor number, tumor size, and macro-vascular invasion.

Our study aims to identify risk factors for the development of HCC among our patients by comparing the clinical features of patients with HCV-LC without HCC and patients with HCV-LC with HCC.

Methods

Clinical:

We retrospectively analysed our database with HCV-LC patients with or without HCC who presented between January 2016 and October 2021. All patients gave their written informed consent to participate in this study examining the natural history of their disease. This study conforms to the ethics guidelines of the Declaration of Helsinki and was approved by University's Ethical Committee under number 24 on December 09 2022.

The study included 40 patients (20 women and 20 men) with HCV-LCwithout HCC and 42 patients (12 women and 30 men) with HCV-LC-HCC. Data on basic demographic characteristics were collected such as age, sex, height and weight, as well as common clinical complete blood count and liver function laboratory parameters and descriptors of the baseline radiological abdominal imaging.

A diagnosis of cirrhosis was based on the results of histological examination and/or elastography: liver stiffness was above 14 kPa. Severity of cirrhosis was assessed using the Child-Pugh classification system[14]. HCC was diagnosed histologically or by the detection of consistent findings using at least two imaging techniques from among US, CT, or MRI according to recommendations of Western guidelines[15,16]. Vascular invasion by tumor was assessed by US, dynamic CT and angiography. HCC characteristics included the number of tumor nodules; the maximum tumor diameter and presence or absence of macroscopic portalvenous invasion.

Hepatitis C virus infection was detected by enzyme linked immunosorbent assay (ELISA). Allpatients were positive for HCV RNA by a quantitative polymerase chain reaction assay. Other baseline laboratory investigations such as CBC (complate blood count), parameters, liver function tests, including serum total bilirubin, ALT, AST, GGT, ALKP, AFP and serum albumin levels, and serum creatinine levels were also measured.

A complete history was obtained, and a physical examination was carried out for all patients. After the initial treatment for HCV, patients with HCV-LC without HCC were scheduled to be monitored regularly at least every 3-6 months via clinical examinations, serum liver function tests, AFP and US. CT or/and MRI were performed in patients with suspected HCC based on tumor markers and/or US.

Statistical:

Analysis was performed with the Statistical Package for Social Sciencesversion 20.0 software (SPSS Inc., Chicago, IL, USA). Patients in both groups were followed until they died and were censored at the time of their last clinic visit. The patient who underwent liver transplantation was followed up until the day of transplantation, which was handled in the same way as the day of death. Categorical data such as gender were presented as frequency and percentage. Quantitative data such as age and laboratory investigations among HCV-cirrhosis and HCV-LC-HCC groups were compared by using student t-test. A P value of 0.05 or less was considered statistically significant.

Results

The study included 40 patients with HCV-related cirrhosis and 42 patients with HCV-related cirrhosis plus HCC. The median age of the patients with HCV-LC without HCC was 54 years (range of 40–78 years), and that of patients with HCV-LC-HCC was 60 years (range of 44–81 years). The HCV-LC-HCC group were predominantly males (71.4%).

Liver and inflammatory tests in cirrhosis versus cirrhosis plus HCC patients.

Table 1 shows a comparison of medians of laboratory parameters between HCV-LC without HCC and HCV-LC-HCC patients. Using the student's t-test, we found that neutrophil counts, NLR, PLR, GLR, as well as serum levels of albumin, ALKP and AFP were statistically significantly different in the HCV-LC-HCC group ($p<0.05$) as compared with HCV-LC without HCC group.

Serum AFP levels.

Serum AFP levels, tumor-associated PVT and MTD are amongst the most-studied aggressiveness factors for HCC behavior. They were then examined individually (Tables 2, 3 and 4). Fourteen (33.3%) patients had AFP values of ≤ 100 IU/ml, eight (19.1%) patients had very high AFP levels (>1000 IU/ml) and the remaining 26.2% of patients had intermediate AFP levels. The clinical characteristics of patients with high versus low AFP levels were then compared (Table 2). Significant differences were found in blood WBC and neutrophil levels, as well as for levels of total bilirubin, albumin and ALKP. There were also large differences in levels of CRP, although it did not achieve statistical significance. Interestingly, the low AFP group had 7.1% of patients having PVT and the high AFP group had 62.1% of patients with PVT ($p= 0.059$).

Incidence of PVT and PVT patient characteristics.

PVT was then examined and 35.7% of HCC cases were PVT positive. PVT positive and negative patients were then compared (Table 3). PVT positive patients had much higher levels of serum total bilirubin, ALKP, AFP, as well as MTD, in comparison to PVT negative patients, although statistical significance was reached only for ALKP and AFP levels. All the other parameters of Table 1 were also examined, but no differences between the 2 groups were found.

Incidence of MTD and clinical differences in small and large MTD patients.

Maximum tumor diameter (MTD) was next evaluated. Only 48.1% of patients had tumors <5cm MTD and the remainder (51.8%) had larger tumors, reflecting an advanced stage of most of our patients (69.1%). The large and small MTD groups were then compared (Table 4) and much higher levels of serum AFP were found, as well as percent of patients with PVT, in the large size tumor patients (no PVT in small MTD group). All the other parameters of Table 1 were additionally examined, but no differences between the 2 groups were found.

Discussion

In this study the comparison of clinical and laboratory parameters between HCV-LC patients with and without HCC revealed the following: 1) HCC is more common in elderly men; 2). NLR, PLR, GLR, ALKP and AFP levels are significantly increased and albumin level is decreased in HCC patients; 3). the ALKP levels are high and AFP levels are very high in patients with portal venous thrombosis and presence of large (≥5 cm) tumors.

Chronic HCV infection is the most common underlying liver disease among patients with HCC. Several factors may be responsible for the development of HCC, but viral hepatitis (HBV and HCV) predominate[17,18].

Several sociodemographic characteristics have been associated with HCC, particularly in patients with cirrhosis. In our study, the age of HCV-related HCC patients prevailed over the age of HCV-related cirrhosis. Our results are consistent with those of previous studies[19,20]and are based on the duration of development and progression of cirrhosis and HCC. In addition, HCC is predominant among men (male to female ratio 2–3:1), which is likely due to differences in sex hormones[21]. This fact is also observed in our study; thus, this ratio was also 2.5:1.

One of the traditional predictors of HCC is AFP, which is a fetal component protein produced by the liver[22]. But it is not accurate enough for screening and diagnosing of HCC. Thus, in our study, 33.3% of patients with HCC had AFP levels ≤ 100 IU/ml, and 19.1% patients had very high AFP levels (>1000 IU/ml), which indicates a more aggressive subclass of HCC[21,22].

Some studies have indicated an association between inflammation and tumor malignancy[23]. Systemic inflammatory markers, such as NLR, PLR and GLR, can be

predictors of HCC development[23]and of poor prognosis. In this study we confirmed that the NLR, PLR and GLR are statistically significantly increased in the HCC patients.

We also focused on the descriptive statistics of 2 important HCC biology indices, namely MTD and PVT. PVT is the most frequent form of macrovascular invasion that occurs in 44.0% – 62.2% of HCC patients[24]. The development of PVT in HCC is observed at the advanced stage of the disease with large tumor sizes. This fact also negatively affects the prognosis of the disease. We show that most of our cases are advanced (≥50% had MTD >5cm) and that they have a large percentage of patients with PVT. Also 62.1% of patients with PVT had high levels AFP and significantly higher ALKP levels, as noted by others [25].

This study has several limitations. The first is the small number of patients. Secondly, the dynamics of the investigated laboratory parameters have not been clarified. We believe that there is a need for extensive and multi-center research in this field in the future

Conclusion

Elderly age, male sex, increased levels of NLR, PLR, GLR, ALKP, AFP and decreased level albumin in HCV-related cirrhosis increase the risk of HCC, as well very high level of AFP (>1000 IU/ml) increase suspicion of portal vein thrombosis and presence of large (≥5 cm) tumors. Consideration of these indicators in routine monitoring may be useful in early diagnosis and treatment of HCC in HCV-related cirrhosis.

Main points

- Elderly age and male sex increase the risk of HCC in HCV-LC.
- Increased levels of NLR, PLR, GLR, ALKP, AFP and decreased level of albumin increase the probabilityof HCC in HCV-LC.
- High AFP level raises a suspicion of portal vein thrombosis and presence of large tumors.
- **Conflict of Interest Statement**
- The authors declare no conflict of interest. All authors have read and agree with the contents of this paper

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- **Ethical Approval and Consent**
- This work complies with the guidelines of the World Medical Association, Declaration of Helsinki. This work was approved by our institution's IRB as documented in the methods section.All patients gave their written informed consent to participate in this study examining the natural history of their disease.

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Table 1. Laboratory data of HCV-LC versus HCV-LC-HCC patients.

Laboratory variables	***HCV + cirrhosis***	***HCV-LC + HCC***	***P value***

NE# (2.1-6.1) x 10^3/μL	3.1	3.03	0.009
LY# (1.3-3.5) x 10^3/μL	1.6	0.76	0.121
NLR	2	3.3	0.005
PLR	68.9	131.7	0.010
GLR	47.5	124.9	0.002
GPR	0.83	1.11	0.454
Alb/CRP	0.98	0.35	0.173
CRP (1-10) mg/L	3.7	8.64	0.409
WBC (4.3-10.3)x 10^3/μL	5.9	5.8	0.473
Hgb (13.6-17.2) g/L	13	12.2	0.311
Platelets(150-400) x 10^3/μL	112	128	0.429
T.Bil. (0.5-1.2) mg/dL	1.08	1.6	0.465
Albumin (3.4-4.8) g/dL	4.01	3.45	0.009
CRE (0.72-1.25) mg/dL	0.76	0.84	0.120
AST (0-34) U/L	53	51	0.609
ALT (0-55) U/L	42	39	0.505
GGT (9-64) U/L	75	125	0.132
ALKP (40-150) U/L	105	146	0.023
AFP (0-8) IU/mL	4.5	211	0.019

HCV – hepatitis C virus; **LC** – liver cirrhosis; **HCC** – Hepatocellular carcinoma; **NE** – neutrophils; **LY** – lymphocytes; **NLR** – neutrophil-to-lymphocyte ratio; **PLR** – platelet-to-lymphocyte ratio; **GLR** –gamma-glutamyl transpeptidase-tolymphocyte ratio; **GPR** – gamma-glutamyl transpeptidase-to-platelet ratio; **Alb** – albumin; **CRP** – C-reactive protein; **WBC** – White blood cells; **Hgb –** Hemoglobin; **T.Bil.** – total bilirubin; **CRE** – creatinine; **AST** – aspartate aminotransferase; **ALT** – alanine aminotransferase; **GGT** – gamma-glutamyl transferase; **ALKP** – alkaline phosphatase; **AFP** – alpha fetoprotein.

Table 2. Comparison of low AFP (≤100) with high AFP (>100) patients.

Laboratory variables	***AFP ≤100IU/mL***	***AFP >100IU/mL***	***P value***
NE# (2.1-6.1) x 10^3/μL	2.4	7.0	0.028
LY# (1.3-3.5) x 10^3/μL	0.7	2.54	0.064
CRP (1-10) mg/L	4.5	50	1.042
WBC (4.3-10.3) x 10^3/μL	5.6	11.4	0.004
Hgb (13.6-17.2) g/L	12.1	12.2	0.543
Platelets (150-400) x 10^3/μL	113	225	0.103
PT-INR (0.8-1.2)	1.17	1.32	0.300
T.Bil. (0.5-1.2) mg/dL	0.69	2.1	0.026
Albumin (3.4-4.8) g/dL	3.7	3.0	0.023
AST (0-34) U/L	51	102	0.170
ALT (0-55) U/L	39	67	0.362
GGT (9-64) U/L	108	227	0.619
ALKP (40-150) U/L	140	282	0.020

MTD (mm)	40	65	0.282
PVT	1 (7.1%)	5 (62.5%)	0.059
Multifocality	9 (60%)	13 (68.4%)	0.222

AFP – alpha fetoprotein; **NE** – neutrophils; **LY** – lymphocytes; **CRP** – C-reactive protein; **WBC** – White blood cells; **Hgb –** Hemoglobin; **PT-INR** – prothrombin time-international normalized ratio; **T.Bil.** – total bilirubin; **AST** – aspartate aminotransferase; **ALT** – alanine aminotransferase; **GGT** – gamma-glutamyl transferase; **ALKP** – alkaline phosphatase**; MTD** – maximum tumor diameter; **PVT** – Portal vein thrombosis.

Table 3. Comparison of PVT positive and PVT negative patients.

Laboratory variables	***PVT -***	***PVT +***	***P value***
T.Bil. (0.5-1.2) mg/dL	0.73	2.05	0.776
ALKP (40-150) U/L	133	300	0.010
AFP (0-8) IU/mL	64	1500	0.069
MTD (mm)	42	70	0.070
Multifocality	11 (40.7%)	5 (33.3%)	0.703

PVT – Portal vein thrombosis; **T.Bil.** – total bilirubin;**ALKP** – alkaline phosphatase**; AFP** – alpha fetoprotein;**MTD** – maximum tumor diameter.

Table 4. Comparison of small (≤50mm MTD) with large (>50mm MTD) patients.

Significant parameters	***MTD ≤50***	***MTD >50***	***P value***
T.Bil. (0.5 – 1.2) mg/dL	0.73	2.05	0.055
Albumin (3.4 – 4.8) g/dL	3.35	3.50	0.578
AFP (0 – 8) IU/mL	61	511	0.040
PVT	0	8 (57.1%)	0.017
Multifocality	6 (46.2%)	5 (35.7%)	0.659

MTD – maximum tumor diameter; **T.Bil.** – total bilirubin;**AFP** – alpha fetoprotein;**PVT** – Portal vein thrombosis.

Abbreviations:

AFP – alpha fetoprotein; **ALKP** – alkaline phosphatase; **ALT** – alanine aminotransferase; **AST** – aspartate aminotransferase; **CBC** - complate blood count; **CRE** – creatinine; **CRP** – C-reactive protein; **CT** – computed tomography; **GGT** – gamma-glutamyl transferase; **GLR –**gamma-glutamyl transpeptidase-tolymphocyte ratio; **GPR** – gamma-glutamyl transpeptidase-to-platelet ratio; **HBV** –hepatitis B virus; **HCC** – Hepatocellular carcinoma;

HCV – hepatitis C virus; HCV-LC – hepatitis C virus related cirrhosis; HCV-LC-HCC- hepatitis C virus related cirrhosis plus HCC; **Hgb –** Hemoglobin; **kPa** – kilopascal; **LC** – liver cirrhosis; **LY** – lymphocytes; **MRI** – magnetic resonance imaging; **MTD** – maximum tumor diameter; **NE** – neutrophils; **NLR** – neutrophil-to-lymphocyte ratio; **PLR** – platelet-to-lymphocyte ratio; **PVT** – Portal vein thrombosis; **RNA** – Ribonucleic acid; **T.Bil.** – total bilirubin; **US** – ultrasound; **WBC** – White blood cells.