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CHARACTERISTICS AND RISK FACTORS FOR PRIMARY HEPATOCELLULAR CARCINOMA

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ABSTRACT

Background: Primary hepatocellular carcinoma (HCC) is the most common liver malignancy.
Aim: To identify the clinical characteristics of patients with HCC and the factors for its occurrence.
Methods: A total of 110 hospitalized patients with HCC was investigated. Histological diagnosis of underlying liver disease was done in 93 patients. Biochemical and hematological parameters were obtained by routine biochemical and hematological techniques. Serum markers for hepatitis B and C viruses and autoantibodies were determined.
SPSS version 17.0 was used for statistical analyses and $p<0.05$ was considered significant.
Results: Patients were aged from 48 to 82 years (65,8 ± 7). Males dominated (74 pts). Solitary HCC pattern was observed in 87 patients. Viral etiology was proven in 61 patients while alcoholic liver disease was detected in 21 patients. Increased AFP values (≥ 20 ng/ml) were obtained in 88 pts; values ≥200 ng/ml in 71 pts, and values ≥400 ng/ml in 57 pts. The clinical presentation was dominated by fatigue, gynecomastia, and bone aches. The most common comorbidities were diabetes mellitus type 2 (53 pts), chronic cardiomyopathy/arterial hypertension (48 pts), and obesity (29 pts).
Comparison of patients with and without cirrhosis (66/110) showed no difference in age, gender, tumor pattern, and AFP values ≥20 ng/ml. Those from the first group had higher AFP, larger solitary tumors, greater liver damage and more pronounced clinical symptoms.
Conclusions: HCC is mostly detected in liver cirrhosis. Other risk factors include hepatitis B and C viruses, heavy alcohol abuse, older age, male gender, metabolic and chronic cardiovascular diseases.

Keywords: Hepatocellular carcinoma, risk factors, characteristics, liver cirrhosis

INTRODUCTION

Primary hepatocellular carcinoma (HCC) accounts for about 90% of liver cancers and thus constitutes a major health problem [1]. The incidence of HCC has been rising in recent years, especially in countries with a high prevalence of chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infections [2]. In addition, an increased incidence of HCC is also observed in patients with alcohol-related liver disease (ALD) as well as non-alcoholic fatty liver disease (NAFLD) [3]. Generally, in the majority of cases, HCC is associated with end-stage liver disease (ESLD), such as liver cirrhosis (LC). Overall, one-third of patients with LC will develop HCC during their lifetime [4].
The aim of the study was to determine the main clinical characteristics of HCC patients with chronic liver disease (CLD) as well as the most common risk factors for its occurrence.

PATIENTS AND METHODS

The study included total of 110 patients with HCC who had been treated in the General Hospital in Uzice, the main health center in western Serbia, from January 01, 1999 to December 31, 2019. Diagnosis of HCC and underlying liver disease was made on the basis of clinical, radiological, biochemical and histological findings. Imaging techniques were obtained by ultrasonography and multiphasic computed tomography (CT). Histological diagnosis of underlying liver disease was made in 93 (84,5%) patients and interpreted by an expert pathologist

based on accepted international recommendations. Diagnosis of ALD was made upon data of long-term alcohol abuse of >30 g/d in males and >20 g/d in females.

Serum alpha fetoprotein (AFP) level was measured using an automated micro particle enzyme-linked immunoassay (EIA) with normal value <20 ng/ml. Markers for HBV and HCV in sera were detected by EIA using commercial kits (Cobas Core HBsAg II, Cobas HCV test, Roche). Viral genomes in sera (RNA and DNA) were detected by reverse transcription-polymerase chain reaction (RT-PCR) (Cobas TaqMan test, Roche). Non-organ specific autoantibodies (anti-nuclear, anti-mitochondrial, anti-smooth muscle, anti-liver-kidney microsome1, and anti-neutrophil cytoplasmic) were detected by indirect immunofluorescence method. Additional examinations of the patients included esophagoduodenoscopy, colonoscopy, endoscopic retrograde cholangiopancreatography and assessment for Kayser-Fleischer ring.

STATISTICAL ANALYSIS

All collected data were analyzed retrospectively.

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL.US) version 17.0 was used for statistical analyses with two-side tests. Parametric and non-parametric tests were performed to identify significant different variables The results were expressed as means ± SD and as percentages. Non-normal data were compared using non-parametric tests (Mann-Whitney test). Non-parametric variables were calculated by Chi-Square or Fisher's exact test. Significant variables were entered into a univariate and multivariate logistic regression model. Results are presented with 95% confidence interval (95% CI). A probability value of $P < .05$ was considered significant.

RESULTS

The investigated patients were aged from 48-82 years (65,8 ± 7). The most frequent was the group included individuals in the seventh decade from 61-70 years old (50/110 patients). Four patients formed the group younger than 40 years old and four of them were over 80 years old.

In relation to the etiology among all 110 examinees, 61 patients had a chronic viral disease (CH-VD), with B and C viruses. All underlying CLD are listed in Table 1.

Table 1. Etiology of underlying liver disease in HCC patients

***P* was calculated for ≥5 cases**
Legend: [1, 2]viral; [3]PSC, primary sclerosing cholangitis; [4]PBC, primary biliary cirrhosis ; [5]AIH, autoimmune hepatitis; [6]ALD, alcoholic liver disease); [7]NAFLD, non-alcoholic fatty liver disease ; [8]cryptogenic cirrhosis

Etiology	1[1]	2[2]	3[3]	4[4]	5[5]	6[6]	7[7]	8[8]	Total	*P
Non-Cirrhosis	21	13	1	1	0	4	1	3	44	
Cirrhosis	15	12	1	3	8	17	4	6	66	.016
Total	36	25	2	4	8	21	5	9	110	

The main characteristics of examined patients with HCC are presented in Figure 1.

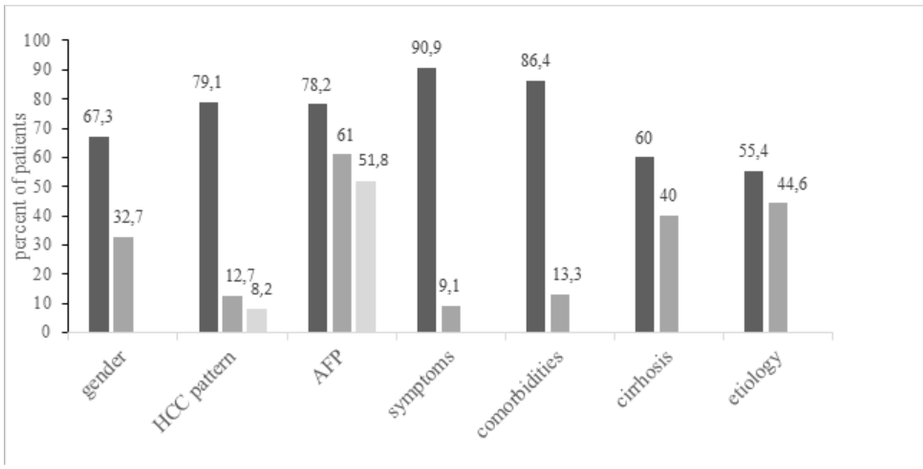

Figure.1 The main comparative characteristics of total patients with HCC

P was calculated for ≥5 cases
Gender: male vs. female, *P*< .001; HCC patterns: solitary, multifocal, diffuse, *P*, 1,2,3 <0.001; AFP ≥ 20 ng/ml, ≥200 ng/ml, ≥ 400 ng/ml, P_1<.001;P_2 =.002;P_3>.05; symptoms (present vs. absent); *P* <.001); comorbidities (present vs. absent) *P* <.001; cirrhosis (present vs. absent), *P*= .04; etiology (viral vs. non-viral) *P*>.05

Seventy-four/110 patients with HCC were males.
The solitary HCC pattern was observed significantly more often (87 patients) than multifocal and diffuse patterns (14 and 9 patients, respectively). Measurement the maximum diameter of solitary tumor was 31,4 mm ± 11,4 (range: 12 mm-70 mm).
The clinical presentation was manifested in most patients by fatigue, gynecomastia and bone pain in 80, 58 and 50 patients, respectively. The following symptoms were less common: weight loss, jaundice, itching, bleeding from digestive tract/urine/nose, abdominal pain, and diarrhea in 36, 35, 32, 30, 28, and 18 patients, respectively. Two patients were febrile.
AFP was elevated (≥20 ng/ml) in 88 patients indicating a significantly higher frequency than for patients with normal values. Concentrations ≥200 ng/ml and ≥400 ng/ml were noted in 71 and 57 patients, respectively.
Comorbidities were observed in 95 subjects most commonly manifested as diabetes mellitus (DM) type 2 (53 pts), followed by chronic cardiomyopathy (CH-CMP) such as arterial hypertension (53 pts), and obesity in 29 pts. Heavy alcohol consumption was demonstrated in 25 pts without reliable histological evidence for alcoholic liver disease. Other associated diseases were manifested as psychiatric disorders (PD) most often as depression (19 pts), gastrointestinal disorders (GID), mostly as diarrhea -18 pts (plus vomiting -9 pts). Chronic pulmonary disorders (CH-CPD) was diagnosed manly as obstructive pulmonary disease in 8 pts.
Biochemical and hematological findings showed high frequencies of patients for the following parameters: moderately elevated values of aminotransferases (ALT and AST) in 107 and 91 pts respectively and gamma-glutamyl transferase (GGT) in 102 pts. Significant decreases of serum albumin level were found in 72 pts. Higher than normal values of conjugated bilirubin were observed in 35 individuals. Clotting disorders with low platelet (PLT) counts and abnormally prolonged partial thromboplastin times (APTT) were noted in 99 pts. There were 66 participants with moderate anemia. Formed liver cirrhosis was noted in 66 HCC patients, which is statistically more significant than the number of patients with other stages of chronic liver diseases. The LC score was estimated according to Child-Turccote-Pugh criteria and is presented in Table 2.

Table 2. Child-Turccote-Pugh Score in patients with LC

Score	Number of Patients (%)
A	25 (37.87)
B	38 (57.57)
C	3 (4.5)
Total	66 (100)

The most patients had score B (57.6%). Histological assessment of 93 liver tissue showed that 44.2% of non-LC patients had severe fibrosis, while 9.1% had moderate fibrosis. No fibrosis was detected in three patients. Concerning tumor biopsy, we followed the recommendations of the EASL expert group. As well, some of the patients had undoupted contraindications to this type of invasive procedure.
A comparative analysis of the characteristics of the HCC patients with and without cirrhosis showed the following results as shown in Table 3 and Figure 2.

Table 3. Comparative characteristics of the patients with HCC in relation to the presence/abscence of liver cirrhosis

P* **was calculated for ≥5 patients
****Not significant**
*** **Not determined**

Abbreviations: BMI, body mass index; CH-CMP, chronic cardiomyopathy; CH-PD, chromic pulmorary disease; DM, diabetes mellitus; GID, gastrointestinal disease; HBV, hepatitis B virus; HCV, hepatitis C virus; PD, psychiatric disease

Variable		HCC in LC 66 (%) patients	HCC in non-LC 44 (%) patients	*P**
Age group	41-50	2	2	NS**
	51-60	14 (21.2)	15 (21.2)	
	61-70	30 (43.9)	20 (45.5)	
	71-80	17	6 (13.6)	
	>80	3	1	

Variable		HCC in LC 66 (%) patients	HCC in non-LC 44 (%) patients	*P**
Male gender	74 (67.3)	43 (65.1)	31 (70.4)	NS
AFP value	≥ 20 ng/ml	53 (80.3)	35 (79.5)	NS
	≥200 ng/ml	61 (92.4)	10 (27.7)	< .001
	≥ 400 ng/ml	48 (72.7)	9 (20.4)	< .001
HCC pattern	Soltary	55 (83,3)	32 (72.7)	NS
	Multifocal (≥ 3 lesions)			
	Diffuse	6 (9.1)	8 (18.2)	NS
		5 (7.6)	4	ND***
Tu diameter (mm)	Solitary	34.4 ± 11,4 (20-70)	25.9 ±9 (12-50)	<.001
Comorbidity	Obesity (> 30 BMI)	5 (7.6)	24 (56.8)	< .001
	DM type 2	31 (47)	22 (50)	< .001
	CH-CMP	22 (33.3)	21 (47.7)	NS
	CH-PD	2	6	ND
	GID (diarrhea)	5 (7.6)	13 (29.5)	.003
	PD	6 (9.1)	13 (29.5)	.009
	Haevy alcohol abuse	21 (31.8)	4 (9.1)	.005
Viral etiology	HBV, HCV	27 (40.1)	34 (77.3)	.036
Laboratory data	RBC<3.6 x10^{12} /l	64 (96.9)	2	ND
	HBG<120 g/l	64 (96.9)	2	ND
	PLT<150×10^9/l	66 (100)	0	ND
	Glycaemia<3.9mg/dl	51 (77.3)	29 (65.9)	NS
	Albumin<3.4 mg/dl	32 (42.5)	10 (22.7)	.002
	Conjugated bilirubin>5 µmoll/l AST>38/U/l	27 (40.9)	8 (18.2)	.003
	U/l ALT>41 U/l	65 (98.5)	30 (86.5)	NS
	GGT>35 U/l AF>270	66 (100)	30 (68.2)	NS
	U/l APTT> 25.6 sec	66 (100)	4	ND
		66 (100)	36 (81.8)	NS
		3	4	ND
		66 (100)	4	ND

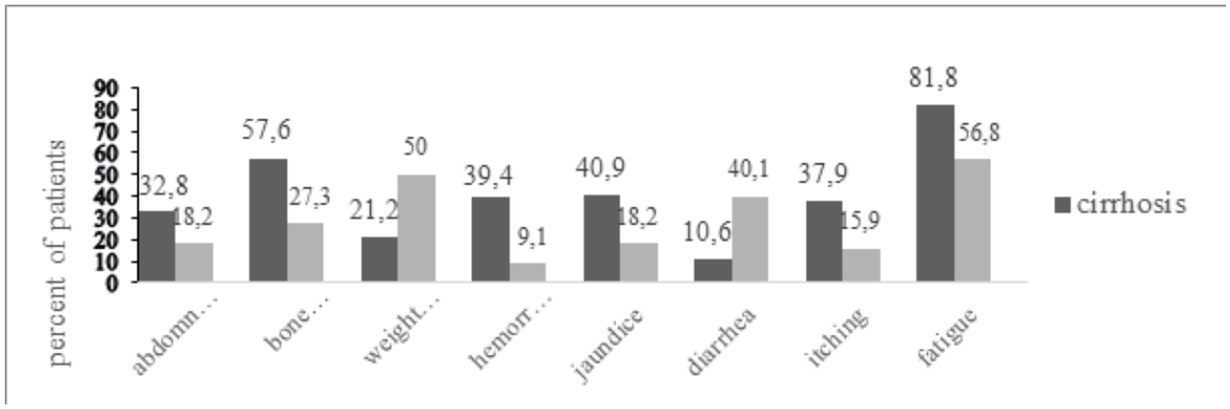

Figure 2. Comparative characteristics of symptoms/signs of patiens with and without liver cirrhosis

P was calculated for ≥5 patients.
Abdominal pain, P= .02; bone pain, P= <.001; weight loss, P= .002; fatigue, P= .002; diarrhea, P< .001; gynecomastia, P= .04; jaundice, P= .001; itching, P=.002; hemorrhage, P=ND

In general, there was no difference in the distribution of gender, age, tumor pattern, and AFP values ≥20 ng/ml. The majority of HCC patients from the both groups were 61-70 years old (45.4%). Relatively more patients in the LC group were aged from 71-80 years compared to those in the non-LC group (25.8% vs. 13.6%) but it was not statistically significant.
The maximum diameter of solitary tumor was larger in LC than in non-LC (34,4 mm vs. 25,9 mm).
AFP ≥200 ng/ml and ≥400 ng/ml showed a higher frequency for both values in HCC patients with LC than in non-LC patients.
Fatigue, bone aches, abdominal pain, hemorrhage (epistaxis/GI/UT), jaundice, itching, and heavy alcohol consumption were more common in HCC patients with LC, while obesity, DM type 2, viral etiology, diarrhea, weight loss, and PD were more frequent in non-LC patients. No significant difference was noted between the frequencies of patients with CH-CMP and CH-CPD. Unfavorable hematological parameters (anemia, thrombocytopenia, prolonged APTT), and hypoalbuminemia were significantly more frequent in patients with LC. Two HCC non-cirrhotic patients had fever.
Calculation using binary regression analysis for both groups revealed values for AFP ≥200ng/ml, ≥400 ng/ml and heavy alcohol consumption as positive predictors for HCC patients with LC, while obesity, DM type 2, and viral etiology were positive predictors for HCC patients with non-LC (Figure 3).

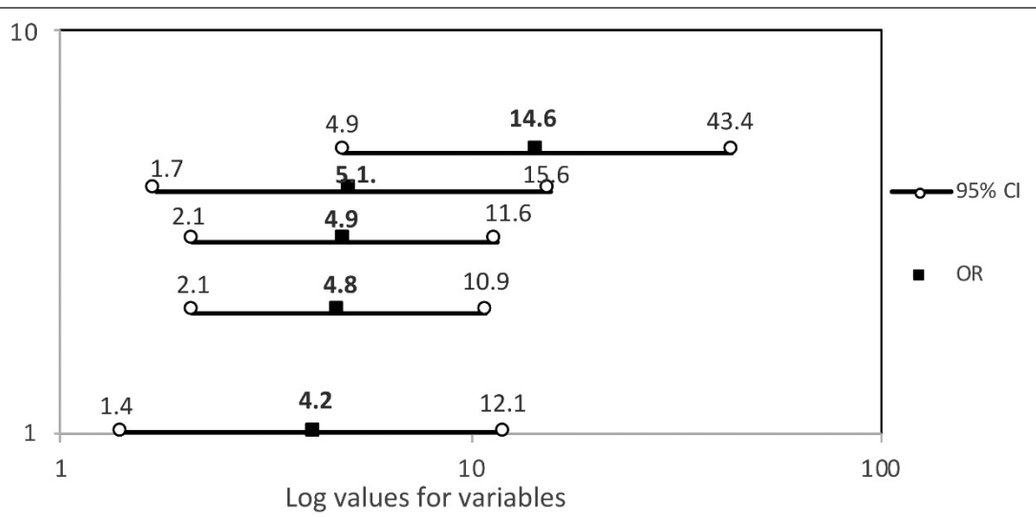

Figure 3. Positive predictors for non-cirrhosis

Binary logistic regression: 14.6, OR for obesity; 5.1, OR for GID; 4.9, OR for viruses; 4.8, OR for DM; 4.2 R for PD
Abbreviations: DM, Diabetes mellitus; GID, Gastrointestinal disease; PD, Psychiatric disease;

Concerning associated diseases and symptoms, PD and GID (diarrhea) were revealed as positive predictors for HCC patients with non-LC. Multivariate regression analysis revealed AFP values ≥200ng/ml as the most important positive predictor for LC (*P* <.001; Exp(B) = 41.480; 13.101- 131.338), while obesity was the most important predictor for HCC patients with non-LC (*P* <.001; Exp(B) =14.4; 4.3-48.2).

DISCUSSION

In this study we have attempted to evaluate the major characteristics of HCC as well as possible causes of its emergences.

Our investigation shows that HCC is more often seen in already formed LC. According to the literature, its earlier occurrence ranges from 7%-54%, depending on the geographic region and some of risk factors. While in Western countries about 15%-20% of cases develop this malignancy at pre-cirrhotic stages, in our study the proportion was significantly higher and amounted to 40% [5,6]. In this regard, the greater presence of B virus in non-LC group which has been found to be directly carcinogenic, may participate in its earlier development. Also, some of unidentified hereditary diseases, toxic substances and extremely alcohol abuse may have impact on the time-based occurrence of HCC.

Concerning the etiology, a common of underlying liver disease in our investigation was of viral origin as well as related to excessive alcohol intake (cumulative 74%) as were reported in other studies [7,8]. Further analysis of these patients with viral etiology showed that those with HBV were more numerous which is in contrast to the authors from Western countries where HCV is more actual. However, in Asian countries, such as China and Japan, the majority of HCC patients suffer from chronic HBV infection due to high incidence of this virus [9,10]. Also, recent data from Turkey show that HBV have 60,5% of HCC non-cirrhotic patients with no difference in frequency compared to LC [11].

In addition to the above data, an increased the incidence of NAFLD in many countries is nowadays very factual real and explains the high incidence of cirrhosis and HCC in these patients [12]. It should also be noted that this metabolic dysfunction-associated non-alcoholic liver diseases (MAFLD) and its increase in incidence, particularly in US, encouraged the group of hepatologists to propose the new nomenclature [13]. Furthermore, the presence of aflatoxin should be mention also as important etiological risk factor in certain geographical areas, which was not the subject of our investigation this time [14].

Unfortunately, we were unable to discover the etiology of underlying liver disease in 8.2% of HCC patients. A similar prevalence of "cryptogenic" cirrhosis has been described in other studies also [15,16]. Difficulties in diagnosing this condition occurs for several reasons. First of all, histological examination of the liver tissue in complete formed cirrhosis is not crucial for revealing the etiology. Then, negative viral serological tests only, and even PCR in serum, are not always reliable as evidence for actual or previous viral hepatitis. It should be noted that some patients may lose the virus in the blood but it still retains in the hepatocytes. However, even though the virus is no longer present in hepatocytes, it is possible that chronic inflammation continues and that cirrhosis and HCC develop "per se". In addition to mentioned above reasons, many other liver diseases such as autoimmune, inherited, metabolic and toxic, etc. which we have not verified, may cause this unidentified liver disease also.

The age of the patients in our study showed that elderly was the most numerous reaching a peak at 61 to 70-year group which is in accordance with the findings of other researchers. Also, male gender, as was the case in our study, has been proven to be dominant in many other studies [17].

Regarding the appearance of the tumor, the most often seen was a solitary alteration (80%) using applied methods (US and CT), although magnetic resonance imaging (MRI) is generally accepted as the most reliable visual examination for an HCC diagnosis [18,19]. Unfortunately, this visual method was not available to us.

The AFP findings are interesting and only partially agrees with other authors. Namely, we found an elevation in AFP (≥20 ng/ml) in the most of our patients (up to about 80%). It is well known that this marker had been used for decades as an appropriate test for surveillance and prognosis of HCC [20]. Although, as the AFP may be moderately elevated in cirrhosis without HCC and because some tumors do not secrete this protein, some authors have suggested a higher cut-off value than ≥200 ng/ml or even higher than 400 ng/ml. Therefore, other markers (PIVKA II, micro RNA, etc.) had been proposed as a more appropriate for tumor diagnosis, but these tests still are not routinely used [21]. As a minority of examined HCC patients may have normal or slightly elevated AFP values (20% <20 ng/ml), caution is advised for its use. Nevertheless, our investigation has shown the AFP ≥200 ng/ml as the most important predictive factor for HCC which can reestablish its diagnostic significance. At the same time, value ≥400 ng/ml founded in about half of our HCC cases was significant but less suggestive than the previous ones.

Clinical presentation of our patients was dominated by the usual manifestations of advanced liver disease with malignancy such as fatigue, abdominal pain and incidence of bone aches [22,23]. Observed enlarged lymph nodes observed radiologically and frequency of bone aches, could indirectly indicate the existence of metastases.

Associated with the illness, a significant number of patients had also gynecomastia and weight loss, accompanied by a tendency for bleeding, obstructive jaundice, itching and diarrhea. Also, a tumor necrosis or microbial infection may have been the reason for fever occurring in two patients.

Further, numerous comorbidities (metabolic-induced or organic) are generally observed in the vast majority of cirrhotic and non-cirrhotic patients, most commonly as DM type 2 and obesity. Also, the progressive impact of chronic obstructive pulmonary disease (COPD) has been described by other authors [24]. Our findings did not confirm this disease as a significant risk factor for HCC (8 patients). Additionally, numerous psychiatric diseases in cirrhosis can be caused by metabolic imbalances, drugs or their abuse, especially in drug addicts [25]. Significnt presence of psihyatric illiness in our study confirms similar results obtained in an earlier study of patients with HCC in Serbia [26].

Liver functional test (LFT) results in patients with HCC generally did not differ from those founded in patients with LC alone. Most patients in both groups were mildly anemic, have hypoalbuminemia, mild icterus and thrombocytopenia. However, the occurrence of pronounced jaundice, hypoglycemia, erythrocytosis, thrombocytosis and hypocalcemia are often signals for malignant progression and a poor prognosis and thus are more common in advanced liver disease or the development of malignancy [27]. In the background of cirrhosis and HCC may be many processes such as chronic inflammation with production of numerous cytokines, endotoxins, oxidative stress, free radicals etc. Also, many other conditions such obesity, have been associated with an increased risk of developing HCC which we also confirmed [28]. Thus, it is realistically expected that HCC patients with cirrhosis have greater impairment in LFTs as well as more discomfort manifested as comorbidities.

Comparing the characteristics of HCC patients with and without cirrhosis, we found some similarities and some differences between both groups. In contrast to observations in Germany who showed that older age dominated in non-LC over LC patients, in our study, elderly HCC patients were more common in LC [29]. We disagree also with the findings concerning the tumor size, male dominance as well as in the frequency of exposure to alcohol abuse and viral etiology. The lack of gender difference in our study could be explained by the fact that in both groups women were in post-menopause (in the 6^{th} and 7^{th} decade) when the protective role of estrogen ceases [30].

Finally, our investigation has some limitations. First of all, regarding diagnosis without MRI and scintigraphy examinations, the probability of revealing more details about the tumor (its size, abundance and appearance), and as well as detection of possible metastases were not ideal. Also, in diagnosis of viral markers, RNA and DNA were not determined in liver tissue which may have reduced the total number of "cryptogenic cirrhosis" cases regardless of serum viral markers. Although measurement of AFP (≥200 ng/ml) gave relatively favorable results, the use of recently suggested novel tumor markers would certainly provide a more precise diagnosis. At the end, lack of examination of many other congenital metabolic diseases and other hereditary conditions in our study that could manifest in the liver would also contribute to that.

CONCLUSIONS

The risks of development primary HCC are numerous. It mostly occurs in the final stage of liver disease, although a significant number can develop in an earlier stage. Most cases are related to the etiology of liver disease, mainly viral, older age, male gender, and other factors including chronic inflammation, excessive alcohol abuse, various metabolic and hormonal disturbances. Regular supervision of patients with chronic liver disease including AFP measurement and visual methods contributes to earlier detection of HCC and thus more successful treatment.

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