Demographic, etiological, clinical features, and laboratory features of hepatocellular carcinoma; a single center experience

Tuba Ersal[1], Fatma Laika Karabulut[2]

[1]Department of Hematology, Bursa Uludag University Scholl of Medicine, Bursa, Türkiye
[2]Department of Internal Medicine, Endomer Health Service, Istanbul, Türkiye

Journal of Bursa Faculty of Medicine
e-ISSN:

Original Article

Hematology

Received
November 30, 2021
Accepted
December 24, 2021
Published online
January 04, 2023

J Bursa Med 2023;1(1) 26-32

ABSTRACT

Objectives: Hepatocellular carcinoma (HCC) is our country's most commonly encountered cancer. This study examined demographic, etiologic, clinical characteristics, and biochemical and serological findings of patients with HCC.

Methods: We retrospectively analyzed 207 HCC patients followed by gastroenterology and medical oncology departments.

Results: It was established that, in the demographic analysis, HCC was more common in the elderly population, especially in men. The positive hepatitis B virus surface antigen rate was 65.5%, anti-delta was 2%, and hepatitis C virus antibody was determined to be 15%. The rate of alcohol users was 11.1%, and that of tobacco users was 68.2%. Serum alkaline phosphatase, gamma-glutamyl transferase, and serum alpha-fetoprotein (AFP) levels were above average in 75.6%, 86.3%, and 72.6% of patients, respectively. Approximately 63% of patients had cirrhosis at presentation. Ultrasonography (USG) was the primary diagnostic method in 57% of the patients. Histopathological diagnosis was made by ultrasound-guided biopsy in 67.6% of the patients.

Conclusion: Chronic hepatitis B was the most common etiological factor for HCC, and chronic hepatitis C was observed at a significant rate of 15%. The majority of the patients developed HCC on the cirrhotic ground. Most of the patients had high levels of AFP. In 58% of patients, the tumor was located in the right lobe. Routine liver tests and clinical findings varied. Radiologically, it was concluded that USG, computed tomography, and magnetic resonance imaging techniques were complementary and equivalent methods in terms of tumor diameter

Keywords: Hepatocellular carcinoma, Hepatitis B virus, Hepatitis C virus, Alpha-fetoprotein.

Hepatocellular carcinoma (HCC) is a malignant tumor of the liver which originates from hepatocytes. HCC is the fifth most common cause of cancer worldwide and the second leading cause of cancer death, accounting for nearly more than 500,000 deaths annually. HCC is the fifth most common malignancy in men and the eighth in women. The 5-year overall survival rate is below 5% [1]. HCC frequently occurs in the setting of chronic liver disease or cirrhosis. Hepatitis B virus (HBV), hep-

How to cite this article
Ersal T, Lika Karabulut F. Demographic, etiological, clinical features, and laboratory features of hepatocellular carcinoma; a single center experience. J Bursa Med 2023;1(1):26-32

Address for correspondence
Tuba Ersal, M.D., 1Department of Hematology, Bursa Uludag University Scholl of Medicine, Bursa, Türkiye. E-mail: tubaersal@uludag.edu.tr. Phone: +90 506 711 76 85

atitis C virus (HCV), alcoholic liver disease, exposure to toxic chemicals and probably non-alcoholic steatohepatitis are considered amongst the major causes of cirrhosis in patients with HCC. Rarer causes include hereditary hemochromatosis, alpha-1 antitrypsin deficiency, autoimmune hepatitis and some types of porphyria. Globally, HBV is the most common cause of HCC [2]. Most of patients are asymptomatic during the early period. In patients with compensated cirrhosis, acute development of liver failure signs such as ascites, encephalopathy, jaundice or variceal bleeding is important with regard to HCC. These signs may indicate the tumoral invasion of hepatic or portal vein or arteriovenous shunting [3]. The physical examination in HCC patients reveals the findings related to the underlying liver disease (splenomegaly, ascites, jaundice or other findings of decompensated cirrhosis) [4]. 4 Laboratory tests are often non-specific. Thrombocytopenia, hypoalbuminemia, hyperbilirubinemia and hypoprothrombinemia can be identified in most cirrhotic patients with HCC. Serum aminotransferases, alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) levels are often abnormal but are non-specific [5].

In 10-20% of patients, metastatic dissemination is evident at the time of initial diagnosis. Extrahepatic metastasis generally occurs in patients with a tumor size of more than 5 cm in diameter. The most frequent metastasis sites involve lungs, intraabdominal lymph nodes, bone, brain and adrenal glands [6, 7].

The diagnostic delay is common in HCC due to lack of specific pathognomonic signs as well as the presence of a large liver functional reserve, and therefore many patients experience delay in receiving appropriate treatment at the time of diagnosis [8, 9]. The average survival time after the diagnosis ranges from 6 to 20 months [10]. Large tumor size, vascular invasion, deterioration in functional status and nodal metastases are poor prognostic indicators [9, 11]. American Association for the Study of Liver Diseases (AASLD) guidelines recommend further investigation for HCC in patients with underlying liver disease (i.e., cirrhosis, chronic viral hepatitis) who have concomitantly elevated serum alpha-fetoprotein (AFP) levels [11]. In these patients, computed tomography (CT) screening and/or magnetic resonance imaging (MRI) of liver should be performed as the first-line modalities of diagnosis [12]. The definite diagnosis of HCC is often based on the presence of hyper-vascular lesion with T2 hyperintensity and venous invasion characteristics or accompanied with AFP elevation.

AFP levels are commonly used as a marker in HCC. The elevation of AFP levels in cirrhotic patients is suggested to be important in terms of HCC development. Although AFP levels > 500 ng/ml (normal reference range: 10-20 ng/ml) in high-risk patients are considered to be diagnostic for HCC, [13] patients with low AFP levels are also diagnosed with HCC through screening studies [14, 15].

Several imaging modalities such as Ultrasonography (USG), CT, MRI and angiography are used in the diagnosis of HCC. USG, often together with AFP levels, is used for screening purposes.

Cancer staging is of utmost importance for determining the disease prognosis and the appropriate treatment options. Severity of underlying liver disease, tumor size, tumoral invasion to surrounding structures and metastasis status are the four key factors in terms of survival among HCC patients [16-19].

Predicting survival is challenging in HCC due to concomitant presence of cirrhosis and tumor as the two underlying diseases. The residual hepatic functional reserve has been reported to be directly correlated with prognosis in several studies. This indicates the stronger role of cirrhosis rather than tumor size as a main predictor of survival outcome. In newly diagnosed untreated HCC patients, survival is limited to weeks or months [20].

Several factors have been associated with poor survival outcome including male gender, advanced age, etiological factor (poorer prognosis in HCV vs. HBV), presence of multiple risk factors, number and volume doubling time of nodules, vascular invasion and distant metastasis [21].

Liver transplantation is considered a curative treatment for HCC. Curative resection is one of the treatment options in HCC [22, 23]. However, in most of cases, the tumor is beyond the resection limits at the time of diagnosis along with failure to meet inclusion criteria for transplantation programs. In such cases, nonsurgical treatment modalities can be utilized such as local ablation (ethanol, acetic acid, radiofrequency, cryoablation), trans-arterial chemoembolization, radioactive iodine and lipiodol therapy [24-26].

In patients with advanced HCC, routine use of chemotherapy is not possible due to several reasons. HCC is a relatively chemotherapy-resistant tumor. Chemotherapy cannot be tolerated by patients with severe hepatic dysfunction, while efficacy of chemotherapy is also low among patients with significant cirrhosis. Comparative efficacy of several chemotherapeutics including doxorubicin, tamoxifen, megestrol, interferon

alpha, antiandrogens and sorafenib have been reported in the randomized controlled studies. Apart from sorafenib, use of these agents caused marked toxicity with no significant survival benefit or improvement in complete response rates [27, 28].

In this study, we aimed to evaluate patients diagnosed with HCC in our hospital in terms of etiological, demographic, clinical and laboratory characteristics.

METHODS

A total of 207 patients diagnosed with HCC based on clinical, radiological and pathological findings were included in this retrospective study conducted at tertiary care gastroenterology and oncology clinics of ***** Training and Research Hospital in 2010.

Data on patient demographics (age, gender), biochemical and serological parameters, etiology, symptoms and physical examination findings at initial admission, presence of paraneoplastic syndrome, diagnostic and screening tests and final diagnosis after biopsy and tests were retrieved from hospital records. Smoking status and alcohol consumption as well as the concomitant medications such as oral contraceptive, androgen or steroids were also recorded.

RESULTS

Overall, 77.29% of patients were males. Median patient age in males and females were 58 years and 64 years, respectively. HBV positivity was noted in 68.2% of patients, while 16.6% of patients were HCV positive. History of regular alcohol consumption and smoking were noted in 11.1% and 62.8% of patients, respectively. Alcohol was considered the isolated risk factor only in 3 patients. The most frequent symptoms on initial admission were abdominal pain (30%), weight loss (19%), fatigue (16%) and jaundice (11%). Physical examination on initial admission revealed hepatomegaly and splenomegaly in 61.84% of patients, while ascites was noted in 70% of patients. Paraneoplastic syndrome was evident in 73 patients at the time of initial admission. The most common laboratory abnormalities included hyperlipidemia (65%) followed by hyperuricemia (17%), hypoglycemia (8%) and hypercalcemia (5%). Chronic hepatitis and cirrhosis were evident in 78% and 64% of patients, respectively at the time of initial diagnosis, while HCC was the first diagnosis in 33 patients without chronic hepatitis or cirrhosis. USG was the first diagnostic test used in 54% of patients. Assessment regarding the localization of the tumor revealed the tumor to be

Table 1. Demographic and clinical characteristics

Patient characteristics	n	%
Total	207	100
Gender		
Male	160	77.29
Female	47	22.71
Age		
Median	59	
Min-max	17-84	
Clinical Presentation		
Stomachache	103	30
Weight loss	65	19
Weakness	55	16
Jaundice	36	11
Swelling	28	8
Nausea-vomiting	16	5
Fever	14	4
Anorexia	10	3
Other (anorexia, constipation, itching, bone pain, cough)	14	4
Physical examination findings		
Hepatomegaly	128	61.8

Table 1 *contunied*. Demographic and clinical characteristics

Splenomegaly	107	51.7
Ascites	63	30
Icterus	39	18.8
Palmar erythema	32	15.4
Spider angioma	11	5.3
Venous collateral	10	4.8
Gynecomastia	3	1.5
Hepatitis serology		
HBV	120	58
HCV	23	11.1
HBV+HCV	7	3,4
HBV+HDV	4	1.9
Negative	46	22.2
Paraneoplastic syndrome		
Hyperlipidemia	48	65
Hyperuricemia	13	17
Hypoglycemia	11	15
Hypercalcemia	6	8
Hypokalemia	5	7
Gynecomastia	5	7
Hypothyroidism	3	4
Alcohol abuse	23	11.1
Tobacco abuse	130	62.8
OS (month)		
Median	7	
Min-max	0.25-48	

Abbreviations: HBV: hepatitis B virus, HCV: hepatitis C virus, HDV: hepatitis D virus, TNM: Tumor, lymph node, metastasis, OS: Overall survival.

located in the right lobe in 85% of cases. The HCC was diagnosed pathologically with biopsy in 68% of patients, while 32% of patients were diagnosed on the basis of clinic and radiological findings. According to TNM staging, 34% of patient had stage 3A disease, followed by stage 4A (17%), stage 3C (13%), stage 1 (12%), stage 4B (11%), stage 2 (9%) and stage 3B (4%) (Table 1). Excluding the 18 patients with no data available on AFP levels, AFP levels were found to be higher than normal in 78.8% of patients. AFP levels were > 100 ng/mL in 114 patients, > 400 ng/mL in 69 patients and > 1000 ng/mL in 39 patients (Table 2).

DISCUSSION

Although decrease in the incidence of HCC has been expected in relation to improved living standards in certain societies, the studies revealed paradoxical results indicating the incidence of HCC to be still on the unpreventable rise. In an autopsy study from Japan, assessment of 19357 autopsies revealed HCC rates to increase from 1.91% in years 1958-1959 to 7.66% in years 1986-1987 [29]. HBV infection is the etiological factor in nearly 80% of HCC cases and

HCC incidence correlates with HBV carrier rates [30].

In the current study, HBV positivity was noted in 68.2% of patients. Overall, 9 patients were HBsAg negative anti-HBc IgG positive. In a study by Matsuzaki *et al*. among Japanese patients with no serological findings related to HBV and HCV in the recent past, HBV-DNA was demonstrated to be integrated to the host genome, indicating that previous history of

Table 2. Baseline laboratory results of patients

Variables	Median	Min-max	Standard deviation
ALP (mg/dL)	389	33-3500	459.8
GGT (mg/dL)	221	16-1295	228.4
Bilirubin (mg/dL)	2.6	0.2-48	5.2
Albumin (g/dL)	3.2	1.2-5.1	0.9
LDH (U/L)	455.4	2-2215	294.5
AFP (ng/mL)	4376	0.6-54000	12912.3

Abbreviations: ALP: alkaline phosphatase, GGT: gamma glutamyl transferase, LDH: lactate dehydrogenase, AFP: fetoprotein.

HBV infection may also have a role in the neoplastic development [31].

HCV positivity was noted in 16.6% of our patients. This rate seems to emphasize a need for further investigation on HCV positivity, given that it represents the second most common risk factor in developing HCC, while in a study with 54 patients with primary liver tumor, none of patients had HCV positivity [32].

In our study, regular alcohol consumption rate was 11.1%. However, only 6 patients reported heavy alcohol consumption with at least 60 g daily alcohol consumption for more than 10 years. In addition, of 22 cirrhotic patients with alcohol consumption, alcohol was the isolated risk factor only in 3 patients who were also heavy alcohol consumers, while HBV positivity was evident in 16 patient, HCV positivity in 2 patients and HBV plus HCV positivity in 1 patient. Accordingly, on the basis of this rate, alcohol should be considered as a severe risk factor which also has a synergistic effect, in combination with the other concomitant etiologic factors, in progression to cirrhosis.

Although at initial admission, chronic hepatitis and cirrhosis diagnoses were noted in 78% and 64% of our patients, respectively; further clinical, laboratory, radiological and endoscopic evaluation after hospitalization of patients revealed the cirrhosis rate of 80.6%. This seems in accordance with the literature findings indicating 80-90% of HCC cases to be due to underlying cirrhosis.

Considering the localization of the tumor, our findings revealed the tumor to be located in the right lobe in 85% of cases. This finding is in agreement with distribution of tumor localization reported in a study by Özdemir *et al*. [32]

The USG was the first diagnostic test used in 54% of our patients, while CT and MRI were used as supportive diagnostic modalities. Amongst diagnostic tests, USG seems to be effective method in initial diagnosis, as a simple, cost-effective imaging modality with no side effects. When used as a screening test, USG has been associated with specificity of > 90% and sensitivity of 65-80% [33].

CT and MRI are used as complementary imaging modalities when USG alone is not sufficient. Despite the remarkable advances in imaging technology, there is no ideal method that can be used alone in HCC screening and to discriminate malignant and benign nodules. In such nodules, with inability to make malignant vs. benign discrimination, USG-guided liver biopsy is considered an important method supporting the diagnosis. In a study by Caturelli *et al*. on USG-guided fine needle aspiration biopsy of 294 newly detected nodules, findings revealed HCC diagnosis in 87.6% of patients who had < 2 cm nodules and could not be diagnosed via AFP, while in those with nodules smaller than 1 cm HCC diagnosis rate was 68.7% [33].

Elevated AFP levels have been reported in approximately 60-70% of patient with HCC across USA and Europe [34].

CONCLUSION

Our findings related to retrospective evaluation of 207 patients with HCC revealed the higher incidence of HCC at an advanced age and particularly in males, while the viral etiology, HBV (68.2%) and HCV (16.6%) in particular, remains to be the most important risk factor in our country. Chronic liver parenchyma disease at cirrhotic stage was evident in 64% of our patients, while imaging on tumor localization and size revealed the right lob location and a tumor size of > 7 cm in majority of patients. AFP levels, which were significantly over the normal range, may be a complementary marker aiding in diagnosis but have no significant impact on disease progression or survival. Other laboratory findings seem to be abnormal

but nonspecific. Metastasis sites involved lung (35%), lymph node (21%), bone (21%), abdominal wall (4%) and adrenal glands (4%).

Despite the provision of all treatment options, survival was poor with 7 months on average, while the longest and shortest survival time was 48 months and 1 week, respectively. As recommended, patients at high risk of HCC development should be included in a follow up program and be assessed in terms of USG findings and AFP levels every 6-12 months.

Authors' Contribution

Study Conception: FLK,; Study Design: FLH, TE,; Supervision: FLK,; Materials: TE,; Data Collection and/or Processing: TE,; Statistical Analysis and/or Data Interpretation: FLK, TE,; Literature Review: TE,; Manuscript Preparation: TE and Critical Review: FLK, TE.

Acknowledgement

The authors thank all those who participated in the recruitment of patients and helped to gather

REFERENCES

1. Parkin DM. Global cancer statistics in the year 2000. Lancet Oncol. 2001;2(9):533-43 DOI: 10.1016/s1470-2045(01)00486-7.

2. McMahon BJ, Alberts SR, Wainwright RB, Bulkow L, Lanier AP. Hepatitis B-related sequelae. Prospective study in 1400 hepatitis B surface antigen-positive Alaska native carriers. Arch Intern Med. 1990;150(5):1051-4 DOI: 10.1001/archinte.150.5.1051.

3. Sugano S, Miyoshi K, Suzuki T, Kawafune T, Kubota M. Intrahepatic arteriovenous shunting due to hepatocellular carcinoma and cirrhosis, and its change by transcatheter arterial embolization. Am J Gastroenterol. 1994;89(2):184-8 https://pubmed.ncbi.nlm.nih.gov/8304300/

4. MC. K. Tumors of the liver. In: Zakim D. BT, editor. HEPATOLOGY: A TEXTBOOK OF THE LİVER DİSEASE. PHILADELPHIA: WB Saunders Company; 1996. p. 1513.

5. Lai CL, Ng RP, Lok AS. The diagnostic value of the ratio of serum gamma-glutamyl transpeptidase to alkaline phosphatase in alcoholic liver disease. Scand J Gastroenterol. 1982;17(1):41-7 DOI: 10.3109/00365528209181042.

6. Yoon KT, Kim JK, Kim DY, Ahn SH, Lee JD, Yun M, et al. Role of ¹⁸FFluorodeoxyglucose Positron Emission Tomography in Detecting Extrahepatic Metastasis in Pretreatment Staging of Hepatocellular Carcinoma. Oncology. 2007;72(suppl 1)(Suppl. 1):10410 DOI: 10.1159/000111715.

7. Yuki K, Hirohashi S, Sakamoto M, Kanai T, Shimosato Y. Growth and spread of hepatocellular carcinoma. A review of 240 consecutive autopsy cases. Cancer. 1990;66(10):2174-9 DOI: 10.1002/1097 0142(19901115)66:10<2174::aidcncr2820661022>3.0.co;2-a.

8. Kew MC, Dos Santos HA, Sherlock S. Diagnosis of primary cancer of the liver. Br Med J. 1971;4(5784):408-11 DOI: 10.1136/bmj.4.5784.408.

9. Llovet JM, Bustamante J, Castells A, Vilana R, Ayuso Mdel C, Sala M, et al. Natural history of untreated nonsurgical hepatocellular carcinoma: rationale for the design and evaluation of therapeutic trials. Hepatology. 1999;29(1):62-7 DOI: 10.1002/hep.510290145.

10. A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. Hepatology. 1998;28(3):751-5 DOI: 10.1002/hep.510280322.

11. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42(5):1208-36 DOI: 10.1002/hep.20933.

12. Gogel BM, Goldstein RM, Kuhn JA, McCarty TM, Donahoe A, Glastad K. Diagnostic evaluation of hepatocellular carcinoma in a cirrhotic liver. Oncology (Williston Park). 2000;14(6 Suppl 3):15-20

13. Wu JT. Serum alpha-fetoprotein and its lectin reactivity in liver diseases: a review. Ann Clin Lab Sci. 1990;20(2):98-105 https://pubmed.ncbi.nlm.nih.gov/1691611/

14. Collier J, Sherman M. Screening for hepatocellular carcinoma. Hepatology. 1998;27(1):273-8 DOI: 10.1002/hep.510270140.

15. Lok AS, Lai CL. alpha-Fetoprotein monitoring in Chinese patients with chronic hepatitis B virus infection: role in the early detection of hepatocellular carcinoma. Hepatology. 1989;9(1):110-5 DOI: 10.1002/hep.1840090119.

16. Okuda K, Ohtsuki T, Obata H, Tomimatsu M, Okazaki N, Hasegawa H, et al. Natural history of hepatocellular carcinoma and prognosis in relation to treatment. Study of 850 patients. Cancer. 1985;56(4):918-28 DOI: 10.1002/1097-0142(19850815)56:4<918::aidcncr2820560437>3.0.co;2-e.

17. Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. The Cancer of the Liver Italian Program (CLIP) Investigators. Hepatology. 2000;31(4):840-5 DOI: 10.1053/he.2000.5628.

18. Primack A, Vogel CL, Kyalwazi SK, Ziegler JL, Simon R, Anthony PP. A staging system for hepatocellular carcinoma: prognostic factors in Ugandan patients. Cancer. 1975;35(5):135764 DOI: 10.1002/1097-0142(197505)35:5<1357::aid-cncr2820350518>3.0.co;2-8.

19. Lai CL, Lam KC, Wong KP, Wu PC, Todd D. Clinical features of hepatocellular carcinoma: review of 211 patients in Hong Kong. Cancer. 1981;47(11):2746-55 DOI: 10.1002/10970142(19810601)47:11<2746::aid-cncr2820471134>3.0.co;2-k.

20. Okuda K. Natural history of hepatocellular carcinoma including fibrolamellar and hepatocholangiocarcinoma variants. J Gastroenterol Hepatol. 2002;17(4):401-5 DOI: 10.1046/j.14401746.2002.02734.x.

21. Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis. 1999;19(3):329-38 DOI: 10.1055/s-2007-1007122.

22. Belghiti J, Hiramatsu K, Benoist S, Massault P, Sauvanet A, Farges O. Seven hundred fortyseven hepatectomies in the 1990s: an update to evaluate the actual risk of liver resection. J Am Coll Surg. 2000;191(1):38-46 DOI: 10.1016/s1072-7515(00)00261-1.

23. Bralet MP, Régimbeau JM, Pineau P, Dubois S, Loas G, Degos F, et al. Hepatocellular carcinoma occurring in nonfibrotic liver: epidemiologic and histopathologic analysis of 80 French cases.

Hepatology. 2000;32(2):200-4 DOI: 10.1053/jhep.2000.9033.
24. Hasegawa S, Yamasaki N, Hiwaki T, Sako K, Komorizono Y, Baba Y, et al. Factors that predict intrahepatic recurrence of hepatocellular carcinoma in 81 patients initially treated by percutaneous ethanol injection. Cancer. 1999;86(9):1682-90 DOI: 10.1002/(sici)10970142(19991101)86:9<1682::aid-cncr9>3.0.co;2-6.
25. Shiina S, Tagawa K, Niwa Y, Unuma T, Komatsu Y, Yoshiura K, et al. Percutaneous ethanol injection therapy for hepatocellular carcinoma: results in 146 patients. AJR Am J Roentgenol. 1993;160(5):1023-8 DOI: 10.2214/ajr.160.5.7682378.
26. Llovet JM, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. Hepatology. 2003;37(2):429-42 DOI: 10.1053/jhep.2003.50047.
27. Burroughs A, Hochhauser D, Meyer T. Systemic treatment and liver transplantation for hepatocellular carcinoma: two ends of the therapeutic spectrum. Lancet Oncol. 2004;5(7):40918 DOI: 10.1016/s1470-2045(04)01508-6.
28. Mathurin P, Rixe O, Carbonell N, Bernard B, Cluzel P, Bellin MF, et al. Review article: Overview of medical treatments in unresectable hepatocellular carcinoma--an impossible meta-analysis? Aliment Pharmacol Ther. 1998;12(2):111-26 DOI: 10.1046/j.13652036.1998.00286.x.
29. Okuda K KM, Okuda H. Neoplasms of the liver. In: Schiff L SE, editor. Diseases of the Liver. 7 ed. Philadelphia, PA: JB Lippincott Co; 1993. p. 1236-96.
30. El-Serag HB, Mason AC. Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med. 2000;160(21):3227-30 DOI: 10.1001/archinte.160.21.3227.
31HBV genome integration and genetic instability in HBsAg-negative and anti-HCV-positive hepatocellular carcinoma in Japan. Cancer Letters. 1997;119(1):53-61 DOI: https://doi.org/10.1016/S0304-3835(97)00249-8.
32. Özdemir S ST, Şentürk H. Primary liver tumors. Turkish Gastroenterology. 1993(4):398401
33. Caturelli E, Solmi L, Anti M, Fusilli S, Roselli P, Andriulli A, et al. Ultrasound guided fine needle biopsy of early hepatocellular carcinoma complicating liver cirrhosis: a multicentre study. Gut. 2004;53(9):1356-62 DOI: 10.1136/gut.2003.032359.
34 Bruix J, Barrera JM, Calvet X, Ercilla G, Costa J, Sanchez-Tapias JM, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. Lancet. 1989;2(8670):1004-6 DOI: 10.1016/s0140-6736(89)91015-5.