Erciyes Med J 2012; 34(2): 44-9 • DOI: 10.5152/etd.2012.15

Can the Presence of C-Kit-Positive Hepatic Progenitor Cells in Chronic Hepatitis C Have a Role in the Follow-Up of the Disease?

Kronik Hepatit C'de C-Kit Pozitif Hepatik Progenitör Hücrelerinin Hastalığın Takibinde Rolü Var mı?

ORIGINAL INVESTIGATION
ÖZGÜN ARAŞTIRMA

Süda Tekin Koruk[1], İlyas Özardalı[2], Dilnur Dinçoğlu[2], Muhammed Emin Güldür[2], Celal Çalışır[1]

ABSTRACT
ÖZET

Objective: The aim of this study was to compare the clinical and pathological properties of cases of hepatitis C virus (HCV) infection with respect to the presence or absence of hepatic progenitor cells positive for the proto-oncogene c-Kit (CD117) in hepatic tissue.

Material and Methods: The study was performed on hepatic biopsy specimens from 61 cases with HCV infection. Stained hepatic biopsy slides were examined. Immunohistochemical staining was performed using an anti-c-Kit antibody. The cases were divided into four groups (minimal, mild, moderate and severe according to their total hepatic activity index and into two groups (none/mild, moderate/severe) according to fibrosis grade.

Results: c-Kit-positive oval cells were detected in 30 cases (49.1%). There was no statistically significant difference in c-Kit positivity status with respect to the ALT, AST or HCV RNA results. c-Kit positivity increased with increasing hepatitis grade ($p<0.001$). In cases with moderate/severe fibrosis, c-Kit positivity was found to be higher than in cases with no fibrosis or low-grade fibrosis ($p<0.02$).

Conclusion: It was found that HCV patients with more inflammation and fibrosis were more likely to present c-Kit-positive hepatic progenitor cells. c-Kit expression in hepatic progenitor cells could possibly be used in the follow-up of patients with HCV. Larger patient cohorts are needed to confirm these results.

Key words: CD117 antigen, hepatitis c virus

Amaç: Bu çalışmanın amacı, kronik C hepatiti (KHC) tanısı almış olan olguların klinik ve patolojik özelliklerinin, karaciğer dokusundaki protoonkogen c-Kit (CD117) pozitif hepatik progenitör hücrelerin varlığı açısından karşılaştırılması idi.

Gereç ve Yöntemler: Çalışma, 61 kronik hepatit C enfeksiyonu olgusunun karaciğer biyopsi örneklerinde yapıldı. Boyalı karaciğer biyopsi lamları incelendi. Anti c-Kit antikoru kullanılarak immunhistokimyasal boyama yapıldı. Olgular almış oldukları toplam hepatik aktive indeksi puanına göre oluşturulmuş 4 gruba (minimal, mild, modereta, severe) ve fibrozis derecesine göre 2 gruba (no-mild, moderate to severe) ayrıldı.

Bulgular: Çalışmada olguların 30'unda (%49,1) c-Kit pozitif oval hücreler saptandı. C-Kit boyanma durumu ile alanin aminotransferaz, aspartat aminotransferaz ve HCV RNA düzeyleri arasında istatistiksel olarak anlamlı farklılık bulunmadı. Hepatit derecesi arttıkça c-Kit pozitifliği artmakta idi ($p<0,001$). Orta-ağır derecede fibrozis gösteren olgularda, fibrozis göstermeyen ve hafif derecede fibrozis gösteren olgulara göre c-Kit pozitifliği daha yüksek bulundu ($p<0,02$).

Sonuç: Çalışmamızdaki olgularda, artan inflamasyon ve fibrozis derecesi ile c-Kit pozitif hepatik progenitör hücre sayısında artış saptanmıştır. Bulgularımız, hepatit C olgularında c-Kit pozitif oval hücre varlığının hastaların takibinde bir kriter olabileceğini düşündürmektedir. Ancak sonuçların doğrulanması için geniş çalışmalara ihtiyaç vardır.

Anahtar kelimeler: CD 117 antijeni, hepatitis c virüsü

[1]Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Harran University, Şanlıurfa, Turkey

[2]Department of Pathology, Faculty of Medicine, Harran University, Şanlıurfa, Turkey

Submitted/Geliş Tarihi
04.08.2011

Accepted/Kabul Tarihi
28.03.2012

Correspondance/Yazışma
Dr. Süda Tekin Koruk
Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Harran University, 63100, Şanlıurfa, Turkey
Phone: +90 414 318 31 20
e.mail: suda_tekinkoruk@yahoo.com

Introduction

The most important life-threatening consequence of chronic HCV infection is the development of hepatic fibrosis, which is often followed by cirrhosis and hepatocellular carcinoma (HCC) (1). HCV infections are usually asymptomatic. Serological and molecular tests are useful for the diagnosis of the disease; however, liver biopsy is considered the most direct way of visualising the necroinflammatory status of the liver (2). Hepatic biopsy reveals the underlying causes of the hepatic disease as well as the grade of fibrosis and degree of necroinflammation (3).

The proto-oncogene c-Kit (tyrosine-protein kinase Kit or CD117) is a protein that in humans is encoded by the KIT gene. c-Kit is primarily expressed in haematopoietic stem cells, basal cells of the skin, melanocytes, germ cells and Cajal cells in the intestinal tract (4). It has been reported that this receptor has roles in cellular apoptosis, cell differentiation, proliferation, chemotaxis and the regulation of cellular adhesion (5). c-Kit overexpression is observed in many human malignancies, notably in gastrointestinal malignancies, colon cancer, and neuroblastomas (6, 7).

Progenitor cells (oval cells) of the liver are small, narrow cells with a basophilic cytoplasm and an oval nucleus, and they appear after all kinds of liver damage. These oval cells are bipotential and have the ability to differentiate into either hepatocytes or biliary epithelial cells. c-Kit is considered to be a determinant marker for these cells (8). In addi-

tion, it has also been reported in several studies that oval cells may be the progenitor cells of HCC and cholangiocarcinoma (8-10).

c-Kit expression has been mainly studied in HCC patients in previous publications. In the present study, the aim was to compare the clinical and pathological properties of cases diagnosed as chronic hepatitis C infection with respect to the presence or absence of c-Kit-positive hepatic progenitor cells in hepatic tissue.

Material and Methods

Patients

The study was performed on hepatic biopsy specimens of 61 cases of hepatitis C infection whose treatment and follow-up were ongoing between the dates of January 2008 and December 2009 in the Department of Infectious Diseases and Clinical Microbiology. The diagnosis and treatment approach were in accordance with the guideline criteria (11). HCV genotyping was performed with a line probe assay (Inno-LiPA HCV II, Bayer Diagnostics, USA) or with an in-house method (12, 13). Pre-treatment levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyl transferase, urea, creatinine, bilirubin, platelets, complete blood count, HBsAg, anti-HBs, anti-HDV, anti-HIV and autoantibodies were determined in these patients.

Liver biopsies were taken from patients who were anti-HCV and HCV RNA-positive by reverse transcriptase- polymerase chain reaction (RT-PCR) (Amplicor; Roche Diagnostic Systems, USA) after obtaining their written informed consent. Patients with chronic liver disease (decompensated cirrhosis, hemochromatosis, Wilson's disease and autoimmune hepatitis), patients presenting with findings of HCC by ultrasonography or computerised tomography, a history of alcohol consumption and those receiving immunosuppressive treatments were excluded from the study. The clinical and laboratory data of the cases were obtained from the follow-up records. The study was conducted upon review and approval of Harran University Medical Faculty Ethics Committee.

The histopathological evaluation of the biopsy specimens taken from the patients was performed in the Department of Pathology. For this purpose, slides stained with haematoxylin-eosin, reticulin and Masson's trichrome were examined and the inflammation density and fibrosis grade were determined in each case. Specimens containing less than four portals in this evaluation were not included in the study. Inflammation density and fibrosis grades were performed according to the Modified Knodell system suggested by Ishak (14).

Accordingly, all the scores obtained in each case for periportal inflammation, interface hepatitis, confluent necrosis and lobular necrosis were summed and thus a total hepatic activity index (grade) was calculated. The cases were classified into four groups with respect to inflammation density according to their hepatic activity index score and were considered minimal if 1-3, mild if between 4-8, moderate if between 9-12 or severe if between 13-18. Each case was evaluated again with respect to the fibrosis grade according to the system suggested by Ishak. After this evaluation, the cases were divided in two groups in which cases with 0-2 points were designated none/mild fibrosis, while those with 3-6 points were designated moderate/severe fibrosis.

Immunohistochemical Staining

Paraffin blocks were taken from the archives for immunohistochemical examination and 4 μm-thick cross-sections were taken. The sections were deparaffinised and rehydrated by passing them through an alcohol series. Sections were treated in a 1 mmol/L EDTA (pH 8.0) solution in a microwave oven for 20 minutes for antigen retrieval. The sections were incubated for 5 minutes in 3% hydrogen peroxide solution for the inactivation of endogenous peroxidase. c-Kit (Dako, polyclonal rabbit-antihuman, code no: A4502) was applied as the primary antibody for 80 minutes. Then, biotinylated anti-mouse anti-rabbit IgG was applied to the sections, streptavidin-conjugated peroxidase was applied and the sections were stained with 3,3'diaminobenzidine tetrahydrochloride. Haematoxylin was used for contrast staining. In prepared specimens, oval round cells stained with c-Kit were evaluated by light microscopy. Accordingly, the cases were divided into two groups, i.e., cases containing c-Kit -positive oval cells and cases not containing them.

Statistical Analysis

Continuous variables were not normally distributed by the Kolmogorov-Smirnov test. Therefore, the Mann-Whitney U test and the Chi-square test were used in the statistical analysis. $P<0.05$ was taken as the significance level.

Results

In this study, 60.7% (n=37) of the participants were male, 39.3% (n=24) were female, and the mean age was 48.3±12.8 (range 15-66) years. The average alanine aminotransferase (ALT) and aspartate aminotransferase (AST) values of the cases were found to be 70.0±57.1 (range 19-264) U/L and 53.8±46.4 (range 16-228) U/L, respectively. The average HCV RNA level was $4.8x10^6±5.8x10^6$ (range $2.7x10^4$ -$3.4x10^7$) IU/mL. The genotype distribution of the cases was 77.0% (n=47) genotype 1 (1, 1a, 1b) and 23.0% (n=14) genotype 2 (2, 2a, 2b). a-Fetoprotein (AFP) levels in patients were within normal levels (≤10 ng/mL), and abdominal ultrasound results were normal.

The distribution of the age, ALT, AST and HCV RNA levels of the cases according to their fibrosis grade is presented in Table I. No statistically significant differences were observed between the fibrosis grade groups with respect to age, ALT, AST or HCV RNA ($p>0.05$) (Table 1).

The distribution of the ALT, AST and HCV RNA levels of the cases according to the c-Kit staining status of tissue sections is presented in Table 2. No statistically significant differences were observed between the c-Kit staining status groups with respect to ALT, AST or HCV RNA ($p>0.05$).

The distribution of the cases according to genotype and the presence of c-Kit positive cells is presented in Table 3. Although there were fewer cases containing c-Kit positive cells among the genotype 2 patients, there were no statistically significant differences between the two groups.

Table 1. Distribution of age, ALT, AST and HCV RNA levels of cases according to the fibrosis grade in tissue sections

	None/mild fibrosis median (min-max) (n=46)	Moderate/severe fibrosis median (min-max) (n=15)	U	p
Age (years)	53 (15-66)	54 (16-64)	344.0	0.98
ALT (IU/mL)	53.0 (19-264)	59.0 (21-243)	294.5	0.39
AST (IU/mL)	35.5 (16-228)	48.0 (20-196)	249.5	0.11
HCV RNA (IU/mLx10^3)	2100.0 (27-34000)	3820.0 (119-12000)	311.0	0.56

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatit C Virus

Table 2. Distribution of ALT, AST and HCV RNA levels of cases according to the c-Kit staining status of tissue sections

	C-Kit		U	p
	Negative (n=31)	Positive (n=30)		
ALT (IU/L)	44.0 (19-264)	58.0 (22-243)	389.9	0.27
AST (IU/L)	33.0 (16-228)	45.0 (20-196)	332.5	0.06
HCV RNA (IU/mLx10^3)	3100.0 (27-34000)	1865.0 (119-11000)	411.5	0.44

Values are median (min-max). ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, HCV: Hepatit C Virus

Table 3. Distribution the cases according to genotype and the presence of c-Kit positive cells

	c-Kit				Chi-square	p
Genotype	Negative		Positive			
	n	(%)	n	(%)		
1	21	44.7	26	55.3	2.11	0.14
2	10	71.4	4	28.6		

The distribution of the fibrosis and inflammation grades of the tissue section according to the presence of c-Kit positive cells is presented in Table 4. c-Kit positivity was found to be higher in cases with moderate/severe fibrosis than in cases with none/mild fibrosis ($p<0.02$). A higher inflammation grade was found to be associated with higher c-Kit positivity ($p<0.001$).

c-Kit-positive oval cells were found in 30 of the 61 cases (49.1%) included in the study. All these cells were observed in the portal areas or in the lobular border of the portal area. The vast majority of c-Kit positive narrow cytoplasmic cells found had formed small cellular clusters (Figure 1). In addition, c-Kit positivity of variable grades was also seen in biliary epithelial cells in the vast majority of the cases with moderate/severe fibrosis that contained c-Kit -positive oval cells (Figure 2).

Significant differences were found between the four different total hepatic activity index groups and between the two different fibrosis grade groups with respect to c-Kit oval cell positivity.

Discussion

Liver histology is the best prognostic indicator for chronic HCV infection as aminotransferase levels are normal in one-third of HCV patients. Histological damage is found to be advanced in some normal cases (1). In our study, the enzyme levels in moderate/severe fibrosis cases were found to be similar to those in none/mild fibrosis cases. Thus, the importance of liver histology rather than aminotransferase levels for HCV infection follow-up is once again confirmed.

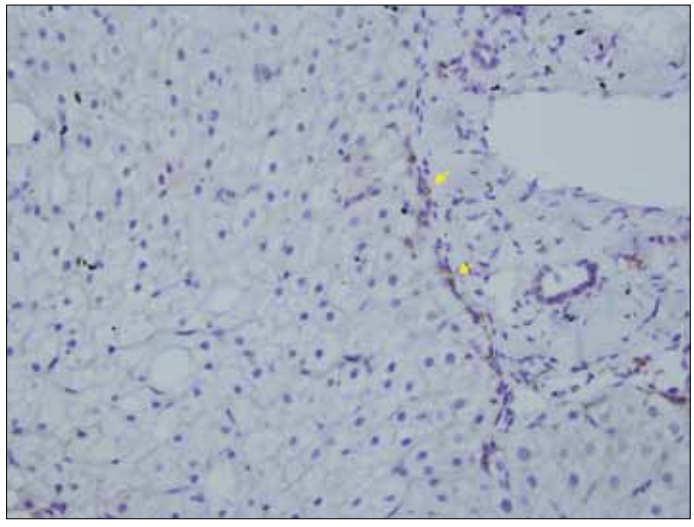

Figure 1. Immunohistochemical staining using a polyclonal rabbit antibody against c-Kit. Positive c-Kit stained oval cells (marked by arrows) were seen at the border of the portal area and the lobulus in a biopsy showing mild hepatitis and moderate fibrosis (magnification 400)

HCV RNA determination is the most sensitive method for the diagnosis of HCV infection (1). Although serum ALT levels can show fluctuations, serum HCV RNA levels remain constant (15). However, HCV RNA levels are not indicative of the fibrosis grade, and therefore cannot provide a prognosis of the disease (16). In our study, HCV RNA levels were found to be high in advanced-stage hepatitis; however, there was no significant difference between this stage and the other stages.

Table 4. Distribution of the fibrosis and inflammation grades of tissue sections according to the presence of c-Kit-positive cells

		c-Kit					
		Negative		Positive			
		n	%	n	%	Chi-square	p
Fibrosis	None/mild fibrosis	29	63.0	17	37.0	9.2	0.02
	Moderate/severe fibrosis	2	13.3	13	86.7		
Grade	Minimal[a]	13	92.9	1	7.1	16.9	0.001
	Mild[a]	17	45.9	20	54.1		
	Moderate[a]	1	10.0	9	90.0		

[a]All three groups make a difference. As the grade increased, the percentage of C-kit positivity also increased

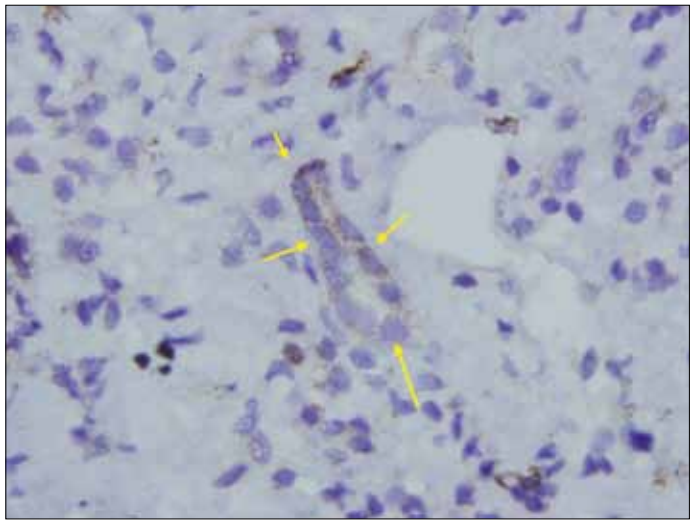

Figure 2. Positive c-Kit staining of biliary epithelial cells(marked by arrows) in the biliary ductus in the portal area (magnification 1000)

Hepatic fibrosis occurs in response to all forms of liver injury, in which collagen and other proteins are deposited between hepatocytes and the sinusoidal endothelium. In viral hepatitis, fibrosis starts in the periportal area, expands in the form of septa and is observed as bridge formation between the portal areas (17). Primary HCC is a late complication of chronic HCV infection, and it is generally seen in patients with cirrhosis (18). In a study done on patients diagnosed with liver cancer, HCV was found in 71% and HBV infection in 15% of patients (19). Therefore, histopathological examination of the liver is an important method in the follow-up of patients diagnosed with chronic HCV infection for staging the disease, defining it with respect to cirrhosis, and for diagnosing HCC which is the most important complication of this disease.

One of the factors that positively affects the response to treatment in chronic HCV patients is infection with genotype 2 or 3 among the six identified genotypes (20). In our study, we found that staining with c-Kit in HCV genotype 2 cases was less but not statistically different than in genotype 1 cases. We did not find any study in the literature in which both genotype and c-Kit positivity were studied. However, taking into consideration that genotype is an important prognostic factor, our opinion is that c-Kit positivity will be correlated with genotype in evaluations done with a larger number of cases.

Oval cells, which are small cells with a characteristic ovoid nucleus, are one of the important origins of liver stem cells. They appear in the periportal region and then infiltrate along the bile canaliculi (21). Ma et al. (22) observed that, in human livers with chronic viral hepatitis, some c-Kit-positive cells with an oval-like morphology were present, and an antibody against c-Kit was useful in detecting them. These oval cells were characterised by ovoid nuclei (7 µm×9 µm to 12µm×17 µm), small size and scant cytoplasm. They were located predominantly in the periportal region, and were often found in close association with inflammatory cells in chronic active hepatitis (22). As mentioned in the literature, in prepared specimens, oval round cells stained with c-Kit were evaluated in the present study. Lee et al. (8) stated that expression of c-Kit is also noted in mast cells with a distinctive membranous pattern of staining. In addition to morphological differences, leukocyte common antigen (CD45) immunostaining in consecutive sections allowed the separation of mast cells from c-Kit positive progenitor cells.

c-Kit belongs to the type III family of receptor tyrosine kinases expressed in normal cells, such as hematopoietic stem cells, germ cells and ductal breast epithelium (23, 24). c-Kit has also been found to be expressed in 46 different tumour types, including gastrointestinal tumours (25). Mansuroglu et al. (26) showed that, compared with the normal liver, stem cell factor (SCF) and/or c-Kit gene expression increased in a rat model of cirrhosis and cholangiocarcinoma, which is the second most common primary hepatic neoplasia. They concluded that SCF and its receptor c-Kit may contribute to tumour development. In a study done by Medinger et al. (27) in cancer patients, c-Kit immunopositivity was found in 22% of cases, although positivity was found in only 17% of patients with HCC. Although patients with chronic hepatitis C were included in this study, patients who had developed HCC were not included. However, c-Kit positivity has also been shown in the early stages of the disease. There are publications in which c-Kit positivity in HCC cases and its relationship with chronic hepatitis were investigated, but few studies have addressed hepatitis grade and c-Kit density in chronic hepatitis C infection.

A close correlation between the degree of progenitor cell activation and the severity of inflammation and fibrosis has been shown in chronic hepatitis (28). In chronic hepatitis B patients, c-Kit positivity was very significantly associated with both necroinflammatory activity grade and the stage of the disease (9). In our study, the re-

sults were similar to those obtained in chronic hepatitis B patients. Although there was no group of patients with HCC, the c-Kit staining ratio was found to be higher in advanced stages of the disease.

In some cases in which c-Kit-positive oval cells were found, c-Kit positivity was also found in biliary epithelial cells. The results of previous studies show that c-Kit-positive cells are bipotential cells that can differentiate into either hepatocytes or biliary epithelium (29).

Some discrepancies exist between the results of studies in which c-Kit positivity was investigated in HCC cases. Although c-Kit positivity in HCC was observed in some studies, c-Kit positivity was not observed in other studies (9). Finding c-Kit expression in tumor cells supports the theory that progenitor cell activation develops as a result of chronic liver damage due to hepatitis and contributes to the formation of HCC, because c-Kit expression is a feature of progenitor cells. If this theory is proven to be correct, then hepatitis cases in which c-Kit positivity is seen at early stages bear a higher rate risk of developing carcinoma (HCC). However, we think that advanced studies investigating genetic and molecular changes in hepatitis C and HCC cases are required.

Conclusion

A statistical correlation was not found between biochemical parameters and c-Kit positivity in hepatitis C cases in our study. However, a significant relationship was found between fibrosis stage (which is one of the most important prognostic determinants) and c-Kit positivity and inflammation density. Therefore, c-Kit expression in hepatic progenitor cells could possibly be used as a marker in the follow-up of patients with HCV.

Acknowledgements
This manuscript was presented as a poster at the 15th Congress of Turkish Clinical Microbiology and Infectious Diseases (KLIMIK), 23-27 March 2011, Antalya, Turkey.

Conflict of interest
No conflict of interest was declared by the authors.

Authors' contributions: Conceived and designed the experiments or case: STK, IO, CC. Performed the experiments/examined the case: STK, IO, DD, MEG. Analysed the data: STK. Wrote the paper: STK, IO. All authors read and approved the final manuscript.

References

1. Ray SC, Thomas DL. Hepatitis C. In: Mandell GL, Benett JE and Dolin R, eds. Principles and Practice of Infectious Diseases. 7th ed. Philadelphia, Churchil Livingstone; 2010. p. 2157-85. [CrossRef]
2. Fontaine H, Nalpas B, Poullet B, Carnot F, Zylberberg H, Brechot C, et al. Hepatitis activity index is a key factor in determining the natural history of chronic viral hepatitis C. Hum Pathol 2001; 32(9): 904-9. [CrossRef]
3. Marcus EL, Tur-Kaspa R. Chronic hepatitis C virus infection in older adults. Clin Infect Dis 2005; 41(11): 1606-11. [CrossRef]
4. Gibson PC, Cooper K. CD117 (KIT): a diverse protein with selective applications in surgical pathology. Adv Anat Pathol 2002; 9(1): 65-9. [CrossRef]
5. Sakuma Y, Sakurai S, Oguni S, Hironaka M, Saito K. Alterations of the c-Kit gene in testicular germ cell tumors. Cancer Sci 2003; 94(6): 486-91. [CrossRef]
6. Lux ML, Rubin BP, Biase TL, Chen CJ, Maclure T, Demetri G, et al. KIT extracellular kinase domain mutations in gastrointestinal tumors. Am J Pathol 2000; 156(3): 791-5. [CrossRef]
7. Chung CY, Yeh KT, Hsu NC, Chang JH, Lin JT, Horng HC, et al. Expression of c-Kit protooncogene in human hepatocellular carcinoma. Cancer Lett 2005; 217(2): 231-6. [CrossRef]
8. Lee ES, Han EM, Kim YS, Shin BK, Kim CH, Kim HK, et al. Occurrence of c-Kit + tumor cells in hepatitis B virus-associated hepatocellular carcinoma. Am J Clin Pathol 2005; 124(1): 31-6. [CrossRef]
9. Kara B, Doran F, Kara IO, Akkiz H, Sandikci M. Expression of c-Kit protooncogen in hepatitis B virus-induced chronic hepatitis, cirrhosis and hepatocellular carcinoma: has it a diagnostic role? Int J Clin Pract 2008; 62(8): 1206-11. [CrossRef]
10. Gordon GJ, Coleman WB, Grisham JW. Temporal analysis of hepatocyte differentiation by small hepatocyte-like progenitor cells during liver regeneration in retrorsine exposed rats. Am J Pathol 2000; 157(3): 771-86. [CrossRef]
11. National Institutes of Health. National Institude of Health Consensus Development Conferece Statement: Management of hepatitis C: 2002. Hepatology 2002; 36(5 Suppl 1): S3-20. [CrossRef]
12. Stuyver L, Wyseur A, van Arnhem W, Hernandez F, Maertens G. Second generation line probe assay for hepatitis C virus genotyping. J Clin Microbiol 1996; 34(9): 2259-66.
13. Yun Z, Lara C, Johansson B, Lorenzana de Rivera I, Sonnerborg A. Discrepancy of hepatitis C virus genotypes as determined by phylogenetic analysis of partial NS5 and core sequences. J Med Virol 1996; 49(3): 155-60. [CrossRef]
14. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995; 22(6): 696-9. [CrossRef]
15. Inglesby TV, Rai R, Astemborski J, Gruskin L, Nelson KE, Vlahov D, et al. A prospective, community based evaluation of liver enzymes in individuals with hepatitis C after drug use. Hepatology 1999: 29(2): 590-6. [CrossRef]
16. Chevaliez S, Pawlotsky JM. Hepatitis C virus serologic and virologic tests and clinical diagosis of HCV- related liver disease. Int J Med Sci 2006; 3(2): 35-40. [CrossRef]
17. Goodman ZD, Ishak KG. Histopathology of hepatitis C virus infection. Semin Liver Dis 1995; 15(1): 70-81. [CrossRef]
18. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, et al. Hepatitis C virus genotypes and risk of hepatocellular carsinoma in cirrhosis: a prospective study. Hepatology 1997; 25(3): 754-8. [CrossRef]
19. Simonetti RG, Cammà C, Fiorello F, Cottone M, Rapicetta M, Marino L, et al. Hepatitis C virus infection as a risk factor for hepatocellular carsinoma in patients with cirrhosis. A case-control study. An Intern Med 1992; 116(2): 97-102.
20. Asian Pacific Association for the Study of the Liver (APASL) Hepatitis C Working Party, McCaughan GW, Omata M, Amarapurkar D, Bowden S, Chow WC, et al. Asian pasific assocaiton for the study of the liver consensus statements on the diagnosis, management and treatment of hepatitis C virus infection. J Gastroenterol Hepatol 2007; 22(5): 615-33. [CrossRef]
21. Marrero JA. Hepatocellular carcinoma. Curr Opin Gastroenterol 2005; 21(3): 308-12. [CrossRef]
22. Ma X, Qiu DK, Peng YS. Immunohistochemical study of hepatic oval cells in human chronic viral hepatitis. World J Gastroenterol 2001; 7(2): 238-42.
23. Arber DA, Tamayo R, Weiss LM. Paraffin section detection of the c-Kit gene product (CD117) in human tissues. Value in the diagnosis of mast cell disorders. Hum Pathol 1998; 29(5): 498-504. [CrossRef]
24. Lammie A, Drobnjak M, Gerald W, Saad A, Cote R, Cordon-Cardo C. Expression of c-Kit and kit ligand proteins in normal human tissues. J Histochem Cytochem 1994; 42(11): 1417-25. [CrossRef]
25. Went PT, Dirnhofer S, Bundi M, Mirlacher M, Schraml P, Mangialaio S, et al . Prevalence of Kit expression in human tumors. J Clin Oncol 2004; 22(22): 4514-22. [CrossRef]

26. Mansuroglu T, Ramadori P, Dudás J, Malik I, Hammerich K, Füzesi L, et al. Expression of stem cell factor and its receptor c-Kit during the development of intrahepatic cholangiocarcinoma. Lab Invest 2009; 89(5): 562-74. [CrossRef]
27. Medinger M, Kleinschmidt M, Mross K, Wehmeyer B, Unger C, Schaefer HE, et al. C-Kit (CD117) Expression in Human Tumors and its Prognostic Value: An Immunohistochemical Analysis. Pathol Oncol Res 2010; 16(3): 295-301. [CrossRef]
28. Lee TK, Castilho A, Ma S, Ng IO. Liver cancer stem cells: implications for a new therapeutic target. Liver International 2009; 29(7): 955-65. [CrossRef]
29. Crosby HA, Kelly DA, Strain AJ. Human hepatic stem-like cells isolated using c-Kit or CD34 can differentiate into biliary epithelium. Gastroenterology 2001; 120(2): 534-44. [CrossRef]