Hepatocellular carcinoma in Nigeria - A review

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Hepatocellular carcinoma (HCC), a malignant tumour of the hepatocytes, is one of the 7 most common solid malignancies of man[1, 2]. It continues to be one of the most lethal human malignancies with a mortality index of 0.94. Approximately one million new cases of HCC are diagnosed per year world-wide. Its highest incidence is Africa, south of the Sahara, and South-East Asia, where the hepatitis B virus (HBV), aflatoxin contamination of foods and certain hepatotoxic medicinal herbs are recognised aetiological risk factors[3 4 5 6]. HCC accounts for more than half of all malignancies in some countries and is the leading cause of death in many populations[7]. In Nigeria, it is 3rd commonest cancer cause of death among adult males in the western Ife-Ijesha zone[8]. It is currently the tumour with the second highest increase in incidence and the one with the highest increase in death rates over the last 10 years in the United State[9].

Elsewhere, the occurrence of HCC is rising first from other causes, principally Hepatitis C Virus and also from the recent high international population mobility that is causing a rise in HBV in those parts. For example, in the United States, it has been estimated that the number of cases of HCC will continue to increase by 81% (from a baseline of about 13,000/yr) by the year 2020, primarily because of the hepatitis C epidemic[10]

The general outlook for HCC is dismal indeed. In fact, despite advances in medical technology, the 5-year survival between 1981 and 1998 improved only 3%, probably because most patients who have HCC are diagnosed at advanced stages leading to an overall 1-year survival of 25% in the United States (4) The uniformly catastrophic course and outcome for HCC is especially so in the high incidence cases where the disease appears to run a more rapid course. In Nigeria, Ndububa *et al*[11], while carrying out a 13–year prospective study of their cases found a mean survival period from diagnosis to be 14 weeks.

Epidemiology

Globally the distribution of HCC is closely linked to hepatotropic viral infections such as hepatitis C and B. Thus it is most common among the southeast Asians and sub-Saharan Africans and in these places, this has been linked with the high incidence of childhood infection with hepatitis B. HCC is more common in men than in women being the fifth most common cancer in men and the eighth in women worldwide. In the USA 74% of cases are men, in high risk areas (China, Japan, Africa) the difference in male: female ratio is as high as 8:1

In Nigeria, we are confronted with the archetypal high-incidence HCC as is the case in most of sub-Saharan Africa[12 13]. It is

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commoner among males but more female cases are being diagnosed and the age at diagnosis rising, probably because of recent improvements in health indices and life expectancy in the country[14 15].

Geographic distribution determines the age at diagnosis. In most parts of the world HCC is rarely diagnosed before the age of 40, however in Africa and Asia, the age of diagnosis is usually substantially lower. In Nigeria, teenagers have been diagnosed with the condition in our experience and, sometimes, extremely protean and unexpected modes of presentation have been reported[16 17].

Risk factors

Several risk factors have been associated with HCC. As mentioned above, the strongest is the increased incidence of viral hepatitis B (HBV) infections, as well as viral hepatitis C (HCV) infections. The overall incidence of HCC worldwide ranges based on the incidence of viral hepatitis infections. In the United States with a relatively low prevalence of viral hepatitis, the incidence of HCC is approximately 3 per 100,000[18]. In parts of Asia and Africa where viral hepatitis is endemic, the incidence of HCC is 30 per 100,000.

Viral hepatitis B is endemic in southern Africa and Southeast Asia, affecting approximately 6-28% of the population. Central and South America as well as northern Africa, eastern Europe and Asia have incidences of approximately 2-5%, while the incidence is less than 0.5% in North America. Viral hepatitis B carriers have a 5% increased risk of developing HCC[19]. In Southeast Asia nearly 100% of adults have serologic evidence of viral hepatitis B infections; vertical transmission accounts for greater than 60% of all new viral hepatitis B infections[20]. In Nigeria, chronic HBV infection is a major risk factor and aetiology for HCC. This stems from the high endemicity of infection by the virus as clearly demonstrated by Gashau *et al*[21], who reported anti-HBc seroprevalence of 80% and 84.4% among HCC patients and controls respectively. The latency period for development of HCC and viral hepatitis B is estimated at 10-20 years. The viral DNA inserts into the host DNA at multiple sites inducing alterations in genus control and cell growth. This causes non-specific cytotoxic effects, persistent hepatocyte damage and hepatocyte regeneration. Also, there is inappropriate activation of oncogenes, including HBV-X (HBX) activating gene as a possible cause for increased viral transmission. HBX protein acts as a transactivator of cellular and viral promoters and can complex with the C-terminus of the p53 gene causing functional mutation[22].

The incidence of viral hepatitis C is rapidly increasing throughout the world[20]. Hepatitis C is a risk factor for HCC with a latency period of 15-25 years. The hepatitis C virus is a blood-born virus that was found in 7-10% of all patients who received transfusions as late as the 1980s[23]. HCC has increased seventeen fold in HBV and HCV-infected patients[24]. 5 to 8% of patients with HCV will develop a chronic infection and carrier state. 20 to 30% will develop cirrhosis. Again, there are no specific mechanisms related to chronic hepatocellular damage and regeneration. However, it is thought that it may well be that the hepatitis C virus may possess a yet unrecognised oncogene within its genome. Hepatitis C virus does not appear to be an important cause of chronic liver disease in Nigeria at present[25].

Dietary hepatotoxic agents, principally aflatoxin B_1 (a by-product of fungal contamination of foodstuffs) have long been known to be a cause of HCC. Aflatoxin is produced by moulds and its products are intercalated into human DNA to form mutagenic adducts with guanosine of the p53 gene, a tumour suppressor gene. A rather peculiar codon 249 mutation of the p53 gene, common among liver cancers has been shown to be strongly associated with Aflatoxin B1 toxicity. The microsomal enzymes responsible for detoxifying aflatoxins are mutated in high

risk areas. In areas of Africa and Asia up to 60% of food is contaminated by aflatoxin and a good correlation exists between the level of contamination of foods with aflatoxins and the incidence of liver cancer[26]. A Nigerian study has shown a low incidence of codon 249 hotspot mutation among our HCCs probably signifying a supreme role for the HBV in liver carcinogenesis here[27]

Essentially, any disease process which causes cirrhosis of the liver will predispose a person to HCC. This is because the cirrhotic state, owing to its associated enhanced hepatocellular proliferation and turnover, is pre-malignant[28]. Sixty to 90% of patients with HCC have underlying cirrhosis with higher rates recorded in Western countries[29 30]. At autopsy, 20 to 40% of cirrhotics are found to have previously undiagnosed HCC. Aetiological factors for cirrhosis include viral hepatitis infections, chronic alcoholism, primary biliary cirrhosis, primary sclerosing cholangitis, haemochromatosis[31], alpha 1-antitrypsin deficiency, glycogen storage disease, and Wilson's disease. While in some countries of the northern Hemisphere such as France 92% of HCC is related to alcoholic cirrhosis[32], Alcohol is not an important cause risk factor for the disease among the generality of Nigerians.

Certain known hepatotoxic industrial chemicals such as thorotrast, polyvinyl chloride, and tetrachloride are also implicated in hepato-carcinogenesis[33]. These chemicals are not widely available in Nigeria.

Pathogenesis

The molecular pathogenesis of high-incidence HCC, as we have in Nigeria, has been strongly linked to chronic viral hepatitis, particularly HBV, since chronic inflammation is associated with presence of viral genotoxic products, host cytokine production, and increased hepatocellular turnover. Chronic HBV infection is associated with integration of its DNA into the host's genome and binding of the product of its transactivating X gene. This leads to activation of oncogenes and proto-oncogenes. The end result is disruption of normal cell cycle regulation. The hepatitis C viral protein putatively causes malignancy by disrupting normal cell signalling pathways. This affects cell growth regulation and apoptosis.

Morphology

Grossly, HCCs can be expansive, infiltrative, or diffuse. The expansive tumours are usually associated with liver cirrhosis, are usually singular and frequently have a fibrous capsule made up of apposition of the reticulin fibres found between the liver trabeculae. A minority of tumours are pedunculated, attached to the liver by a more or less wide implantation base.

The infiltrative HCCs may occur on cirrhotic or non-cirrhotic livers, and are so characterised because their boundaries with the surrounding liver parenchyma are smudgy and imprecise. They may be constituted of a single tumour

Fig. 1

focus, but more frequently there are multiple confluent nodules (Fig 1).

The diffuse carcinomas are seen as multiple, discrete nodules of 0.5 to 1cm diameter, which are homogenously distributed all over the liver, and which are always associated with cirrhosis (Fig 2).

Fig. 2

Microscopically, the fundamental histological characteristic of HCCs is the resemblance between the neoplastic cells and normal hepatocytes, and which are differentiated only by the nuclear irregularity of the former. The arrangement of the tumour cells in the form of trabeculae is also similar to the normal liver plates, even though there are some special structural patterns as well. Usually they lack reticulin stroma.

A gradation of I to IV is used to indicate the degree of differentiation of the tumour, even though areas with different degrees of differentiation could be observed in the same tumour. Cellular cohesion is lost in the less differentiated tumours.

Some HCCs may present inclusion bodies in the cytoplasm of a variable number of cells. Some of these are indistinguishable from hyaline of Mallory which is observed in alcoholic hepatitis. Other inclusion bodies have a spherical appearance and are PAS positive and diastase resistant. Some of these correspond to deposits of alpha 1-antitrypsin while others correspond to other proteins synthesized by the tumour cells which are not secreted in blood. In some HCCs one can observe cells with ground glass cytoplasm, similar to those of the hepatocytes laden with HBsAg but which are not reactive to anti-HBs. The presence of bile within the tumour cells and in the lumina of intercellular biliary canaliculi is an infrequent observation even though it is specific for HCC. Some tumours contain fat and glycogen vacuoles.

The cells of HCCs are arranged in various patterns which constitute the subtypes: trabecular, acinar, solid or compact, clear cell, pelioid, sarcomatoid, and sclerosing. The most common subtype is trabecular, which usually consists of broad 10-20 cells thick trabeculae of hepatocytes with a predominantly well-differentiated pattern, polyhedral cells, and large hyperchromatic nuclei with prominent, occasionally multiple nucleoli. The chromatin is clumped and attached to the nuclear membranes. The pseudoglandular type (or simply acinar) shares the cytological characteristics of the trabecular subtype but exhibits lumens usually filled with bile and rarely with mucin. The trabecular pattern is the most frequent and occurs in about 43% of cases, followed by the pseudoacinar occurring in 13%[34,35]. The compact subtype is characterised by small round or spindle-shaped

hepatocytes with hyperchromatic small nuclei without distinct nucleoli, but with very abundant chromatin and little cytoplasm. It adopts rarely a pattern reminiscent of neuroendocrine neoplasms, smoothly infiltrating the neighbouring normal hepatic parenchyma and occasionally grows within the sinusoids and associating with areas of hepatic dysplasia. The clear cell subtype appears to be a variant of the trabecular type in terms of general pattern of growth and arrangement of the neoplastic hepatocytes in cords with small peripheral nuclei, abundant clear cytoplasm, and ballooning of the tumour cells. The pelioid variant is characterised by blood-filled cystic spaces within the tumour while the sarcomatoid variant is a carcinosarcoma. The sclerosing variant is associated with hypercalcaemia and is characterised by extensive fibrosis in a cirrhotic or non-cirrhotic liver.

A special variant is fibrolamellar carcinoma, made up of large polygonal cells with abundant eosinophilic cytoplasm frequently containing inclusion bodies of the hyaline of Mallory type as well as bile. These cells are surrounded by a fibrous acellular stroma which has a laminar appearance. This variant of HCC is more common in young persons of both sexes and is not associated with liver cirrhosis. Alpha foeto-protein (AFP) is normal, but there are elevated levels of the transport protein of vitamin B12 in the blood. Multinucleation of tumour cells is a characteristic found occasionally in both clear cell and fibrolamellar subtypes (4% of autopsy cases) and almost never in the trabecular, well-differentiated tumours.

In most cases there is amplification of the c-met oncogene and positivity for the p53 protein, but in both cases their role is unknown, since this does not correlate with any other known morphological, prognostic, or biological parameter. Other independent pathways are marked by key mutations in the b-catenin gene, aberrant expressions of AFP[36], caveolin-1 and thrombospondin-1[37].

A large, probably multicentre, study of the morphological variety and clinical characteristic of HCC remains to be undertaken in Nigeria.

Prognosis and metastasis

HCC frequently invades the intrahepatic branches and the principal trunk of the portal vein and the hepatic veins, thereby constituting tumour thrombi. The thrombosis in the portal vein is less frequent in uncomplicated cirrhosis. On some occasions the portal tumour thrombus may grow toward the oesophageal varices. The tumour invasion of the hepatic veins is less frequent than the tumour occupation of the branches of the portal vein and both could even coexist. When the tumour occupation of the hepatic veins extends into the inferior vena cava it is clinically converted into the Budd-Chiari syndrome. Intravascular free-floating tumour clusters (IvCs) found histologically in the vicinity of the tumour is another interesting oddity of HCC. Thrombus formation is not seen morphologically in association with these IvCs, which are usually covered by endothelium. It is proposed that the endothelial-lined trabecular structure of HCC everts, frondlike, via vascular structures within the tumour capsule into peritumoral vascular lumens without destruction of the endothelial coating. This may protect these HCC tumour projections from thrombus formation but may also act as a barrier to tumour extravasation, and this may be exploited from a therapeutic point of view[38].

The invasion of the intrahepatic biliary system is less frequent (9.2% of autopsy cases). When this happens in the hilar region it precipitates the occlusion of the lumen of the common hepatic and/or common bile ducts which results in obstructive jaundice.

The incidence of metastasis ranges from 50 to 75% of autopsy cases without relation to the

cirrhotic or non-cirrhotic state of the underlying liver. Tumour spread can take place by the haematogenous route (56%), lymphatic route (26%), and contiguous spread (21%).

In Nigeria, owing to an aggressive course of the disease and tendency to late presentation, more than half of patients have pulmonary metastasis at death[12 14].

Treatment

Available options for the treatment of HCC include surgical and chemotherapeutic methods. Which is applicable in the individual case depends on a number of factors; these include the size, number and location of the tumour, presence or absence of cirrhosis, patency of the portal vein, and presence of metastasis, presence of other co-morbid diseases and the overall mental and physical health status of the patient.

Surgical resection and organ transplantation have dramatically improved the chances of cure and/or improved 5- year survival rates worldwide. The prognostic factors for a resectable tumour are the size and the functional reserve of the uninvolved residual liver. Chemoembolisation, radiofrequency ablation, ethanol ablation, cryoablation, radiotherapy or even chemotherapy may be useful as other forms of therapy.

Systemic chemotherapy is the mainstay of treatment of patients who are not candidates for surgical resection, transplantation or localized tumour excision. However, HCC is relatively chemotherapy resistant. This resistance is attributed to the universal expression of the multi-drug resistance gene protein on the surface of the malignant cells. This has been said to lead to active efflux of the chemical agents. Relatively young patients respond better to chemotherapy especially when the complications of cirrhosis have been adequately managed, than older patient.

Chemotherapy may either be in the form of single drug therapy, combination chemotherapy or chemoimmunotherapy.

- Single agent chemotherapy*:* Tested drugs include doxorubicin, cisplastin, and fluorouracil. gemcitabine, capecitabine. The most active drugs are fluorouracil, cisplastin and doxorubicin. Response rates for gemcitabine and capecitabine have been low and short term.
- Combination therapy has also been studied with cisplastin based combination therapy having a higher therapeutic rate than the others. Studies have shown that very little or no difference at all exists in the response rate between double/triple drug regimens. It must however be noted that some combination therapy do more harm than good
- Chemo immunotherapy simply refers to the combined therapy of chemical agents and immunomodulatory agents. *PLAF* is a combination of cisplastin, interferon-alpha,doxorubicin and infusional 5-fluorouracil.It has been associated with a high response rate which has been attributed to interferon-alpha(immuno-modulator). Unfortunately treatment related toxicity is quite high. Chemoimmunotherapy is best used for young patients with no cirrhosis and normal bilirubin levels.
- Some agents work by disrupting the lifelines of the tumour. This they do by preventing angiogenesis, they are therefore termed *Antiangiogenesis agents.* This class of drugs has proved to be quite promising since HCC is a highly vascular tumor. An example of an anti-angiogenesis agent is *bevacizumab.*

Chemoembolisation is also a form of chemotherapy. Here the chemotherapeutic agents are delivered directly to the tumour and

most of its blood supply. Embolising agents such as cellulose, gelatin foam particles, and microspheres are used to deliver intra-arterial chemotherapy. Chemoembolisation is highly contraindicated in patients with portal vein thrombosis, encephalopathy, or biliary obstruction.

Surgical resection is aimed at a possible cure for HCC. However resection is only possible in a small percentage of patients. It is best done when the HCC lesions are solitary and confined to the liver without vascular involvement and a greater percentage of the hepatic functional reserve sill intact. The procedure is called partial hepatectomy. There are no strict criteria in terms of tumour size but most surgeons use less than 5cm as their cut-off. The survival time and rate for respectable lesions vary widely. The time is usually 5-10years and the rates vary from 40% to about 90%.

Statistics show that Fibro lamellar HCC may have a better prognosis for survival after surgical resection because of a more favourable size, predominantly left lobe location, and the absence of cirrhosis in the unaffected portion of the liver. Appropriate evaluation of patients prior to resection is crucial since intra-operative mortality is doubled in cirrhotic versus non-cirrhotic patients. Preoperative laparoscopic inspection aids in diagnosing both the tumour and extent of cirrhosis

Liver transplantation has the potential for eliminating the cancer the cancer as well as the underlying liver disease, therefore most patients are suitable candidates for transplantation. It involves replacing the damaged liver with a donor liver provided the two individuals are HLA compatible.

Local tumour ablation is reserved for patients who are not candidates for surgical resection yet can have liver directed procedures performed on them. It involves percutaneous injection of ethanol, acetic acid; heat (via radiofrequency, microwave or laser ablation) or cold (with liquid nitrogen).Local tumour ablation can only be used for tumours smaller than 4-5cm.

In a recent work, it was shown that the overwhelming majority of Nigerian HCC patients present in such an advanced state that in 96.1% of them only symptomatic treatment could be offered[11]. Obviously, surgical intervention, local ablation nor transplantation is an available option in this scenario, chemotherapy is unaffordable by most. Thus the misery is complete for the Nigerian HCC patient and his/her carers in the circumstance

References

1. Waterhouse J, Muir C, Correa P, Powell V, editors. Cancer incidence in five continents Vol. III International Agency for Research on Cancer. Lyon, France. WHO, 1976.
2. Gerber MA, Thung SN and Popper H. Viral Hepatitis. *In:* Deinhardt F, Deinhardt, editors. Viral Hepatitis and Hepatocellular carcinoma. New York: Marcel Dekker Inc.; 1983; 327–41.
3. Blumberg BS, Larouze B, Thomas W, *et al.* The relation of infection with the Hepatitis B agent to primary hepatic carcinoma. Am J Pathol 1975; 81: 69–682.
4. Van Rensburg SJ, Cook-Mozaffari P, Van Schalkkwyk DJ, Van DerWatt JJ, Vincent TJ and Purchase IF. Hepatocellular Carcinoma and dietary aflatoxin in Mozambique and Transkei. Brit J Cancer 1985; 51: 713–726.
5. Tsega E. Hepatocellular Carcinoma In Ethiopia: A Prospective Study of 100 Patients. E Afr Med J 1977; 54: 281–291.
6. Beasly RP, Hwang L-Y, Lin C-C and Chien CS. Hepatocellular Carcinoma

and Hepatitis B Virus: A Prospective Study of 22707 Men in Taiwan. Lancet 1981; 2: 1129–1133.
7. Global surveillance and control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J Viral Hepat 1999; 6: 47-55.
8. Ojo OS Unpublished 10-year report of the Ife-Ijesha Cancer Registry. 1999.
9. El-Serag HB, Davila JA., Petersen NJ *et al.,* The continuing increase in the incidence of hepatocellular carcinoma in the United States: An update Ann. Intern Med2003; 139(10): 817 - 823
10. El-Serag HB and Mason AC., Trends in the survival of patients with hepatocellular carcinoma between 1977 and 1997 in the United States. Hepatology 2001; 33: 62 - 65
11. Ndububa DA, Ojo OS, Adeodu OO, Adetiloye VA, Olasode BJ, Famurewa OC, Durosinmi MA and Agbakwuru EA. Primary Liver Cell Carcinoma in Ile-Ife, Nigeria: A Prospective Study of 154 Cases. Nigerian Journal of Medicine 2001; 10 (2): 59 - 63
12. Francis TI and Smith JA Hepatocellular Carcinoma in Nigerians: A Study of 144 autopsy-proven cases (1958- 1968) W Afr 1972; 21: 37–42.
13. Fakunle YM, Ajudkiewicz AB, Greenwood MB and Edington GM. Primary Liver Cell Carcinoma in Northern Guinea Savannah of Nigeria. Trans Roy Soc Trop Med. Hyg 1977; 71: 335–337.
14. Olubuyide IO. The Natural History History of Primary Liver Cell Carcinoma: A Study of 89 Untreated adult Nigerians. Centr Afr J Med 1992; 38: 25–29.
15. Bojuwoye BJ, Anjorin AS, Olatunji PO, Ogunsulire IA and Aliyu RA. Primary Liver Cell Carcinoma in Ilorin, Nigeria. Abstract of paper presented at the conference of the Nigerian Association for the Study of the Liver (NASL), Ile-Ife. 1996: 6.
16. Ojo OS. Hepatocellular carcinoma in Nigerians. Yet Unpublished data.
17. Nganwuchu AM and Ojo OS, Rupture of a nodule of hepatocellular carcinoma simulating uterine rupture in late pregnancy. Tropical Doctor 1991, 21:46-47.
18. Greenlee RT, Murray T, Bolden S and Wingo PA. Cancer statistics, 2000. CA Cancer J Clin 2000; 50(1): 7-33.
19. Beasley RP. Hepatitis B virus. Cancer 1988; 61: 1942-1956.
20. Poovorawan Y, Chatchatee P and Chongsrisawat V. Epidemiology and prophylaxis of viral hepatitis: a global perspective. J Gastroenterol Hepatol 2002; 17 Suppl: S155-166.
21. Gashau W and Mohammed I. Hepatitis B viral markers in Nigerian patients with primary liver cell cancer. Trop Geog Med 1991; 43: 64–67.
22. Levy L, Renard CA, Wei Y and Buendia MA. Genetic alterations and oncogenic pathways in hepatocellular carcinoma. Ann N Y Acad Sci 2002; 963: 21-36.
23. Hassoun Z and Gores GJ. What surgeons should know about viral hepatitis and hepatocellular carcinoma. Surg Oncol Clin N Am 2003; 12(1): 1-11.
24. Donate F, Boffetta P and Puoti M. A meta-analysis of epidemiological studies on the combined effect of hepatitis B and C virus infections in causing hepatocellular carcinoma. Int J Cancer 1998; 75: 347-354.
25. Ojo OS, Thursz M, Thomas HC, Ndububa DA, Adeodu OO, Rotimi O, Lawal AA, Durosinmi MA, Akonai AK, Fatusi AO, and Goldin RD. Hepatitis B Virus markers, Hepatitis D Virus antigen and Hepatitis C Virus

antibodies in Nigerian Patients with Chronic Liver Disease. E Afr Med J. 1995; 72: 32-34.
26. Alpert ME and Davidson CS. Mycotoxins: A possible cause of primary carcinoma of the liver. Am J Med 1969; 46: 325-327.
27. Ndububa DA, Yakicier CM, Ojo OS, Adeodu OO, Rotimi O, Ogunbiyi O and Ozturk M. p53 Codon 249 mutation in hepatocellular carcinoma from Nigeria. Afr J Med Med Sci 2001; 30: 125–127.
28. Montalto G, Cervello M, Giannitrapani L, Dantona F, Terranova A and Castagnetta LA. Epidemiology, risk factors, and natural history of hepatocellular carcinoma. Ann N Y Acad Sci 2002; 963: 13-20.
29. Caporaso N, Romano M, Marmo R, de Sio I, Morisco F, Minerva A, *et al.* Hepatitis C virus infection is an additive risk factor for development of hepatocellular carcinoma in patients with cirrhosis. J Hepatol 1991; 12(3): 367-371.
30. Nzeako UC, Goodman ZD and Ishak KG. Hepatocellular carcinoma in cirrhotic and non-cirrhotic livers. Am I Clin Pathol 1996; 105: 65 -75.
31. Morcos M, Dubois S, Bralet M, Belghiti J, Degott C and Terris B. Primary liver carcinoma in genetic hemochromatosis reveals a broad histologic spectrum. Am J Clin Pathol 2001; 116: 738-743.
32. Poynard T, Aubert A, Lazizi Y, Bedossa P, Hamelin B, Terris B, *et al.* Independent risk factors for hepatocellular carcinoma in French drinkers. Hepatology 1991; 13(5): 896-901.
33. Bosch FX, Ribes J and Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999; 19(3): 271-285.
34. Chedid A, Ryan LM, Dayal Y, Wolf BC and Falkson G. Morphology and other prognostic factors of hepatocellular carcinoma. Arch Pathol Lab Med 1999; 123: 524-528.
35. Okuda K. New trends in hepatocellular carcinoma. Int Clin Lab Res 1993; 23: 173-178.
36. Torbenson M, Kannangai R, Abraham S, Sahin F, Choti M and Wang J. Concurrent evaluation of p53, b-catenin, and alpha-fetoprotein expression in human hepatocellular carcinoma. Am J Clin Pathol 2004; 122(3): 377-382.
37. Yerian LM, Anders RA, Tretiakova M and Hart J. Caveolin and thrombospondin expression during hepatocellular carcinogenesis. Am J Surg Pathol 2004; 28(3): 357-364.
38. Quaglia A, Etessami N, Sim R, Difford J and Dhillon AP. Vascular invasion and herniation by hepatocellular carcinoma in cirrhosis: A wolf in sheep's clothing? Arch Pathol Lab Med 2005; 129(5): 639-644.