Original Article

Hepatocellular Carcinoma in Zaria: An Analysis of the Morphology and Associated Risk Factors

Usman Bello [1], Samaila Modupeola Omotara [2], Iliyasu Yawale [1]

[1]Department of Morbid Anatomy and Forensic Medicine, College of Health Sciences, Usmanu Danfodio University, Sokoto. Nigeria [2]Department of Pathology, Ahmadu Bello University Teaching Hospital Zaria, Kaduna

Corresponding Author: *Dr. Usman Bello, Department of Morbid Anatomy and Forensic Medicine, College of Health Sciences, Usmanu Danfodio University, Sokoto, Nigeria. Tel: +234 803 610 7185, E-mail: bbusman55@yahoo.com*

ABSTRACT

Background: *There is substantial global variation in the prevalence of hepatocellular carcinoma (HCC). This variation depends on the prevailing aetiology and/or risk factors present in any particular locale, thus the need to document our experience with HCC in Zaria.* ***Materials and Methods:*** *This is a 15years morphological assessment of all diagnosed cases of HCC. Microscopic features detailing different pathologies were analysed and graded using the Edmond Steiner grading system.[1] Risks factors documented in accompanying case files were collated, the HBV and HCV statuses were determined by serological assays during patients' initial work up while alcoholism was determined based on quantity consumed per day over specified period.* ***Results:*** *Overall, sixty two cases were studied comprising 39 males and 23 females with a M: F ratio of 1.7:1. The ages ranged from 15 to 74years and a peak in the 5th decade of life with 21cases. The hepatotropic viruses were the commonest risk factors; HBV-35.5%, HCV- 9.7% and HBV/HCV co infection- 12.5%. Overall, chronic hepatitis accounted for 58% of cases. Other risk factors included alcohol consumption and morbid obesity which accounted for 6.5% and 3.2% cases respectively while cirrhosis and steatohepatitis accounted for 46.8% and 25.8% respectively. 66.1% cases were Edmond Steiner grades I and II diseases. Only 2 cases of clear cell HCC and solitary cases of sarcomatoid and sclerosing HCC variants were recorded.*

Conclusion: *The common risk factors for HCC were the hepatotropic viruses.*

Key word: Hepatocellular carcinoma, Hepatitis B infection, Chronic hepatitis, Cirrhosis

INTRODUCTION

Hepatocellular carcinoma (HCC) is a malignant tumour derived from hepatocytes and is a major public health problem worldwide.[2] It is the commonest primary tumour of the liver, the 5th commonest malignancy and also the third leading cause of cancer-related death with varied prevalence the world over.[3] Globally, the estimated incidence of new HCC cases is 500 000-1 000 000 per year, with 600 000 annual mortality. This ranges from 99 per 100,000 persons in Mongolia to less than 5 per 100,000 persons in Scandinavia.[4] HCC shows dynamic temporal trends with marked geographic regional variations, racial and ethnic group differences, gender predilections and the presence of several well-documented environmental potentially preventable risk factors.[5] The growing incidence of HCC worldwide is related to the increased prevalence of the various risk factors in particular, of chronic liver diseases. Chronic infection with Hepatitis B virus (HBV), Hepatitis C virus (HCV) or both is the most common cause globally and in Nigeria, the most important incriminated risk factor is hepatitis B viral hepatitis.[6] However, alcohol-induced liver injury in Western population and dietary ingestion of high levels of aflatoxin in Southern China and sub-Saharan Africa present special hazards, particularly in individuals chronically infected with HBV.[7] Other exogenous factors incriminated include iron overload, long-term use of oral contraceptives and high-dose anabolic steroids. The risk of HCC increases when aetiological agents exist in combination such as HCV infection and alcohol use or HBV infection and exposure to aflatoxin.[8]

Liver cirrhosis following necroinflammatory processes with fibrosis that occurs in chronic

infection with hepatotropic viruses and steatohepatitis associated with alcohol induced liver injuries, morbid obesity and some HCV genotype infections are the most significant risk factors for HCC development.[4] Chronic hepatitis by any aetiologic agent predisposes an individual to the development of HCC irrespective of the progression to cirrhosis. This is demonstrated in HBV chronic hepatitis and non - alcoholic steato-hepatitis (NASH).[9]

MATERIALS AND METHOD

All the liver biopsies submitted to the Department of Pathology, Ahmadu Bello University Teaching Hospital (ABUTH) Shika-Zaria, from 1st January, 2001 to 31st December 2015 formed the study materials. The biopsies were fixed in formalin, processed and embedded in paraffin and stained with hematoxylin and eosin, reticulin, periodic acid Schiff, Masson trichrome and mucicarmine stains. Patients' biodata, clinical history, documented risk factors and other relevant laboratory and radiographic investigations were retrieved from individual case cards. All HCC cases were retrieved, reviewed by authors and graded using the Edmondson and Steiner grading system which uses defined microscopic features as stated below;

Grade I: Well differentiated tumour; small tumour cells arranged in thin trabeculae
Grade II: Larger cells with abnormal nuclei, granular acidophilic cytoplasm and glandular structures may be present
Grade III: Larger and more hyperchromatic nuclei, less granular acidophilic cytoplasm
Grade IV: Poorly differentiated tumour cells, hyperchromatic nuclei and loss of trabecular pattern Known associated risk factors were determined from serological evidence of HBV and HCV, clinical history of significant alcohol consumption of 60–80 g/d of alcohol for at least 10 years in male and 20-40g/d in female and presence of morbid obesity in NAFLD. These were augmented with histological features like presence of ground glass hepatocytes with or without activity and presence of viral inclusion identified with orcein stain in HBV, presence of lymphoid aggregates with steatosis in HCV, while in ALD; steatosis, hepatocytic ballooning, apoptosis, mixed lobular inflammation, Mallory–Denk bodies, megamitochondria, portal lipogranuloma and the unique pattern of perisinusoidal/pericellular fibrosis were looked for. Non alcoholic steatohepatitis (NASH) is a clinico- pathologic syndrome with histological features similar to alcoholic liver disease in the absence of significant alcohol consumption.

RESULTS

Four hundred and thirty eight liver biopsies were received during the study period. Of these, only sixty two cases were HCC which formed 14.2% of all chronic liver diseases (CLDs) and third common cause of CLDs within the study period. There were 39 males and 23 females with a male to female ratio of 1.7:1. The ages ranged from 15 to 74years and peaked in the 5th decade of life. Fifty eight percent (58%) of cases occurred between the ages 31 to 50 years (Table I).

The associated risk factors were hepatotropic viruses comprising hepatitis B virus (HBV) in 22 cases (35.5%), hepatitis C virus (HCV) in six cases (9.7%) and HBV/HCV co infection in eight cases (12.5%). In four cases (6.5%) there was history of significant alcohol consumption confirmed histologically by presence of Mallory hyaline bodies, while the histologic feature in two other cases (3.2%), was that of non- alcoholic steato-hepatitis (NASH). In 32.3% of the cases, there was no evident aetiologic factor or clinic-pathologic correlation. (Figure I).

Overall, chronic hepatitis accounted for 58% (36) of cases and 20 of these cases (55.6%) progressed from cirrhosis to hepatocellular carcinoma. Also there were four cases of alcoholic liver diseases (ALD) cases with HCC and three of these, had background cirrhosis. Cirrhosis alone, accounted for 46.8% of the HCC cases reviewed, while steatohepatitis accounted for 25.8% and was seen in HCC cases with background HCV infection (4), HBV/HCV co infection (6), alcoholic liver disease (4) and non alcoholic liver disease (2) (Table II).
Histologically there were 58 cases of conventional HCC (Figure II) with 18 cases of Edmonson-

Steiner grade I disease, 23cases of grade II, 12 cases of grade III and 5 cases of grade IV disease. The remaining four cases were solitary cases of sarcomatoid and sclerosing HCC variants and two cases of clear cell variant in a 47 year and 53 year old female patients respectively. (Figure III)

Table I: Age and Sex Distribution of Patients with Hepatocellular Carcinoma

Sex	Age group in years							Total
	< 21	21-30	31-40	41-50	51-60	61-70	>70	
Male	3	4	9	12	6	3	2	39
Female	1	1	6	9	2	2	2	23
Total	4	5	15	21	8	5	4	62

Table II: Association of Known Risk Factors of HCC

Known Aetiologic agent	Frequency of the associated Risk factor				Total
	Hepatitis	Steatohepatitis	Cirrhosis	None	
Hepatitis B Virus	22	0	12	0	22
Hepatitis C Virus	6	4	3	0	6
HBV/ HCV co infections	8	6	5	0	8
Alcoholism	0	4	3	0	4
Morbid Obesity	0	2	0	0	2
Unknown	0	0	6	14	20
Total (%)	36 (58.1%)	16 (25.8%)	29 (46.8%)	14 (22.9%)	62 (100%)

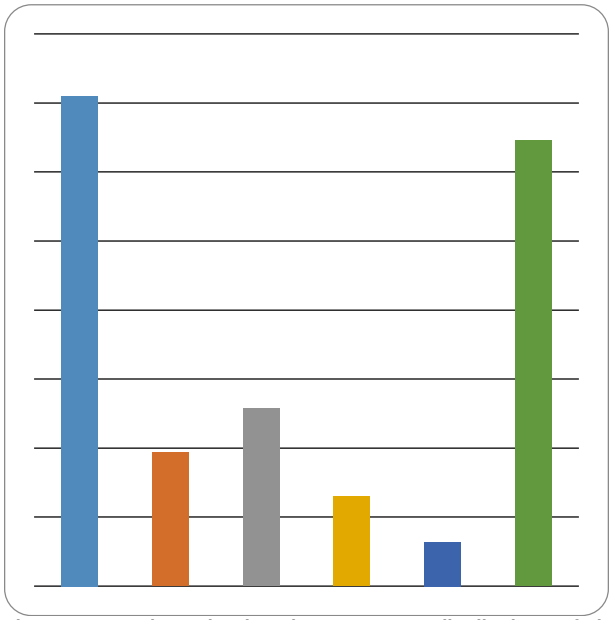

Figure I: Bar chart showing the percentage distributions of the known aetiologic agents of hepatocellular carcinoma

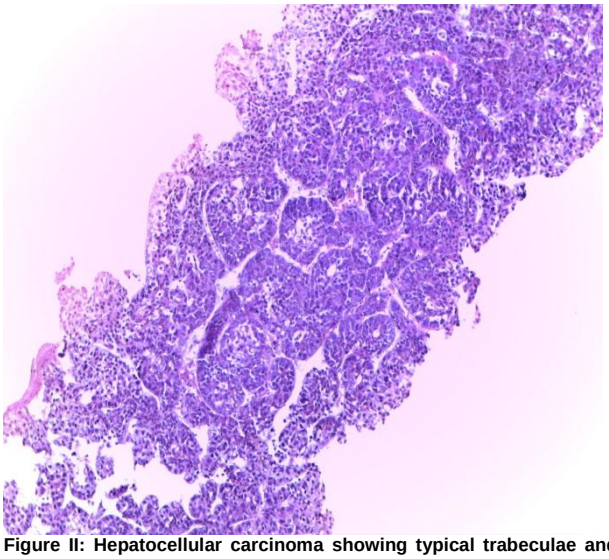

Figure II: Hepatocellular carcinoma showing typical trabeculae and acini formations; Edmonson Steiner grade II differentiations

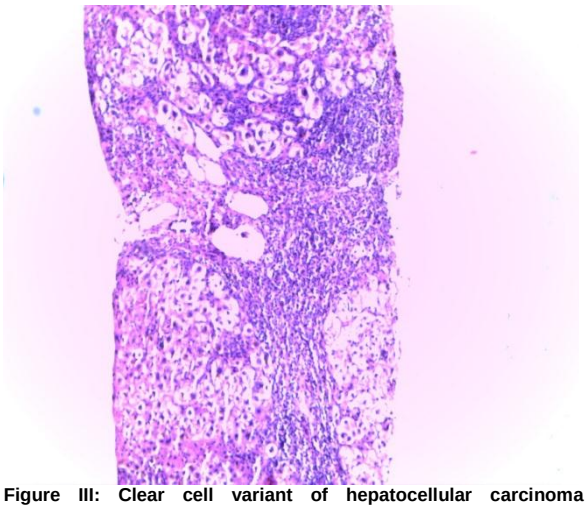

Figure III: Clear cell variant of hepatocellular carcinoma in background cirrhosis

DISCUSSION

HCC constituted 14.2% of all liver pathologies and was the third common cause of chronic liver diseases (CLDs) in this study period. Over sixty five percent of the cases occurred in the third to fifth decades of life with male predominance. This is the life and work force of any economically vibrant society thus negatively impacting on the social and economic well- being of the region. Our male preponderance is similar to reports from Bangladesh, Egypt and Nigeria (Port Harcourt).[10-12] The 14.2% frequency rate for HCC in this study is slightly higher though comparable to the 12.5% rate reported from Benin, South South Nigeria.[13] However, increasingly higher rates of 27.0%, 29.3%, 31.3%, 33.0%, 36.3%, 44.1% and 45.5% were reported in Kano, Ile-Ife, Jos, Lagos, Ilorin,

Enugu and Ibadan respectively, all within Nigeria.[14-20] These rates may be related to other risk factors in these regions. Similarly, Abdulkareem F B et al, in Lagos south west Nigeria reported an 80% frequency in their 118 cases comprehensive review of hepatic neoplastic diseases. [21] This is comparable to our HCC cases which accounted for 72.1% of all liver tumours in this study.

Varied prevalence has been documented within regions and countries. In Nigeria, reports showed different geographic prevalence. It was the 5th commonest cancer in Jos, North central [22] while Nwokediuko et al in Enugu, south east reported a frequency of 9.7% of all cancers[20] and 3.8% of the total malignancies in a report from Port Harcourt, south south.[12] Reports from Ife, Ilorin, Enugu and Ibadan revealed that HCC was the commonest cause of chronic liver disease,[15, 18-20] while in, Kano, Jos and Maiduguri it was second common cause following chronic hepatitis.[14,16,22]

Associated aetiologic factors identified in this study were HBV infection in 22 cases (35.5%), HCV infection in 6 cases (9.7%) and HBV/HCV co-infections in 8 cases (12.5%), while chronic hepatitis and cirrhosis were the most significant risk factors. The 46.8% post cirrhosis cases in this study is high compared to the 30% reported from Lagos,[22] and considerably lower than the 78% from Ile-Ife and 66.2% from Tanzania by Hyasinta J et al.[15,23] There is a strong evidence for the pathogenetic role of hepatotropic viruses in the development of HCC through the cirrhotic liver irrespective of age of the patient.[24] Hepatotropic viral hepatitis is the leading cause of liver cirrhosis associated hepatocellular carcinoma. In one series from Japan, the development rate of hepatocellular carcinomas after a 15-year observation period was 27% in hepatitis B and 75% in hepatitis C.[24] The cirrhotic state of the liver provides a premalignant state due to enhanced hepatocytes proliferation and high hepatocyte turnover in addition to other molecular events associated with the aetiological agents. In HBV hepatitis, viral DNA insert into the host DNA at multiple sites inducing alterations in genus control and cell growth causing non specific cytotoxic effects, persistent hepatocytes damage and regenerations in addition to inappropriate activation of oncogene HBV-X (HBX) gene.[6] HBX protein transactivate cellular and viral promoters and complexes with the C-terminus of p53 gene causing functional mutations.[5]

The incidence of hepatitis B and or hepatitis C surface antigenemia in patients with HCC is over 90% in most developed nations with significant percentages associated with cirrhosis.[4,5] The sero-prevalence of HBsAg in Nigeria ranges from 10% to 40% and that of HCV from 4.5% to 5%.[25]

Alcohol and morbid obesity resulting to ALD and NASH with or without cirrhosis were the least aetiologic factors seen in this study. In developed nations obesity causing NAFLD, alcohol and HCV infection were the leading aetiologic factors in the causation of HCC.[25] Obesity is a global public health problem particularly in developed countries and it is also an important risk factor for NAFLD. Non- alcoholic steatohepatitis (NASH) is a well-recognized clinico- pathologic syndrome that occurs primarily in obese women with diabetes mellitus and it has histological similarities to alcoholic liver disease in the absence of significant alcohol consumption. It is known to progress to cirrhosis and HCC, though the exact mechanism remains obscured.[8,26] Hyper-insulinaemia with insulin resistance and its antecedent molecular consequences are the culprit in the pathogenesis of HCC in NASH.[27] Hyperinsulinemia upregulate the production of insulin-like growth factor-1 (IGF1), activation of insulin receptor substrate-1 (IRS-1), which is involved in cytokine signaling pathways, causing cellular proliferation and inhibition of apoptosis.[9] Adiponectin which inhibit angiogenesis through modulation of apoptosis is decreased in insulin-resistant states.[26] C-Jun amino-terminal kinase 1 (JNK1); a ubiquitously expressed mitogen-activated protein kinase has an abnormally elevated activity in obesity and it phosphorylates IRS-1.[5,26] The net effect of these factors is promotion of uninhibited cell growth and may play significant role in the development of HCC in patients with NASH.[3,9,26] Increasing westernization and adoption of indolent lifestyle may thus pose another danger of increased incidence of HCC in our environment.

Most of our cases were conventional HCC with four exceptions of two clear cells, a sarcomatoid and a sclerosing HCC variants. Clear cell

hepatocellular carcinoma comprises about 9% of all malignant hepatocellular neoplasms and shows a marked female predilection and high association with cirrhosis.[24] Our two cases were in female patients and post cirrhotic. The Edmonson and Steiner grading system though old is still applicable for grading of conventional HCC system of grading however, it has little prognostic impact.[4] Also, a three tier simplified system has been in used in some centres as well differentiated, moderately differentiated and poorly differentiated corresponding to Edmonson Steiner grades I/II, III and IV respectively. The most important prognostic factor of HCC is tumour stage which depends on number and size of nodules, presence of vascular invasion and extra hepatic spread.[6] Tumour size is a major prognostic factor, with a very good prognosis for small or minute carcinomas.[28]

In conclusions, hepatocellular carcinoma is prevalent in our setting. It occurred a decade earlier compared to western population affecting young to middle aged individuals with male predilections. Hepatitis B viruses were the most significant aetiologic agents while chronic viral hepatitis, cirrhosis and steatohepatitis were significant risk factors for HCC development

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