Prevalence of Hepatitis B Virus (HBV) surface antigen and HBV-associated hepatocellular carcinoma in Kenyans of various ages

Geoffrey Zambezi Mutuma[1], Margaret W. Mbuchi [1] , Eberhard Zeyhle[3] , Rana Fasana[4], Fredrick A. Okoth[2] J. Maina Kabanga[1] , Kuria Julius[1] , T., Luseso Shiramba[1], M. Kariuki Njenga[5], Peter. M. Kaiguri[2], Vincent Osidiana[2]

1   Center for Clinical Research, Kenya Medical Research Institute P.O.BOX 20778-00202, Nairobi, Kenya
2   Center for Virus Research, Kenya Medical Research Institute P.O. Box 54628-00200, Nairobi , Kenya
3   African Medical and Research Foundation P. O Box 00506-27691Nairobi Kenya
4   University of Nairobi Pathology Department,College of Health Sciences P. O. Box 19676, Nairobi.
5   Center for Disease Control and Prevention-Kenya, Nairobi, Kenya.

SUMMARY

As a follow-up of an earlier study in Kenya that reported a 5:1 association between chronic liver disease and/or liver cancer with hepatitis B virus surface antigens (HBsAg), we investigated the prevalence of hepatitis B virus surface antigen in an asymptomatic rural normadic population, and among hepatocellular carcinoma patients in a major urban centre. In a rural population of 579 individuals, there was an overall HBsAg prevalence of 8.8%, with the largest number (34%) of those positive being between 5 and 10 years of age. The number of HBsAg positives decreased with advance in age, suggesting either mother to child transmission or early childhood exposure to hepatitis B virus (HBV). In a small group (N = 51) of rural patients seeking medical attention because of clinical symptoms of hepatomegaly and/or splenomegaly, 52% were HBsAg positive. Of the 88 liver cancer biopsies examined from an urban population, 78 (86.6%) were hepatocellular carcinoma (HCC) cases occurring mostly among 41 – 60 year old people, with the remaining being cholangiosarcomas (9%) and hepatoblastomas (2%). More importantly 75% of the HCC cases were HBsAg positive. Among the urban liver cancer cases, there was a male: female ratio of 5:2, which was also reflected by the high number of HBsAg-positive rural cases of patients with hepatomegaly. Taken together, these results indicate a greater than 5:2 association between HCC and HBV infection, and a higher prevalence of HBV exposure in males than females.

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Introduction

Hepatitis B virus (HBV) is a DNA virus belonging to the Hepadnaviridae family that infect humans causing a disease characterised by a strong preference for infecting liver cells. HBV infection is a serious global health problem affecting up to 2 billion people worldwide (1). The HBV-induced disease is the tenth cause of death worldwide with 500,000 to 1.2 million deaths per year due to chronic hepatitis, cirrhosis, and hepatocellular carcinoma (2,3). Hepatocellular carcinoma (HCC) is the fifth most frequent cancer that is responsible for 300,000 to 500,000 deaths annually (4,5,6,7). It is estimated that up to 45% of the world's population lives in areas of high chronic HBV prevalence including the sub-Saharan Africa, the Pacific and Asia (8,2). Non-endemic regions known to have high rates of chronic HBV infection include southeastern and Central Europe, the Amazon basin, the Middle East and the Indian sub-continent.

HBV is present in all body fluids and secretions, including blood, saliva, semen, sweat, breast milk, tears and urine, and therefore, virus is transmitted through various routes, apparently depending on the incidence of the disease in the region. In regions of high HBV incidence, the virus is transmitted vertically from an infected mother, either prepartum or perinatally, with the child having greater than 60% risk of acquiring HBV

infection (9,10,11). The rate of perinatal infections can be particularly high, reaching up to 90% (12,13). In regions of intermediate incidence, transmission occurs during infancy or early childhood, with a lifetime infection risk of 20-60%(10). In regions of low incidence, which include North America and Europe, HBV transmission occurs in adults through intravenous drug use or unprotected sex, with a lifetime risk of less than 20% (13,8).

Africa has the second largest number of chronic HBV carrier rate after Asia, with over 50 million people being lifetime carriers. It has been estimated that over 12 million people will die due to hepatitis B induced liver disease, representing a 25% risk among carriers (14).The sub-Saharan region is highly endemic with HBsAg carrier rates of 9-20%, whereas 56-98% of the adult population shows evidence of past exposure to HBV infection. Studies in Kenya showed a HBsAg carrier rates of 5 - 30% (15,18).The first peak of HBV infection in Kenya appears to be at early school age, whereas the second peak occurs at puberty and childbearing age (18).

HCC is the most common liver cancer, with a higher prevalence in males than females, and is almost always fatal. The risk factors of HCC include HBV infection, hepatitis C virus infection, aflatoxicosis, alcoholism, smoking, and hereditary conditions such as hemochromatosis, alpha-antitrypsin deficiency, tyrosinaemia, anabolic steroids and oestrogen levels (19,20,21, 22, 23). Epidemiological and experimental studies have demonstrated that chronic HBV infection is a major factor contributing to the development of primary liver cancer. For example, a high HCC incidence has been demonstrated in regions with a high seroprevalence for HBV infection (24). In addition, patients with HCC show 70 - 90% seroprevalence of HBV when compared to 10-20% HBV seroprevalence in the entire population in the same regions (25). Additionally, a 10 to 100-fold risk of HCC has been observed in HBsAg carriers compared to non-carriers in different ethnic and social groups (26,27,28).

Animal studies have also demonstrated oncogenicity of the HBV viral proteins including; demonstration of hepatocarcinogenesis associated with upregulated expression of viral proteins in transgenic mice, non-specific transactivation of cellular oncogenes and inactivation of the anti-tumour protein p53 by viral proteins (29,30).

Data on the prevalence of the HCC is obtained primarily from autopsies, and it provides a near accurate incidence of the cancer in the population.

Unfortunately, autopsy and death records in sub-Saharan Africa are poor and therefore inadequate in providing an accurate incidence of the disease. Limited autopsy data indicates that HCC is up to 10 times more prevalent in sub-Saharan Africa than in the western world, being associated with 4% and 5.8% of all deaths in South Africa and Nigeria, respectively (31,32,27). As with other geographical regions, HCC appears to be more common in males than in females with a male to female ratio of 5:1 in Uganda (33). In a study involving autopsies from several African populations, the average at which HCC was detected was 37years, when compared to 53 years in American blacks, and 63 years in American whites (34). The data also showed a strong association between HCC and HBV infection, with 68% and 72% of the people in Uganda and Zambia, respectively, having HCC also positive for HBsAg, when compared to 8% and 12% of controls, respectively (35).

An earlier study in Kenya reported that 77% of patients with liver disease or primary liver cancer were positive for the HBsAg or HBV antibodies, when compared to 15% in a control group (36). Here, we studied the prevalence of HBsAg across various ages of males and females in a rural Kenyan population, and used liver biopsies obtained from a more medically advanced urban population to determine the prevalence of HBV-associated HCC across age groups and gender.

The Turkana study was conducted in the month of July, 1994 in the North Eastern Turkana district and was prompted by an observation of a relatively high number of individuals with jaundice and enlarged liver and spleen, during a hydatid disease survey carried out by the Hydatid disease control program Africa Medical and Research Foundation (AMREF).

Materials and methods

The Turkana study was conducted in the month of July, 1994 in the North Eastern Turkana district.
The people living in this area belong to the Turkana tribe and are nomadic pastoralist highly mobile throughout the year in search for food and water for their livestock. Although there are a few settlement areas with shops, schools and health facilities, the settler population is very small.

Individuals screened included school children, semi settled individuals as well as a completely nomadic group. Their demographic data including age,

sex, and family size, was collected in a questionnaire and later transferred to a computer. The KEMRI-HEP CELL hepatitis B surface Antigen Kit (Kenya Medical Research Institute) based on Reverse Passive Hemagglutination using fixed sheep red blood cells sensitized with highly purified antibody against hepatitis B surface antigen was used to screen and confirm the serum samples according to manufacturers procedures. 51 serum samples from patients seeking medical help at an AMREF clinic in Lokichogio in the Turkana district and presenting with hepatomegally in the year 2004 were also screened for HBsAg at the clinic.

Immunohistological staining

Screening of liver biopsy material was a retrospective study involving Histopathology laboratories of Kenya Medical Research Institute (KEMRI), Kenyatta National Hospital, The Nairobi Hospital and MP Shah Hospital. Available cases of Formalin-fixed paraffin embedded liver cancer biopsies were retrieved from the laboratory achieves from 1991 to 2001. The cases were categorised as hepatocellular carcinoma, cholangiocarcinoma or hepatoblastoma. Using the normal eosin and haematoxylin histological stains, hepatocellular carcinoma cases were further sub-classified to histological patterns, solid, trabecular, clear cell, scirrhous, and pseudoglandular. The information obtained from the study was analysed in terms of age, sex, histological pattern, histoimmunochemical staining and subjected to descriptive statistic of mean, distribution and cross tabulations.

Special stains for Southgate's Mucicarmine, Orcein, Alfa fetal protein, Hepatitis B surface antigen, cytokeratin 7(CK7) and cytokeratin 19(CK19) were done using the standard staining method.

Briefly, 5µm thick tissue sections of formalin-fixed paraffin-embedded tissues of surgical specimens histologically diagnosed as hepatocellular carcinoma were cut using a Microm Cryo-Star microtome (Microm, Walldorf, Germany) and dried onto Poly-L-lysine(Sigma) microscope slides at 60oC for 60 minutes. The sections were deparaffinized in 2 changes of 5 minutes each in xylene and hydrated through descending grades of alcohol from absolute alcohol, 50% alcohol, to water. Sections were treated with 3% hydrogen peroxide in Tris-buffered saline (TBS) for 10 minutes. Sections were put into a high pH Tris-ethylenediaminetetraacetic acid (EDTA) antigen retrieval buffer at 60oC for 60 minutes. Sections were allowed to cool to room temperature and the following primary antibodies applied according to manufacturers

instructions; monoclonal antibodies raised against Hepatitis B surface antigen, human cytokeratin 7, human cytokeratin 19 and polyclonal rabbit anti human cytokeratin 19 (Dako, Inc.) and incubated in a humiditychamber at -4oC overnight. Sections were brought to room temperature and washed in 2 changes of 3 minutes each in TBS.

Appropriately diluted secondary biotinylated antibody (Multi-Link Swine anti-goat/-mouse/-rabbit immunoglobulin, Dako Inc.) was applied to the sections in a humidity chamber for 30 minutes at room temperature. Secondary antibody was washed off the sections in TBS and appropriately diluted streptavidine-peroxidase applied to the sections for 30 minutes at room temperature. After washing in 2 changes of 3 minutes each in TBS, 0.01% diaminobenzidine tetrahydrochloride(Sigma) in 0.03% H_2O_2 in TBS, pH 7.6 was applied as chromogen for 10 minutes at room temperature. Chromogen development was stopped in a gentle stream of tap water for 2 minutes, sections lightly counterstained in haematoxylin, dehydrated in alcohol, cleared in xylene and mounted in PDX mountant.

Microscopic evaluation was done on the light microscope and positive immunoreaction marked by a brown precipitate.

Sections were also stained using modified Taener-Unna Orcein method and Southgate's Mucicarmine method.

Results

Age and sex distribution of HBsAg

Of 579 serum samples from a rural population, 8% (19 of 239) males and 9.41% (32 of 340) females were positive for HBsAg. There was a high prevalence of HBsAg in the pre-adolescent age that declined with age with 59% below 20years of age and 32% between 21-40 years (Figure 1).

Figure 1. Hepatitis B surface antigen prevalence in different age groups of a rural nomadic population in Kenya. The group consisted of 239 men and 340 women
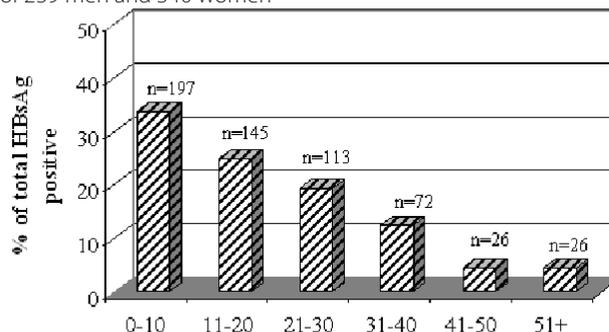
In 51 individuals presenting with suspected hepatomegally and splenomegally, 25% of those positive for HBsAg were 20 years or younger with those above 20 years of age being predominantly male(Figure 2).

Figure 2. Prevalence of HBsAg among Turkana patients (N = 51) showing clinical evidence of splenomegaly and/or hepatomegaly.
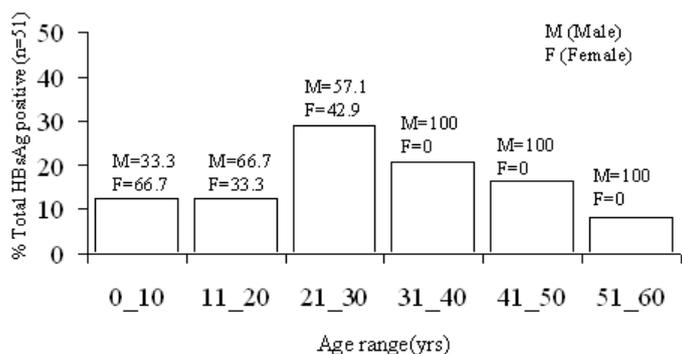

Correlation between HBV and HCC

Of the 88 liver biopsies obtained from four urban hospitals and analysed for histopathology and immunohistochemical changes, hepatocellular carcinoma was the most common liver cancer constituting 89% of all biopsies (Figure 3A).

Figure 3. (A). Frequency of various types of liver cancers observed among urban population in Kenya. (B). Age distribution of HCCs in an urban population in Kenya.
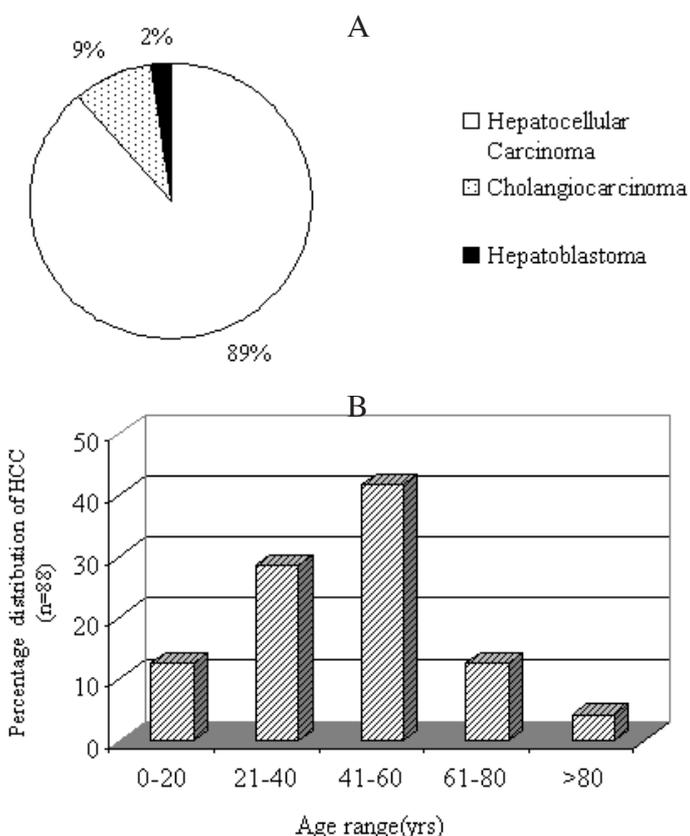
The hepatocellular carcinoma presented as; solid(48.8%), trabecullar(29.5%), clear cell(10.3%), schirrous(7.7%), giant cell(2.5%) and pseudoglandular(1.2%) types (Figures 4 and 5). Whereas HCC was more prevalent at middle age (30-60years) (Figure 3B), the youngest patient with hepatocellular carcinoma was 17 years and the oldest was 83 years with a mean age of 40 yrs.

Figure 4. (A).Histopathologic classification of hepatocellular carcinomas observed in an urban population in Kenya. (B) Ability of special stain to be immunoreactive to various antigens on HCCs. AFP, alfa fetal protein; HbsAg, hepatitis B surface antigen; CK7, cytokeratin 7; CK19, cytokeratin 19.
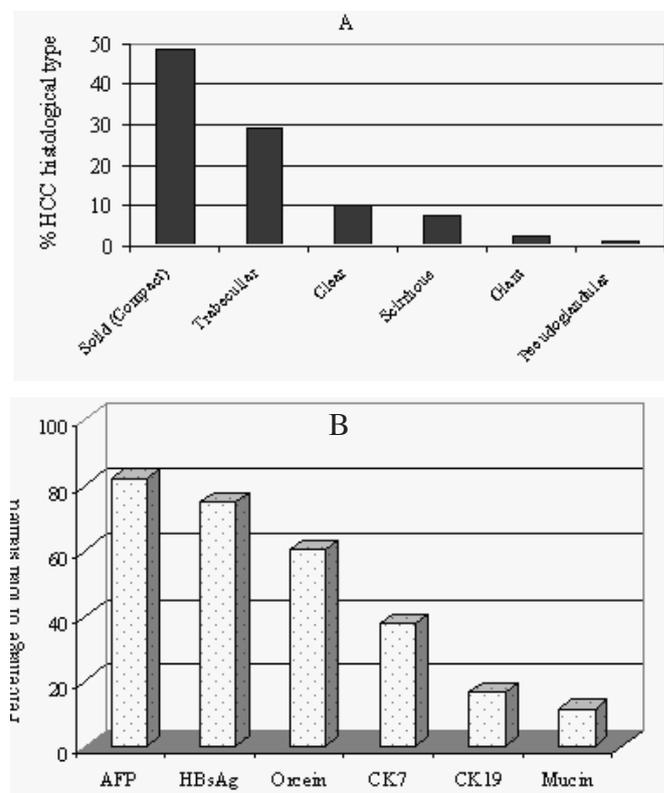
The HCC was predominantly in males who constituted 72% of all cases. More importantly, 75% of HCC biospsies were positive for HBsAg (Figures 4B and 6) demonstrating a strong association of HCC with hepatitis B virus.

This is in agreement with HbsAg data obtained in the rural population where HBsAg was predominantly found in male patients within 20-50 years of age seeking medical help. The immunohistochemical stains showed 60.2% to be positive for Orcein stain, 75% for cytokeratin 7 and 16.7% positive for cytokeratin 19 of all biopsies stained for the respective special stains (Figure 6). There were no parasitic infections detected in all cases of HCC evaluated in this study. This supports the theory that parasitic infections are not precursors to malignancy in the liver.

Figure 5. Demonstration of HCC pathotypes in liver biopsies obtained from hospitals around Nairobi. The HCC types included (A) solid , (B) glandular , (C) clear cell, and (D) scirrhous, (mag = 400) types of HCCs. For histopathology, liver biopsies were fixed in formalin, embedded in paraffin, and 5-µm-thick sections stained in hematoxylin and eosin.
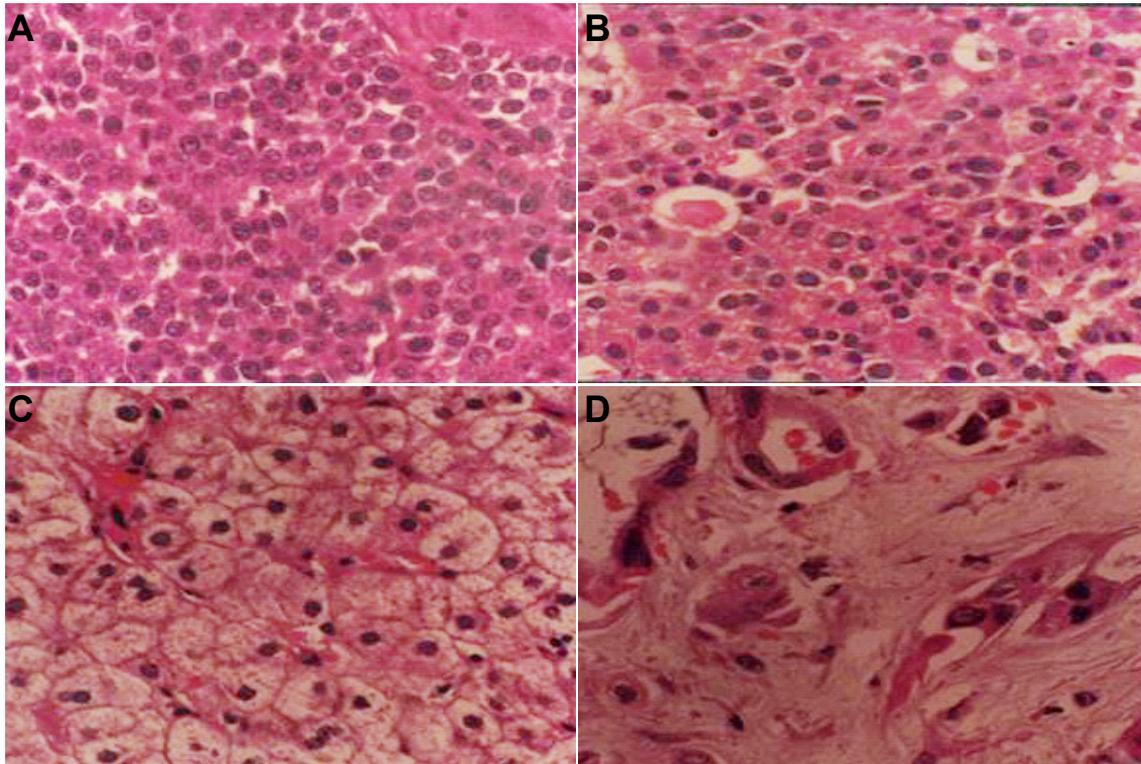

Figure 6. Liver biopsies immunoreative to specific antigens. (A) cytokeratin 7, (B) cytokeratin 19, (C) hepatitis B surface antigen, and (D) alfa fetal protein. Liver biopsies were fixed in formalin, embedded in paraffin, and 5-µm-thick sections cut, mounted on glass slides and stained for specific antigens as described in Materials and Methods. Pictures were taken at a magnification of 400.
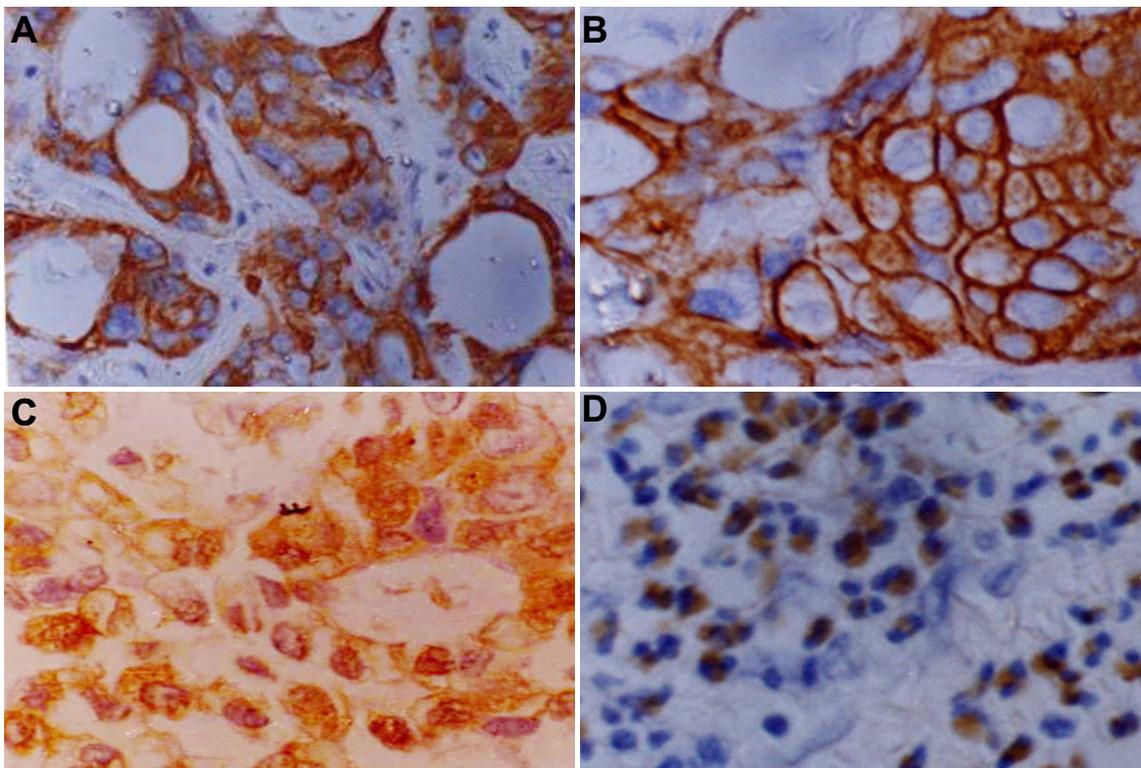
Discussion

Our data confirms earlier reports that Kenya is a high endemicity area for HBV and also confirms the strong association of HBsAg with HCC observed in other HBV endemic regions with up to 75% of HCC showing positive staining for HBsAg.

Although seropositivity of hepatitis B surface antigen was found to be similar in male and female subjects in the rural community reported in our study, there was a high male to female ratio of HBsAg positives among patients attending a clinic with hepatomegally in the same community (Figure 2b). This suggested a male pre-disposition to Hepatitis B virus carrier state which may pre-dispose to hepatocellular carcinoma. This finding is supported by the high male to female ratio observed among HCC cases from biopsies collected from an urban hospital setting. In earlier studies, a HBsAg seroprevalence of 5.1% as determined using immunoelectrophoresis was reported in a rural settler community 50km from Nairobi (15) where HBsAg positivity in all age groups was higher in males than that in females, though the difference was not statistically significant. In this community, significant antigenemia occurred in the 3rd year of life and increased with age, reaching a peak at 14 years followed by a decline with age. Thus the transmission dynamics of HBV in the rural nomadic pastoralist community in our study appears to be similar to that observed in the settler community but with a higher HBsAg prevalence. The high prevalence in individuals aged 10 years and below though relatively higher than that in adults is also within the range previously reported in the Turkana region (16). In a recent study which aimed to establish the seroprevalence of Hepatitis B markers among pregnant women, a prevalence of 15.3% was reported in women at the AMREF Lopiding Static Health facility in Turkana (17). The high prevalence of HBsAg may therefore be related to a high mother to child transmission. Other factors that may contribute to the high prevalence include poor hygienic practices including body scarification as a form of traditional treatment. During the survey, it was common to see small children with body scarifications especially in areas where access to health services was difficult or impossible.

It has been reported that up to 95% of children under five years of age infected with HBV become chronic HBV carriers(10,3) and that one third of chronic carriers will develop progressive liver disease including cirrhosis and primary liver cancer (37) leading to death in 15-25%. In this study, solid type of hepatocellular carcinoma was found to be the commonest followed by trabecular type.

Liver pathology is common in Kenya and most of the pathology is due to hepatitis B virus and hepatitis C virus (HCV). In this study 75% of all hepatoma patients were positive for HBsAg. This confirms previous studies which have demonstrated that 66% of HCC patients were HBsAg positive and up to 98% have at least one maker for past HBV exposure. HBV causes chronic hepatitis that often evolves into liver cirrhosis and hepatocellular carcinoma (38-43).

Geographical areas or populations with high frequency of HCC are also known to be endemic or hyper-endemic for hepatitis type B virus infections with an excessively high proportion of HCC patients exhibiting the presence of a variety of HBV markers. The incidence of HCC in some African populations is nearly 500 times that of developed countries. This may be partly as a result of endemic HBV infection and the persistence of active infection (44,33).

HBV has a major etiologic role in the cirrhosis-associated HCC whereas non-viral carcinogens are implicated in the HCC arising in non-cirrhotic livers. HBV infection with no chronic sequelae may also interplay with other carcinogenic factors in hepatocarcinogenesis (45).

In Zambia and Uganda 72% of the patients with HCC and 41% from USA have serological evidence of current infection, which is 4-6 times more than non-HCC controls. Only 5% of Africans and 26% of Americans with HCC lacked serological evidence of current or past HBV infection an indication that nearly all HCC are exposed (33).These findings were confirmed in this study where 75% of patients with primary liver cancer had evidence of chronic HBV infection, resulting in chronic hepatitis and cirrhosis both of which are closely associated with liver cell dysplasia and hepatocellular carcinoma (44,46).

The relative risk for a HBV carrier dying from HCC is 23, which is higher than the 10-12 relative risk of cigarette smokers dying of lung cancer. Thus, the association with antigenemia appears to be very strong and highly specific as a causative factor of liver cancer (20).

Cholangio-carcinoma comprised of 9.1% of this study. This is one of the primary tumours of the liver which arises from the intrahepatic bile ducts.

Occasionally there are some tumours containing unequivocal elements of both HCC and Cholangio-

carcinoma that are intimately admixed and are defined as combined hepatocellular and cholangiocarcinoma (HCC-Cholangio-carcinoma) (35,47). Whereas in this study there were no cases of combined hepatocellular and cholangiocarcinoma, these types of lesions have been shown to constitute 1.0-4.7% of primary liver cancers in previous studies (48-51).

Cytokeratin 7 and 19 were positive in cancerous glands of Cholangio-carcinoma lesions and negative in HCC. This makes cytokeratin 7 and 19 important stains in differentiating HCC and CC in combined HCC – CC (47). The results from this study showed varying distribution of immunohistochemical stains and interpretation of the results need careful considerations.

HCC was more common in males than females in this study, with a male to female ratio of 5:2 which is in agreement with previous studies in; Uganda, Hong Kong, and Thailand. The average age for the cases with HCC in our study was 41 years compared to Taiwan, where HBV is responsible for 80% of HCC cases and the peak age of patients with HCC for HBsAg cases is 54-55 years(27).

There was no secondary liver pathology (metastatic tumours) in the study cases although this is far more common than primary neoplasm in general practice. This may have been due to the selection criteria since liver pathology was the primary reason for the biopsy.

Since childhood exposure to HBV is associated with chronic hepatitis B virus infection in up to 95% of those infected (10) integration of HBV vaccination in the expanded program for Immunisation in Kenya should reduce liver cancer in the Kenyan population.

Acknowledgements

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