Prevalence of Hepatitis Infections in Patients with Hepatocellular Carcinoma.

*1Junaid Mahmood Alam, 2Ishrat Sultan, 3Rabia Shaheen, 4Laila Kulsoom, 5Syed Riaz Mahmood, 6Hamida Ali and 7Ishrat Bashir Malik

Abstracts:

Chronic exposure to Hepatitis B viral infections, and in few instances hepatitis C also, are strongly suspected of causing hepatocellular carcinoma (HCC). More over, in considerable numbers of HCC cases, the patients found positive for Hepatitis infections. The development of HCC is related to the integration of viral DNA into the genome of host hepatocytes. It is noted that African and far East countries, where HCC is common, have high rates of hepatitis carries, probably with vertical transmission, of viruses from generation to generations. The scope of present study is to evaluate the incidence of HBV or HCV infections in patients with HCC. A brief clinical history of 100 patients (Males; 58%, Females; 42%) with confirmation of HCC along with base-line value of α-fetoprotein (AFP), is taken and cumulated. In all patients, AFP values were found to be elevated ranging from 13.44 to 610 ng/ml in males (mean 184.82 ng/ml) and 13.00 to 576 ng/ml in females (mean 189.54 ng/ml). It was noted that in most of the confirmed cases of HCC, hepatitis infections of HBV and HCV origin is prevalent. In male HCC patients, 24 were diagnosed with HBV whereas 19 with HCV infection. In females 18 HCC patients were HBV positive and 13 with HCV. Remaining patients were investigated thoroughly for any infection, but found devoid of any. However, cirrhosis of biliary origin, haemochromatosis, cystic fibrosis and drug-induced cirrhosis are persistent. It is concluded in present study that HCC patients were discovered with HBV and HCV infections. Nonetheless, this study neither instate the correlation between presence of hepatitis infections and formation of HCC nor ascertain that hepatitis infection are the causative agents of HCC. The results are presented in relation to various risk factors, and clinical and diagnostic characteristic.

Key Words: HCC,AFP, Hepatitis, HBV, HCV. Short Title: Hepatitis infection with HCC.

Introduction

It is now established that chronic exposure to Hepatitis B and C virus cause Hepatocellular carcinoma (HCC) and the development of HCC is related to the integration of viral nuclear material into the genome of host hepatocytes (1-5). Globally, liver diseases caused by HBV are enormous with an estimated 300 million-carrier rate and in USA alone; there are 300,000 new infections of HBV per year (1,4). In addition, HCV is a major cause of liver disease worldwide. About 150,000 to 170,000 new cases of HCV are estimated to occur annually in USA alone (4).

Epidemiological studies show that annual incidence rate of HCC is 3 to 7 cases per 100,000 in North and South America, Northern and Central Europe, and Australia (2,3,6). Moreover, in countries bordering the Mediterranean, an average of 20 cases per 100,000 is reported, whereas the highest rates of 150 cases per 100,000 are found in Taiwan, Mozambique, and Southeast China (1,2,7,8). Several African states also shows considerable incidence of hepatitis-infection related HCC (7). Regarding Cause and Origin, the global distribution of HCC is strongly linked to the prevalence of Hepatitis viral infections, mostly "hepatitis B virus" (1,9,10). It is reported that in infancy, following vertical transmission of virus from infected mother confers a 200 fold increased risk of HCC by adulthood.

In the western world, where HBV is not prevalent, other chronic liver diseases (including HCV infections) are frequent with 85% to 90% exhibiting cirrhosis (1,11,13). Pathogenesis of HCC revealed that there are three major etiological association of HCC; viz, HBV infections, aflatoxin and Cirrhosis (8,11,14).

The actual pathogenesis of HCC may vary between high-incidence population of HBV positive cases and low-incidence population of Western World where other diseases are more represented. In this scenario the scope of present study is to evaluate the incidence of HBV or HCV infections in patients.
with HCC. However, this study neither investigates the correlation between presence of hepatitis infections and extent HCC nor ascertain that hepatitis infection are the causative agent of HCC.

Methods and Research Design

Selection Criteria

The study covers the periods of August 1999 to August 2003. Patients both male and females, admitted in wards or visiting various clinics viz gastroenterology, oncology and hepatology for diagnosis, treatment or recovery regimens were selected through their cas history and lab-diagnosis results of hepatitis profile, AFP and histology. patients were grouped in the age range of >20 yr and <70 yr; patients falling out side this age range were excluded from the study. A brief history of Patients, with confirmed existence of HCC, was taken with clinical symptoms and signs and initial diagnosis. Exclusively patients with CLDs, HCV and HBV or suspected of hepatitis infections with co-existence of HCC or vice versa, were selected and classified according to gender.

A total of 530 patients were screened and verified for HCC and co-existence of Hepatitis viral infections. out of 530, 118 falls fully into the presented criteria. However, during the course of present study 17 patients (11 males 6 females) could not be followed for evaluations or diagnosis as they left the city for their villages or other parts of the country. Two female patients, due to family pressure refuses to provide further cooperation, however, we were able to pursue one of them to provide cooperation. Therefore, the final total number of patients included in the study was 100 (52 males, 48 females).

Exclusion criteria: Patients falling in age range of < 20 > 70 yr, alcoholics, patients' undergone recent surgeries, broncospasmatic (with or with steroid therapies) were included from the study. Patients with other cancers or extensive metastasis to other parts of the body were also excluded to minimize the bias altering the objectives of the study.

Clinical History and related information:
A brief clinical history of all patients was taken, exclusively covering following points.

Confirmation of HCC by:

* Ultra sound/ X-ray
* Histopathology

* AFP results
* Related diagnostic or clinical evaluation

Confirmation of Hepatitis, HBV, HCV

* Hepatitis profile
* PCR diagnosis

Related Hematological, Biochemical and microbiological tests such as CBS, Ferritin, Liver function tests.

Treatments:

* For hepatocellular carcinoma
* For Hepatitis infections

All 117 patients were tested for HBV and HCV hepatitis profile. Out of 117, 23 also have confirmation of HBV or HCV through PCR, 5 of which are those leaving the study. Related tests also includes urine DR, urobilinogen Cultures etc.

Sample Collection:

Blood samples were collected in clot activated tubes for AFP and Hepatitis profiles serum was separated and stored at 20°C until analyzed.

Analysis and Calculation:

For HCC, all AFP analysis was performed in duplicates by Automated ELISA techniques on Cobas-Core and Elecsys 1010 (Roche-Diagnostics) with two-points calibration and controls with definite-off values.

AFP Values greater than 20 ng/ml [range 10 ng/ml] in smokers and greater than 10 ng/ml [range 5 ng/ml] in non-smokers were considered significant.

For hepatitis infections, Hepatitis profile tests were performed on Axsym (Abbott lab, USA) with calibrations and controls. Cut off values for antibodies regarding HCV and HBV are 1.00 ng/ml and 1.00 mIU/L, respectively. All data is presented in the form of Percentage occurrence and significance of data was assessed and verified using students t-test.

Results

The results are presented in Figures 1, 2 and Table 1. Percent distribution pattern of clinical conditions of patients with
elevated AFP and positive Hepatitis profile as fplows;

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCC with HCV origin</td>
<td>32%</td>
</tr>
<tr>
<td>HCC with HBV origin</td>
<td>42%</td>
</tr>
<tr>
<td>HCC of cirrhotic origin</td>
<td>12% no-infection</td>
</tr>
<tr>
<td>HCC Chronic liver disease (CLD) only</td>
<td>14% no-infection</td>
</tr>
</tbody>
</table>

As per clinical conditions and infections, cirrhosis and CLD, the number of male HCC patients with HCV and HBV was 19, 24, and with cirrhotic and CLD, 7 and 8 respectively. Female patients, as per clinical conditions were divided as 13, 18, 5 and 6, respectively, for HCV, HBV cirrhosis and CLD. Collective distribution among two genders is exhibited as; HCV = 59.37% (n=9) in males and 40.62% (n=13) in females, HBV = 57.10% (n=24) and 42.8% (n=18), Cirrhosis = 58.30% (n=7) and 41.66% (n=5) and CLD = 57.14% (n=8) and 42.80% (n=6), respectively (Fig 1). It is noted that 89 patients out of 117 have ultrasound reported confirmation of tumor mass. 22 have additional histopathological biopsy report. Of these 22, 16 are presented in the group whereas remaining 6 were those who left the study. The remaining lot cannot be convinced to allow liver biopsy. Therefore inclusion was done on the basis of X-rays, ultrasound, and AFP. 52 patients have AFP done on initial findings remaining lot was analyzed later. Thus all 117 were AFP tested and were found to be elevated. It is noted that, although hepatocellular carcinoma patients were found infected with HCV and HBV, this doesn’t imply that these infections are the causative agent of the development of HCC.

In all patients, AFP values were found to be elevate ranging from 13.44 to 610 ng/ml in males (X 184.82 ng/ml) and 13.00 to 576 ng/ml in females (X 189.54 ng/ml) (Table 1). However, no significant differences was found between the elevated ranges of two gender groups. In males, 29.3% (n = 17) patients have AFP in the range of > 13.44 ng/ml to < 60 ng/ml; 36.20% (n = 21) in the range of > 60 ng/ml to < 150 ng/ml; 13.79% in the range of > 150ng/ml to < 300 ng/ml and 20.68% (n = 12) in the range of > 300 ng/ml to < 650 ng/ml. In females, it is 3.09.95% (n = 13), 28.57% (n = 12), 21.42% (n = 9) and 19.04% (n = 8) in the same order. Cumulatively, around 34.47% (n = 20) male patients exhibited elevated AFP levels between > 150ng/ml to 650 ng/ml, whereas 40.46% (n = 17) females shows similar pattern. There are 46 patients (28 males, 18 females) who under went therapy for HCC-related clinical complications within last one year to three months. It is also observed that AFP levels are comparatively in lower range of elevation in these patients when those who didn’t went through any treatments. Similarly 16 patients (11 males and 5 females) went through interferon therapy within last one year to 5 months, after diagnosed with hepatitis infection. Interestingly, HCC was diagnosed in these patients in last three months, probably after passage of 3-4 months of therapy procedures-completion. Those HCC patients, who were devoid of any hepatitis viral infections, were diagnosed with either cirrhosis of CLD of unknown origin. Cirrhotic HCC patients were devol of any apparent infections but was originated from biliary complications (three), drug induced (total 7; two patients with paracetamol usage for pain/fever; one Phenytion, four-antibiotics), two with hemochromatosis and cystic fibrosis respectively. Although CLD patients were thoroughly investigated during admittance or clinical visits for any infections, related diseases or accompanying metastasis, yet no evidence were found and had no apparent confirmation of causative agents. Therefore, they were designated as HCC with CLD only with no clinically significant cirrhotic appearance of infections. During consecutive study sessions, we were able to call some of them for further lab as well as clinical investigations, but again fail to detect any evidence of virus, diagnostically significant blood chemistry results (LFTs), or any evidence of other complications through multiple ultrasound tests. Clinical signs and symptoms in all most all patients were fatigue, fever, mild to moderate increase in liver size, few case of ascites and pain.

**Discussion and Conclusions**

Hepatitis infections, especially HCV is an infection that can result in considerable number of morbidity and mortality (15). The sequence of clinical manifestations and complication are cirrhosis, liver failure with ascites, hepatic encephalitis, oesophageal varices and HCC. It is reported that hepatitis infected patients who will progress to cirrhosis within 20 years varies from 2-4% in children and young to 29-30% or more in older population that recieved blood transfusion (15, 16). In addition, 1% to 4% of patients with cirrhosis will develop HCC each year (17,18). A group study study estimated that the number of people living with cirrhosis, new cases of liver failure and HCC and cumulative total of deaths attributed to hepatitis C was likely to elevate by 2020 (19). Moreover, an independent research model estimated that between 2010 and 2019, 165,900 deaths from CLD and 27,200 from HCC would occure (20). The data collected in present study agrees with the studies done previously. From the resulant data, following conclusions were drawn. Hepatitis viral infections, HBV and HCV including cirrhosis are predominantly present in patients with HCC. World wide, there is a clear predominance of males with HCC, ranging
from 8:1 in countries with higher incidence of HCC with 2:1 to 3:1 in population with low frequency (1). AFP test not only seems to be helpful in providing the evidence of metastasis in liver but in cases of viral hepatitis related HCC, the values are significantly high, than those with no infection. However, it should be noted that elevated AFP values neither lead to the details of when, where and for how long the patients have been infected or been a carrier, nor it illustrate that Hepatitis viral infections are the “only” cause of HCC developments. It is reported that many factors other than hepatitis infection, such as age, sex, chemicals, hormone imbalance, alcohol and mutation interact in the development of HCC (1,4,8,9). Moreover, age male sex are found to be the most important factors for the development of HCC in cirrhotic patients (3). Extensive studies revealed that repeated cycles of cell death and regeneration, as seen in chronic hepatitis, are important factors in pathogenesis of HBV (and HCV) associated liver cancer (1). Recent investigations show that genetic polymorphism and mutations are associated with HCC in selected population with HCV infections are the causative agent of the development of HCC. In addition, limitation of study doesn’t allow us to establish correlation between hepatitis infections and formation of HCC. Studies are in progress in our department to elaborate the pathogenesis and biochemical characteristics of hepatitis associated HCC.

References:


**Prevalence of Hepatitis infections in Patients with Hepatocellular Carcinoma**

<table>
<thead>
<tr>
<th>Gender wise distribution of variable and elevated AFP values in HCC patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
</tr>
<tr>
<td>Percent distribution</td>
</tr>
<tr>
<td>29.30%</td>
</tr>
<tr>
<td>36.20%</td>
</tr>
<tr>
<td>13.79%</td>
</tr>
<tr>
<td>20.68%</td>
</tr>
<tr>
<td>30.95%</td>
</tr>
<tr>
<td>28.57%</td>
</tr>
<tr>
<td>21.42%</td>
</tr>
<tr>
<td>19.04%</td>
</tr>
</tbody>
</table>

**Fig 1: Gender wise percent distribution of Clinical Conditions in HCC patients**

**Fig 2: Percent distribution of clinical conditions in HCC patients (n = 100)**

- **Cirrhosis**: 14% (n = 14)
- **CLD**: 12% (n = 12)
- **HCV**: 32% (n = 32)
- **HBV**: 42% (n = 42)