Clinical Studies
Abstract. Cholangiocarcinoma is the second most common primary malignant tumor in the liver. It is a tumor that is characteristically composed of cells resembling those from the bile duct. The disease is difficult to diagnose and is usually fatal due to its late clinical presentation, lack of effective non-operative therapy, and rapid turnover. Most patients have unresectable tumors at the time of presentation and die within 12 months once diagnosis has been made. Prognosis of intrahepatic cholangiocarcinoma (ICC) remains very poor. Currently, there is no established therapy once diagnosis is made. In this report, we provide a case of a patient who presented with ICC and positive history of hepatitis C virus (HCV). The patient also had a strong family history of cancer. Finally, we attempt to review some of the important developments in the study of ICC, with particular attention to recent studies linking hepatitis with the disease.

Case Report

The patient was a 64-year-old Caucasian male who presented with a 6-month history of abdominal pain, loose stool, and reflux unresponsive to medication. On examination, he was found jaundiced, fatigued, and cachetic, with mild tenderness over his abdomen. His past medical history included prostatic enlargement and hypertension, both well controlled medically. Family history included an extensive number of relatives with cancer (Figure 1). The patient’s father died of liver cancer, and his mother from amyotrophic lateral sclerosis (ALS). He had two sisters who both died of ovarian cancer; one niece (living) with cervical cancer; and one nephew who died of a brain tumor.

Investigation of the patient’s liver function revealed abnormal results: total bilirubin 26.9 mg/dL, conjugated bilirubin 18.2 mg/dL; alkaline phosphatase 1879 IU/L; Alanine aminotransferase (ALT) 427 IU/L; aspartate aminotransferase (AST) 245 IU/L. The CA19-9 was mildly elevated to 43 (normal level <37 U/mL). The rest of the other blood tests were within normal limitation. Endoscopy was performed and reported negative findings. Hepatitis A antibody, hepatitis B surface antigen, and hepatitis B core antibody were all negative. Hepatitis C antibody was low positive at 1.19 by chemiluminescent immunoassay (normal lead >1.0). Other rheumatology tests, celiac and primary biliary sclerosis test were negative. The celiac disease screening panel such as endomysial antibodies was negative. Computed tomography scan (CT) revealed multiple intrahepatic lesions. A biopsy of the liver lesion ultimately revealed the presence of moderately differentiated cholangiocarcinoma (Figure 2). The tumor cells were strongly positive for CK-7 and negative for CK-20 and hepatocyte antigen. p53 staining was carried out and was reported as being negative for mutation. After his diagnosis of cholangiocarcinoma, the patient subsequently commenced chemotherapy.

Discussion

Cholangiocarcinoma is the second most common primary malignant tumor in the liver (incidence 5% to 25%) (1). It can be divided into intrahepatic cholangiocarcinoma (ICC) and extrahepatic cholangiocarcinoma (ECC) (1-2).

Globally, ICC is characterized by its wide variability in incidence and risk factors. Areas such as Europe and North America have traditionally had low incidence of ICC, but the number of cases has slowly increased in recent decades (2). Considering its low frequency in these areas, epidemiological studies make ICC etiology very difficult to pinpoint. Nevertheless, several risk factors (primary sclerosing cholangitis, liver fluke infestation, and hepatolithiasis) have been implicated in the development of ICC. Briefly...
speaking, these factors lead to chronic inflammation, predisposing to the risk of ICC.

Chronic inflammatory biliary diseases commonly present with features of chronic inflammation, bile stasis, and increased biliary epithelial cell turnover. Cytokines, which are typically present in inflammatory tissues, have been hypothesized to contribute to epithelial cell carcinogenesis by causing damage to DNA and DNA repair proteins. The overexpression of cyclooxygenase-2 (COX-2) has been noticed in chronic cholangitis and biliary tract carcinoma (3), thus suggesting that this enzyme may play a crucial role in bile duct carcinogenesis and tumor progression. Under chronic inflammation, malignant progression of ICC may be a result of failure in apoptosis activation and in deleting cells with genetic alterations. These alterations in cell signaling and genes have been well studied; some of the notable ones include Bcl-2, tumor growth factor β (TGF-β), tumor necrosis factor, deregulation of ras, and p53.

In addition to the several well-known risk factors that contribute to ICC, recent studies have investigated the association of ICC with viral hepatitis infections. While hepatitis B (HBV) and hepatitis C virus (HCV), and heavy alcohol intake are known causes of hepatocellular carcinoma (HCC), their roles as risk factors in ICC development remain unclear. Several recent studies have proposed possible association between ICC and HCV, whereas the link between HBV infection and ICC is less uniformly demonstrated, with a reported prevalence ranging between 0 and 16.7% of ICC (4-7).

HCV seems to be associated with ICC in regions with relatively low prevalence of HBV infection, such as Japan and the U.S. In contrast, in areas such as Southeast Asia, several studies have identified HBV and HCV nucleic acids and proteins in ICC (Tables I-III).

Differences in etiological factors associated with ICC can be explained by the type of endemic hepatitis virus. Several case-control studies from Korea have found seropositive ICC patients for anti-HCV and hepatitis B surface antigen (HbsAg) at a much higher rate among control studies (6). Likewise, an Italian case-control study found that seropositivity for HbsAg much higher among 26 ICC patients than among 824 controls (11.5% vs. 5.5%) (4). Additionally, several reports from China, USA, and Korea reported cases of ICC that presented with a background of HBV infection, suggesting an etiopathogenetic link between HBV and ICC (6-8). (Table 1)

Studies using molecular tests of ICC tissue samples provide evidence that HBV infection plays a significant risk factor for ICC. In a study from the United States, HBV DNA was present in 2 (18.2%) out of 11 ICC tissue samples.
obtained at the time of surgical resection (7). Hepatitis Bx antigen (HBxAg) and HBV DNA were detected in 80% and 82.5% in cancer and surrounding hepatic tissue, respectively, from 20 patients with ICC (8).

Although the mechanism of HBV/HCV infection in the pathogenesis of ICC has not been entirely explored in detail, the potential oncogenic role of HBx protein, encoded by the HBV X gene, is well known. The HBx protein has been studied and found to be associated with tumor progression and metastasis in various cancer types, including ICC. The HBx protein functions as a transcriptional activator, promoting the expression of oncogenes and inhibiting the expression of tumor suppressor genes, thereby contributing to the development and progression of ICC.

### Table I. Hepatitis B and ICC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Study type</th>
<th>ICC cases</th>
<th>Specimen</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perumal et al.</td>
<td>USA</td>
<td>Case series</td>
<td>11</td>
<td>Fresh frozen tissue</td>
<td>HBV DNA (18.2%)</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>China</td>
<td>Case series</td>
<td>40</td>
<td>Formalin-fixed paraffin-embedded tissue</td>
<td>HBV DNA (82.5%)</td>
</tr>
</tbody>
</table>

### Table II. Hepatitis C and ICC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Study type</th>
<th>ICC cases</th>
<th>Specimen</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kobayashi et al.</td>
<td>Japan</td>
<td>Cohort</td>
<td>14</td>
<td>Serum</td>
<td>ICC incidence 1,000 x higher in anti-HCV+ cirrhosis than HCV- cirrhosis</td>
</tr>
<tr>
<td>Yamamoto et al.</td>
<td>Japan</td>
<td>Case-control</td>
<td>50</td>
<td>Serum</td>
<td>Anti-HCV (OR 6.0, 95 CI, 1.51-24.1)</td>
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</tbody>
</table>

### Table III. Studies comparing HBV and HCV with ICC.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Study type</th>
<th>ICC cases</th>
<th>Specimen</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donato et al.</td>
<td>Italy</td>
<td>Case-control</td>
<td>26</td>
<td>Serum</td>
<td>HBsAg (OR 2.7; 95% CI, 0.4-18.5) Anti-HCV (OR 9.7; 95% CI, 1.6-58.9)</td>
</tr>
<tr>
<td>Zou et al.</td>
<td>China</td>
<td>Case-control</td>
<td>312</td>
<td>Serum</td>
<td>HBsAg (OR 8.86, 95% CI, 6.0-13.2) Anti-HCV (OR 0.93, 95% CI, 0.28-3.1)</td>
</tr>
<tr>
<td>Shin et al.</td>
<td>Korea</td>
<td>Case-control</td>
<td>41</td>
<td>Serum</td>
<td>HBsAg (OR 1.3, 95% CI, 0.3-5.3) Anti-HCV (OR 3.9, 95% CI, 0.9-17.1)</td>
</tr>
<tr>
<td>Shaib et al.</td>
<td>USA</td>
<td>Case-control</td>
<td>83</td>
<td>Serum</td>
<td>Anti-HBc (OR 28.6, 95% CI, 3.9-1,268) Anti-HCV (OR 7.9 95% CI, 1.3-84.5)</td>
</tr>
</tbody>
</table>

Figure 2. Hematoxylin-eosin staining of the tissue biopsy sections from the intrahepatic lesion which was detected by CT scan (original magnification a ×40, b ×200).
shown to activate the transcriptional expression of human telomerase reverse transcriptase, leading to tumorigenesis in cholangiocytes (5). HBx protein has been characterized as a promiscuous activator of many gene promoters in vitro. It binds to p53 protein, thus inhibiting p53-mediated apoptosis in vitro and in vivo (9). It has been discovered that the sera of patients with ICC contained higher anti-HBx with a detection rate of 85.7% (8). Translation of X gene has a transactive expression of genes (10). Studies of transgenic mice show that the HBx might directly induce primary hepatic carcinoma (11).

HBV has also been implicated in the inflammatory process that can lead to ICC. Bile duct lesions in hepatitis encompass a wide range of proliferative, inflammatory, and degenerative changes. In chronic hepatitis B, the HBV may infect the biliary epithelium, which may be subject to immunological attack that can lead to ICC. Thus, ICC may closely be related to HBV infection.

Some argue fewer ICC cases are complicated with liver cirrhosis. In one study, only 12% of ICC patients had associated liver cirrhosis, while 87.8% had chronic active hepatitis and chronic persistent hepatitis (8). This indicates that ICC is not chiefly developed through liver cirrhosis but mainly through persistent hepatitis. The precise relationship between HBV and development of ICC remains to be further elucidated.

Our patient was found to have low positivity for HCV antibody. Research on the link between HCV and ICC still remains ill-defined and deserves attention. Recently, infection with HCV has been suggested as a risk factor for ICC. Several studies have linked chronic viral hepatitis (particularly HCV infection) or cirrhosis with the development of ICC (Table II). These include case reports of ICC detected in patients with HCV infection, as well as studies of ICC patients with high prevalence of anti-HCV antibodies. A case-control study in Korea reported that the prevalence of anti-HCV was 12.5% in patients compared to 3.5% of controls (odds ratio, OR, 3.9) (6). A prospective cohort study from Japan of 600 patients with HCV-related cirrhosis found that 2.3% developed ICC during a mean follow-up of 7.2 years. The risk of developing ICC among patients with HCV related cirrhosis was 1,000 times higher than that of the general population, suggesting that HCV-related cirrhosis is a major risk factor for ICC in the Japanese population (12). This risk is comparably higher than other countries such as Denmark, where studies suggest a 35-fold increase.

A case-control study from Italy that compared 26 patients with ICC with 824 controls found that seropositivity for anti-HCV in ICC cases. A statistically significant increase in the OR was observed for patients with anti-HCV OR 9.7 (4). Similarly, according to a US case-control study of 635 ICC patients, the prevalence of anti-HCV with an adjusted OR of ICC of 6.1 (13). Several mechanisms of cholangiocarcinogenesis in patients with chronic hepatitis C have been proposed. HCV has been proven to cause injury to the epithelial cells of the bile duct, leading to a range of proliferative, inflammatory, and degenerative damage. Like other pathological conditions that lead to ICC, long-standing inflammation, chronic insult, and regenerative hyperplasia of the bile duct epithelium can result to malignant transformation.

Recently, the expression of HCV core gene vector was shown to be present in cholangiocarcinoma cells (14). A possible explanation for this is the survival by liver cells in areas of piecemeal necrosis due to chronic hepatitis C. These cells become hyperplastic and undergo ductal metaplasia where they may lead to ICC.

The mechanism of ICC development secondary to hepatitis (B or C) infection is entirely unknown. Cirrhosis caused by hepatitis certainly can determine the development of ICC. Several studies have found fairly high rates of cirrhosis in patients with ICC, which suggests this notion. Nevertheless, there are other studies in which half or even more of ICC patients had no risk factors related to chronic hepatitis or cirrhosis (4, 7-8). This finding suggests that unlike hepatocellular carcinoma, ICC is not chiefly developed through liver cirrhosis, but through unknown factors, quite possibly persistent hepatitis. The precise relationship of hepatitis or other inflammatory disease and development of ICC remains to be further studied.

In summary, given the poor prognosis of ICC, recognition of risk factors driving the increased incidence is vital. Furthermore, based on the results from literature, testing for HCV and HBV in individuals with ICC appears to be warranted. The precise relationship of chronic hepatitis from non-viral disease with the development of ICC remains to be further studied. Large population-based case-control studies are needed to establish the relationship between chronic viral hepatitis and ICC. The unique family history presented in our patient failed to show any link with p53 mutation but suggested a potential genetic linkage with ICC. This review provides a comprehensive overview of risk factors reported to be associated with intrahepatic cholangiocarcinoma with special attention to hepatitis.

Acknowledgments

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References


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