Hepatitis C virus and other risk factors in hepatocellular carcinoma


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Summary. – Hepatocellular carcinoma (HCC) increased in Egypt in the past years, becoming the most common cancer among men. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are the known primary risk factors for HCC. This study describes the viral profile of HCC in a predominantly rural area in Egypt. We included 148 HCC cases and 148 controls from the Tanta Cancer Center and the Gharbiah Cancer Society in the Nile delta region. Serological (ELISA) and molecular (PCR) analysis for HBV and HCV infection were performed on plasma samples from each subject. Epidemiologic, environmental, and medical histories were collected by interviewing of subjects. Around 90.5% of cases and controls were from rural areas. HCV infection was high in both cases and controls (89.2% and 49.3%, for cases and controls respectively by serology). HCV was the most important HCC risk factor [OR 9.7 (95% CI: 3.3–28.0, P <0.01)], and HBV infection showed marginal tendency of increased risk [OR 5.4 (95% CI: 0.9–31.8, P <0.06)]. Ever worked in farming [OR 2.8 (95% CI: 1.1–7.2, P <0.03)] and history of cirrhosis [OR 3.6 (95% CI: 1.6–8.1, P <0.01)] or blood transfusion [OR 4.2 (95% CI: 0.9–17.8, P <0.05)] were also associated with increased HCC risk. This study in a predominantly rural area in Egypt supports previous reports from other parts of Egypt that HCV infection is the primary HCC risk factor in Egypt. Further understanding of the relationship between infection and other risk factors in the development of HCC could lead to targeted interventions for at-risk individuals.

Keywords: hepatocellular carcinoma; hepatitis; rural; risk factors; Egypt

Introduction

Hepatocellular carcinoma (HCC) is a major source of cancer burden worldwide, and is the third leading cause of cancer-related deaths (Bouchard and Navas-Martin, 2011; Venook et al., 2010). HCC accounts for more than 500,000 new cases per year and nearly as many deaths due to poor disease prognosis (Boyle and Levin, 2008; Parkin et al., 2005). Developing countries have more than 80% of total HCC morbidity and mortality (Boyle and Levin, 2008, Parkin et al., 2005). Trends in HCC incidence have been increasing throughout the world as well as in Egypt (Bosch et al., 2004; Boyle and Levin, 2008; el-Zayadi et al., 2005). Recent estimates of HCC incidence from the Gharbiah population-based cancer registry in Egypt, revealed an Age-Standardized Incidence Rate (ASR) of 20.6–21.7/100,000 among men, more than twice the incidence of HCC among men in the U.S. (Ibrahim et al., 2006; Jamal et al., 2009).

Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) have been clearly established as the primary risk factors for HCC (IARC Working Group on the Evaluation of Carcinogenic Risks to Humans and International Agency for Research on Cancer, 1994). HBV has been implicated as the most important risk factor for HCC, however, the incidence of HBV in Egypt has been
steadily decreasing over the previous two decades while HCV incidence has increased to one of the highest in the world, estimated to average about 14% in the general population (Sievert et al., 2011). HCV incidence has also been reported as high as 25% in the general population of some regions in Egypt (Lehman and Wilson, 2009b) with higher prevalence of HCV than HBV among HCC cases in Cairo (Ezzat et al., 2005; Hassan et al., 2001). Also, most studies that investigated the viral profile of HCC in Egypt were conducted in urban or semi-urban areas in Egypt where the HCV rates were lower than in rural areas (Lehman and Wilson, 2009a,b).

Although viral hepatitis infections are the primary cause of HCC in Egypt, many other environmental and occupational exposures influence the disease etiology. These exposures include but are not limited to pesticide and aflatoxin exposures or unsafe cultural habits that increase risk for hepatitis infection and subsequently HCC development (Anwar et al., 2005; el-Zayadi et al., 2005; Ezzat, 2008).

The aim of the study was to determine the profile of HBV and HCV in HCC patients in a predominantly rural population in the Nile delta region of Egypt, while taking into account a number of environmental, behavioral, and occupational risk factors.

**Materials and Methods**

*Study design and recruitment.* This case-control study was conducted at the Tanta Cancer Center and the Gharbiah Cancer Society, the main cancer hospitals in the central delta region of Egypt, from December 2007 to January 2009. The final study subjects included 148 cases and 148-matched controls after elimination of 2 cases for insufficient blood samples and 12 cases and 14 controls who were unable or refused to give blood samples. Cases were all newly-diagnosed liver cancer patients seen at the recruiting hospitals during the study period without age or sex restriction. Controls were randomly chosen from non-relative visitors of cancer hospitals during the study period without age or sex restriction. A total of 148 matched pairs of cases and controls were included in the analysis. Cases ranged in age from 18–81 years and controls ranged from 17–75 years. Both cases and controls had significantly lower educational levels and were more likely to work in agricultural or industrial occupations than administrative jobs. Cases were also more likely to have worked in farming-related occupations at some point than controls (60.8% vs. 47.3%). Schistosomiasis, cirrhosis, and blood transfusion were also more prevalent among cases than controls.

**Results**

A total of 148 matched pairs of cases and controls were included in the analysis. Cases ranged in age from 18–81 years and controls ranged from 17–75 years. Both cases and controls had sex distributions of 83.3% males and 16.2% females. A high proportion of participants resided for the longest period in rural areas (90.5% of cases, 88.5% of controls) and no significant difference was found between cases and controls. Cases had significantly lower educational levels and were more likely to work in agricultural or industrial occupations than administrative jobs. Cases were also more likely to have worked in farming-related occupations at some point than controls (60.8% vs. 47.3%). Schistosomiasis, cirrhosis, and blood transfusion were also more prevalent among cases than controls.

Cases had a significantly higher prevalence of all hepatitis variables by both methods of testing. Serological results showed that 89.2% of cases were positive for anti-HCV versus 49.3% of controls (Table 1). Chronic HBV infection was detected in 7.4% of cases and 3.4% of controls. Co-infection...
of HBV and HCV was low in both cases and controls, but was higher in cases (2.7%) compared to 1.4% of controls. Molecular results showed that 81.1% of cases and 12.3% of controls were positive for HCV RNA, 10.1% of cases and 0% of controls were positive for HBV DNA, and 6.8% of cases and 0% of controls were positive for both HCV RNA and HBV DNA (Table 1).

Table 1 presents the results of univariate and conditional logistic regression. Results of univariate analysis showed that both HCV [unadjusted OR = 7.6 (95% CI: 3.8–15.1)] and chronic HBV infection [unadjusted OR = 4.1 (95% CI: 1.3–12.9)] were associated with increased risk of HCC, as well as infection with non-chronic HBV infection (i.e. acute, past, and mixed infection, [unadjusted OR = 3.1 (95% CI: 1.8–5.4)]. The increased risk of HCC associated with HCV infection remained significant after adjustment for farming occupation, schistosomiasis, cirrhosis, and blood transfusion [OR = 9.7 (95% CI: 3.3–28.0, P <0.01] using conditional logistic regression analysis. Univariate regression also showed increased risk of HCC associated with ever working in a farming occupation [OR = 2.8 (95% CI: 1.1–7.2, P <0.03], and having liver cirrhosis [OR = 3.6 (95% CI: 1.6–8.1, P <0.01], or blood transfusion [OR = 4.2 (95% CI: 0.99–17.8, P <0.05]. Increased HCC risk associated with farming and cirrhosis remained significant after adjustment for farming, schistosomiasis, cirrhosis, and blood transfusion (Table 2). Likewise, results from the logistic regression model to predict HCC from hepatic infection as determined by molecular analysis of HCV infection, revealed similar pattern. The risk of HCC with HCV infection was [OR = 35.6 (95% CI: 7–180.9, P <0.01]), ever worked in farming [OR = 4.2 (95% CI: 1–14.4, P <0.05]), and cirrhosis [OR = 4.6 (95% CI: 1.4–15.3, P <0.01)]. Although blood transfusion showed a trend of increased risk, it was not statistically significant [OR = 12.9 (95% CI: 0.5–329.7, P <0.12)] (data not shown).

Discussion

A few studies of the association between hepatitis infection and hepatocellular carcinoma have been performed in Egypt over the past 20 years with varying sample sizes (Abdel-Aziz et al., 2000; Abdel-Wahab et al., 2000; Franceschi and Raza, 2009; Sievert et al., 2011). The current study reflects a significantly higher proportion of rural patients than any previous study from Egypt. This study provides further evidence of the role of HCV infection as the most
significant risk factor for development of HCC in Egypt, particularly in rural populations.

The distributions of age and sex in this study are consistent with the study population of all cases of HCC in Gharbiah, Egypt as in previous studies (Lehman and Wilson, 2009b; Soliman et al., 2010). The 90% rural residence of the patient population in this study is unique to the majority of studies on HCC and its risk factors or hepatitis in Egypt. Many previous studies from Egypt included a rural proportion of patients, but not to the degree of this current study.

Strong associations were found between HCC, cirrhosis, and blood transfusion. A study from Cairo also found associations between HCC, schistosomiasis, and blood transfusion (el-Zayadi et al., 2005). Similar to our findings, history of blood transfusion was present in 15.5% of the HCC patients and lower prevalence of schistosomiasis (61.7%) among both HCC cases and controls (el-Zayadi et al., 2005). The higher occurrence of schistosomiasis in our study is likely due to higher proportion of rural cases where higher rates of infection are expected (Frank et al., 2000). Farming occupations were also significantly associated with HCC. This association could be explained by the high level of exposure to carcinogenic pesticides among farm workers (Ezzat et al., 2005). A case-control study of 236 matched cases and controls recruited from Cairo showed a significant association between exposure to pesticides and HCC in male patients from rural regions (Ezzat et al., 2005). Therefore, the association between farming and HCC in our study may be due higher levels of pesticide exposure in patients than controls.

Expected associations between HCC and hepatitis infection were observed in the current study. Serological results showed high prevalence of HCV in both cases and controls (89.2% and 49.3% for cases and controls, respectively). High HCV prevalence among cases is consistent with previous meta-analysis of different studies in Egypt (Lehman and Wilson, 2009a). HCV prevalence has been reported in previous studies at consistently high rates ranging from 53.4–94.1% with most falling in the range of 70–90% (Lehman et al., 2008). The prevalence of HCV among controls in the current study is much higher than estimates from studies of HCV in healthy populations; however, this is likely to be due to the older population in this study. Several studies have reported increasing HCV prevalence in older age groups in Egypt with rates up to 60% (Abdel-Aziz et al., 2000; Darwish et al., 2001; el-Sadawy et al., 2004). The high rates of HCV in both cases and controls in this study are similar to those found in the rural portion of another study from Cairo and other regions of Egypt (Ezzat et al., 2005). In that study, anti-HCV was found positive in about 50% of controls (45.1% in males, 54.1% in females) and around 90% in cases (90.3% in males, 83.8% in females) (Ezzat et al., 2005). Molecular results for HCV in our study are lower in both cases and controls when compared to serological results. This is likely due to the different methods of detection, where HCV DNA measures only current infection and HCV-antibody detection captures previous infection as well.

Previous studies of HBV prevalence in HCC cases have results ranging from 2.4% to 73.7% (Lehman et al., 2008). The current study found similar prevalence of HBV to a previous study from Cairo in which the prevalence of HBV in rural male and female cases was 8.8% and 10.8% respectively, and 2.7% in controls of both sexes (Ezzat et al., 2005). The results of the Cairo study are similar to the current study findings of 7.4% chronic HBV infection among cases, and 3.4% among controls as determined by serological testing and 10.1% and 0% in cases and controls as detected by molecular methods. It is suspected that HBV is underestimated in the current study due to a high proportion of undetected occult HBV, in which HBV infection occurs in HBsAg-negative patients with or without serologic markers of previous infection (Shi et al., 2012). Occult HBV infection has frequently been identified in patients with HCV-related chronic hepatitis (Shi et al., 2012). Because of the high prevalence of HCV in the study population, it is likely that a significant proportion of HBV infection was undetected by the laboratory methods used. Little research has been done in Egypt to determine the prevalence of occult HBV in the HCV-positive population; however studies performed in other populations have reported 15–30% when serum samples are tested (Brechot et al., 2001; Cacciola et al., 1999; El-Sherif et al., 2009). Occult HBV infection is also a possible explanation for the low concordance between serologic and molecular results in this study (Hassan et al., 2011). Molecular results for HBV could not be included in regression models because none of the controls tested positive for HBV and only the main effects of HBV and HCV infection could be determined. Interaction effects of HBV and HCV co-infection could not be assessed because of the low prevalence of co-infection.

The current study had a larger sample size than a majority of the previous studies on hepatitis in HCC patients in Egypt. Previous studies have been performed in other parts of the Nile Delta region, but none in the Gharbiah province. This study population has also a significantly higher proportion of rural participants (both cases and controls) than previous studies.

Our results support previous reports that hepatitis infection is the primary risk factor for hepatocellular carcinoma. Currently, hepatitis B immunization campaigns in Egypt have been successful in vaccinating younger populations; however, this study shows that HBV is not the most significant risk factor for HCC in Egypt even in older populations. Development of successful campaigns targeting hepatitis C could lead to significant reduction in HCC in Egypt.

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References


