Acta virologica 56: 235 – 240, 2012 doi:10.4149/av_2012_03_235

Hepatitis C virus and other risk factors in hepatocellular carcinoma

E. SCHIEFELBEIN[1], A.-R. ZEKRI[2], D. W. NEWTON[1], G. A. SOLIMAN[5], M. BANERJEE[3], CH.-W. HUNG[1], I. A. SEIFELDIN[4], A.-CH. LO[1], A. S. SOLIMAN[1*]

[1]Department of Epidemiology, University of Michigan School of Public Health, Ann Arbor, Michigan, USA; [2]National Cancer Institute, Cairo, Egypt; [3]Department of Biostatistics, University of Michigan School of Public, Ann Arbor, Michigan, USA; [4]Gharbiah Cancer Registry, Tanta, Egypt; [5]Western Michigan University, Kalamazoo, Michigan, USA

Received January 2, 2012; accepted August 30, 2012

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: 0.99–17.8, 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; Jemal *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

*Corresponding author. E-mail: asoliman@umich.edu; phone: +1734-764-5469. E. Schiefelbein and A.-R. Zekri are equal first authors.

Abbreviations: HBV = hepatitis B virus; HCV = hepatitis C virus; HCC = hepatocellular carcinoma; HBsAg = hepatitis B surface antigen; anti-HCV = antibody to hepatitis C virus

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.*, 2008; el-Zayadi *et al.*, 2005; Ezzat *et al.*, 2005).

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 patients admitted to the study hospitals during the study period. Cases and controls were group-matched on sex and age (± 5 years). About 40% of liver cancer cases in the recruiting hospitals were diagnosed by histopathological confirmation while the remaining cases were diagnosed by clinical, biochemical (alpha-fetoprotein), and radiological (ultrasound, triphasicity) confirmation (Ibrahim *et al.*, 2007).

An interviewer-administered questionnaire (37 questions for men, 44 questions for women) that elicited information on epidemiologic, environmental, occupational, lifestyle, reproductive, medical, and family histories was administered to cases and controls. After completing the interviews, participants were asked to donate 5-ml of blood.

Laboratory methods. Plasma and serum were separated from whole blood samples immediately after collection and stored in a -5˚C to -10˚C freezer at the Gharbiah Cancer Society, until weekly transportation to the National Cancer Institute of Cairo University, where samples were divided and stored at both -20˚C and -80˚C. HBV and HCV infections were determined both serologically and by PCR analysis. All samples were tested for hepatitis B surface antigen (HBsAg), antibodies to HBsAg (anti-HBs), antibodies to hepatitis B core antigen (anti-HBc), hepatitis B e antigen (HBeAg), antibodies to HBeAg (anti-HBe), and antibodies to hepatitis C virus (anti-HCV). All serology was tested with ELISA (Adaltis EIAgen HBV kits, Italy). All serologic assays were performed according to manufacturer´s instructions.

HBV-DNA and HCV-RNA were detected with PCR analysis. HBV-DNA and HCV-RNA were extracted from plasma samples using a commercially available viral DNA/RNA purification kit (QIAamp MinElute Virus Spin Kit, USA). Real-Time PCR (TaqMan® Universal PCR Master Mix USA, No AmpErase® UNG, USA) was used to determine the presence of viral DNA and RNA in plasma samples. All genetic analyses were performed according to manufacturer´s instructions.

Statistical methods. Using SAS software [SAS version 9.2 (SAS Institute, NC)], we conducted stratified univariate analyses to determine the demographic and viral profiles of cases and controls. Significant associations between proportions were detected with χ^2-test. Fisher´s exact tests were used for co-infection and molecular HBV variables. Results of HBV serology were characterized as no infection, chronic infection, and other infection (i.e. acute, past, and mixed infection). Conditional logistic regression was used to identify the main effects of hepatitis infection on HCC adjusted for other risk factors. The outcome variable was HCC and independent variables were HCV, HBV, farming, schistosomiasis, cirrhosis, and transfusion.

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 2 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 <0.010], 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 = 3.8 (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).

Table 1. Viral profile of the study subjects by serologic and molecular analysis

Variables by level (n)	Case (n = 148)		Control (n = 148)		P
	No.	%	No.	%	
Serology results					
HCV					
Negative	16	10.8	75	50.7	<0.01
Positive	132	89.2	73	49.3	
HBV(148,147)					
No infection	43	29.1	80	54.4	<0.01
Chronic infection	11	7.4	5	3.4	
Other infection*	94	63.5	62	42.2	
Co-infection(148,147)‡					
HBV-/HCV-	9	6.1	72	49.0	<0.01†
HBV-/HCV+	128	86.5	70	47.6	
HBV+/HCV-	7	4.7	3	2.0	
HBV+/HCV+	4	2.7	2	1.4	
Molecular results					
HCV(148,114)					
Negative	28	18.9	100	87.7	<0.01
Positive	120	81.1	14	12.3	
HBV					
Negative	133	89.9	148	100.0	<0.01†
Positive	15	10.1	0	0.0	
Co-infection(148,114)					
HBV-/HCV-	23	15.5	100	87.7	<0.01†
HBV-/HCV+	110	74.3	14	12.3	
HBV+/HCV-	5	3.4	0	0.0	
HBV+/HCV+	10	6.8	0	0.0	

†Fisher´s exact test; ‡chronic infection = HBV+ ;*includes acute, past, and mixed infection.

Table 2. Conditional logistic regression model to predict hepatocellular carcinoma from hepatitis infection as determined by serology

	Unadjusted OR (95% CI)	Adjusted OR (95% CI)	P (adjusted)
HCV			
Negative	1	1	
Positive	7.6 (3.8–15.1)	9.7 (3.3–28.0)	<0.01
HBV			
No infection	1	1	
Chronic infection	4.1 (1.3–12.9)	5.4 (0.9–31.8)	0.06
Other infection	3.1 (1.8–5.4)	2.5 (1.05–6.0)	0.04
Ever Worked in Farming			
No	1	1	
Yes	1.9 (1.1–3.2)	2.8 (1.1–7.2)	0.03
Schistosomiasis			
No	1	1	
	3.1 (1.6–5.9)	1.6 (0.6–4.4)	0.33
Cirrhosis			
	1	1	
	4.7 (2.5–9.0)	3.6 (1.6–8.1)	<0.01
Blood Transfusion			
	1	1	
	6.5 (2.3–18.6)	4.2 (0.99–17.8)	0.05

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.

Acknowledgements. E. Schiefelbein was supported by the Cancer Epidemiology Education in Special Populations Program of the University of Michigan (R25 CA112383).

References

Abdel-Aziz F, Habib M, Mohamed MK, Abdel-Hamid M, Gamil F, Madkour S, Mikhail NN, Thomas D, Fix AD, Strickland GT, Anwar W, Sallam I (2000): Hepatitis C virus (HCV) infection in a community in the Nile Delta: population description and HCV prevalence. Hepatology 32, 111–115. http://dx.doi.org/10.1053/jhep.2000.8438

Abdel-Wahab M, el-Enein AA, Abou-Zeid M, el-Fiky A, Abdallah T, Fawzy M, Fouad A, Sultan A, Fathy O, el-Ebidy G, elghawalby N, Ezzat F (2000): Hepatocellular carcinoma in Mansoura-Egypt: experience of 385 patients at a single center. Hepatogastroenterology 47, 663–668.

Anwar WA, Khaled HM, Amra HA, El-Nezami H, Loffredo CA (2008): Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: possibilities for prevention. Mutat Res. 659, 176–184. http://dx.doi.org/10.1016/j.mrrev.2008.01.005

Bosch FX, Ribes J, Diaz M, Cleries R (2004): Primary liver cancer: worldwide incidence and trends. Gastroenterology 127 (Suppl. 1), S5–S16. http://dx.doi.org/10.1053/j.gastro.2004.09.011

Bouchard MJ, Navas-Martin S (2011): Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. Cancer Lett. 305, 123–143. http://dx.doi.org/10.1016/j.canlet.2010.11.014

Boyle P, Levin B (Eds) (2008): World Cancer Report 2008. International Agency for Research on Cancer (IARC) : Lyon, France.

Brechot C, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Brechot P (2001): Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely „occult"? Hepatology 34, 194–203.

Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G (1999): Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. N. Engl. J. Med. 341, 22–26. http://dx.doi.org/10.1056/NEJM199907013410104

Darwish MA, Faris R, Darwish N, Shouman A, Gadallah M, El-Sharkawy MS, Edelman R, Grumbach K, Rao MR, Clemens JD (2001): Hepatitis c and cirrhotic liver disease in the Nile delta of Egypt: a community-based study. Am. J. Trop. Med. Hyg. 64, 147–153.

el-Sadawy M, Ragab H, el-Toukhy H, el-Mor A, Mangoud AM, Eissa MH, Afefy AF, el-Shorbagy E, Ibrahem IA, Mahrous S, Abdel-Monem A, Sabee EI, Ismail A, Morsy TA, Etewa S, Nor Edin E, Mostafa Y, Abouel-Magd Y, Hassan MI, Lakouz K, Abdel-Aziz K, el-Hady G, Saber M (2004): Hepatitis C virus infection at Sharkia Governorate, Egypt: seroprevalence and associated risk factors. J. Egypt Soc. Parasitol. 34 (1 Suppl.), 367–384.

El-Sherif A, Abou-Shady M, Abou-Zeid H, Elwassief A, Elbahrawy A, Ueda Y, Chiba T, Hosney AM (2009): Antibody to hepatitis B core antigen as a screening test for occult hepatitis B virus infection in Egyptian chronic hepatitis C patients. J. Gastroenterol. 44, 359–364. http://dx.doi.org/10.1007/s00535-009-0020-3

el-Zayadi AR, Badran HM, Barakat EM, Attia M, Shawky S, Mohamed MK, Selim O, Saeid A (2005): Hepatocellular carcinoma in Egypt: a single center study over a decade. World J. Gastroenterol. 11, 5193–5198.

Ezzat S, Abdel-Hamid M, Eissa SA, Mokhtar N, Labib NA, El-Ghorory L, Mikhail NN, Abdel-Hamid A, Hifnawy T, Strickland GT, Loffredo CA (2005): Associations of pesticides, HCV, HBV, and hepatocellular carcinoma in Egypt. Int. J. Hyg. Environ. Health 208, 329–339. http://dx.doi.org/10.1016/j.ijheh.2005.04.003

Franceschi S, Raza SA (2009): Epidemiology and prevention of hepatocellular carcinoma. Cancer Lett. 286, 5–8. http://dx.doi.org/10.1016/j.canlet.2008.10.046

Frank C, Mohamed MK, Strickland GT, Lavanchy D, Arthur RR, Magder LS, El Khoby T, Abdel-Wahab Y, Aly Ohn ES, Anwar W, Sallam I (2000): The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 355, 887–891. http://dx.doi.org/10.1016/S0140-6736(99)06527-7

Hassan MM, Zaghloul AS, El-Serag HB, Soliman O, Patt YZ, Chappell CL, Beasley RP, Hwang LY (2001): The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients. J. Clin. Gastroenterol. 33, 123–126. http://dx.doi.org/10.1097/00004836-200108000-00006

Hassan ZK, Hafez MM, Mansor TM, Zekri AR (2011): Occult HBV infection among Egyptian hepatocellular carcinoma patients. Virol. J. 8, 90. http://dx.doi.org/10.1186/1743-422X-8-90

IARC Working Group on the Evaluation of Carcinogenic Risks to Humans & International Agency for Research on Cancer (1994): Hepatitis Viruses. International Agency for Research on Cancer, Lyon, France.

Ibrahim AS, Seif-Eldein IAB, Ismail K, Hablas A, Hussein H, Elhamzawy H, Ramadan M (Eds) (2007): Cancer in Egypt, Gharbiah: Triennial Report of 2000–2002, Gharbiah Population-Based Cancer Registry. Gharbiah Population-Based Cancer Registry, Tanta, Egypt.

Ibrahim SA, Abdelwahab SF, Mohamed MM, Osman AM, Fathy E, Al-Badry KS, Al-Kady N, Esmat GE, Al-Sherbiny MM (2006): T cells are depleted in HCV-induced hepatocellular carcinoma patients: possible role of apoptosis and p53. Egypt J. Immunol. 13, 11–22.

Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ (2009): Cancer statistics, 2009. CA Cancer J. Clin. 59, 225–249. http://dx.doi.org/10.3322/caac.20006

Lehman EM, Soliman AS, Ismail K, Hablas A, Seifeldin IA, Ramadan M, El-Hamzawy H, Shoushtari CS, Wilson ML (2008): Patterns of hepatocellular carcinoma incidence in Egypt from a population-based cancer registry. Hepatol. Res. 38, 465–473. http://dx.doi.org/10.1111/j.1872-034X.2007.00299.x

Lehman EM, Wilson ML (2009a): Epidemic hepatitis C virus infection in Egypt: estimates of past incidence and future morbidity and mortality. J. Viral Hepat. 16, 650–658. http://dx.doi.org/10.1111/j.1365-2893.2009.01115.x

Lehman EM, Wilson ML (2009b): Epidemiology of hepatitis viruses among hepatocellular carcinoma cases and healthy people in Egypt: a systematic review and meta-analysis.

Int. J. Cancer 124, 690–697. http://dx.doi.org/10.1002/ijc.23937

Parkin DM, Bray F, Ferlay J, Pisani P (2005): Global cancer statistics, 2002. CA Cancer J. Clin. 55, 74–108. http://dx.doi.org/10.3322/canjclin.55.2.74

Shi Y, Wu YH, Wu W, Zhang WJ, Yang J, Chen Z (2012): Association between occult hepatitis B infection and the risk of hepatocellular carcinoma: a meta-analysis. Liver Int. 32, 231–240. http://dx.doi.org/10.1111/j.1478-3231.2011.02481.x

Sievert W, Altraif I, Razavi HA, Abdo A, Ahmed EA, Alomair A, Amarapurkar D, Chen CH, Dou X, El Khayat H, Elshazly M, Esmat G, Guan R, Han KH, Koike K, Largen A, McCaughan G, Mogawer S, Monis A, Nawaz A, Piratvisuth T, Sanai FM, Sharara AI, Sibbel S, Sood A, Suh DJ, Wallace C, Young K, Negro F (2011): A systematic review of hepatitis C virus epidemiology in Asia, Australia and Egypt. Liver Int. 31 (Suppl. 2), 61–80. http://dx.doi.org/10.1111/j.1478-3231.2011.02540.x

Soliman AS, Hung CW, Tsodikov A, Seifeldin IA, Ramadan M, Al-Gamal D, Schiefelbein EL, Thummalapally P, Dey S, Ismail K (2010): Epidemiologic risk factors of hepatocellular carcinoma in a rural region of Egypt. Hepatol. Int. 4, 681–690. http://dx.doi.org/10.1007/s12072-010-9187-1

Venook AP, Papandreou C, Furuse J, de Guevara LL (2010): The incidence and epidemiology of hepatocellular carcinoma: a global and regional perspective. Oncologist 15 (Suppl. 4), 5–13. http://dx.doi.org/10.1634/theoncologist.2010-S4-05