

DYNAMICS OF LIVER DISEASE IN EGYPT:  
SHIFTING PARADIGMS OF A COMPLEX ETIOLOGY

by

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A dissertation submitted in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
(Epidemiological Science)  
in The University of Michigan  
2008

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Peter Paul Rubens (c. 1612), Philadelphia Museum of Art

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Dedicated to the principle that complete physical,  
mental, and social wellbeing is a basic human  
right deserved by all, regardless of gender,  
race, religion, or socioeconomic status

## ACKNOWLEDGEMENTS

I am extremely grateful to my committee, from whom I received invaluable insights and learned the process of conducting and reporting quality scientific research. My advisor, Dr. Mark Wilson, gave me the freedom to explore my own ideas and encouraged my development as a professional and responsible scientist. The cumulative experience and expertise of Drs. Palmer Beasley, Susan Murray, and Zhenhua Yang provided me with an immeasurable resource I was blessed to have. I am also grateful to my many Egyptian collaborators. In particular, I thank Dr. Mohamed Husein for his guidance during study design and troubleshooting while abroad. I also appreciate the members of the Gharbiah Cancer Registry for their help during my active research. I would especially like to acknowledge Drs. Mohamed Ramadan and Hoda Omar Gad for their tireless efforts in maintaining the highest standards for data quality.

This dissertation was financially supported by a US National Science Foundation Graduate Research Fellowship, a Sigma Xi Grant-in-Aid of Research, and grants from University of Michigan's Rackham Graduate School, Department of Epidemiology, and Global Health Program in the School of Public Health.

Lastly, I am grateful to my family, not only for supplying the necessary genetic material, but also for providing constant intellectual support and setting examples as professionals in their own fields as well as honorable citizens. I am also deeply indebted to my beloved Jamie for sharing my vision and enabling me to reach my goals.

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## LIST OF ABBREVIATIONS

Anti-HBc	Hepatitis B virus core antigen antibody
Anti-HBs	Hepatitis B virus surface antigen antibody
Anti-HCV	Hepatitis C virus antibody
ASR	Age-standardized incidence rate
CAPMAS	Central Agency for Public Mobilization and Statistics
CDC	Centers for Disease Control and Prevention
CI	Cumulative incidence
EB	Empirical Bayes
EIA	Enzyme immuno-assay
ELISA	Enzyme-linked immunosorbent assay
GPCR	Gharbiah Population-based Cancer Registry
HBsAg	Hepatitis B virus surface antigen
HBV	Hepatitis B virus
HBX	Hepatitis B virus X gene
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HPW	Healthy pregnant women
IARC	International Association for Research on Cancer
ICD	International Classification of Diseases
IR	Incidence rate
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MECC	Middle East Cancer Consortium
NCI	National Cancer Institute
PAT	Parenteral antischistosomiasis therapy
PCR	Polymerase chain reaction
PLC	Primary liver cancer
PY	Person-years
RBD	Replacement blood donors
RR	Relative rate
SCC-B	Squamous cell carcinoma of the bladder
SEER	Surveillance Epidemiology and End Results
US	United States
VBD	Voluntary blood donors
WHO	World Health Organization

## ABSTRACT

The burden of liver disease in Egypt is exceptionally high, maintaining the highest prevalence of hepatitis C virus (HCV) worldwide, as well as rising rates of hepatocellular carcinoma (HCC). The foundation of the HCV epidemic in Egypt is generally attributed to a mass public health campaign to eliminate schistosomiasis during the 1960's-1980's. Questions remain regarding the precise incidence of HCV during this campaign, the future burden of chronic disease those affected will experience, and the future direction of HCV and liver disease now that this campaign has ended. This dissertation offers a series of studies designed to precisely define the nature of HCV infections and HCC in Egypt, spatially and temporally, as well as predict the future burden and impact on the Egyptian population. Specific methods included analyses of HCC case data collected from the Gharbiah Population-based Cancer Registry (GPCR) to define demographic and spatial trends in the occurrence of HCC in Egypt, in addition to a meta-analysis and the construction of two mathematical models designed to calculate historic incidence of HCV and project future HCV-related health complications. Results identified significant heterogeneity in HCC occurrence with respect to sex and district of residence. More in-depth investigation identified significant spatial clustering of HCC associated with clusters of squamous cell carcinoma of the bladder (a proxy measure for schistosomiasis burden). Meta-analysis revealed the HCV epidemic is marked by a three-way interaction between time, geographic region, and whether individuals reside in

urban or rural environments. Modeling techniques confirmed the presence of a cohort effect among those affected by the public health campaign, identified by a spike in incidence among those presently aged 30-50 years. The natural history model predicted Egypt will experience significant morbidity and mortality over the next 20 years due to the HCV epidemic. Our findings highlight the significance of developing an integrated strategy for the prevention of HCV infection. Unquestionably, additional factors contributing to liver disease burden remain to be elucidated. This information is crucial and should help define the complex etiology of liver disease in Egypt, enabling policy makers to create targeted, more efficient prevention and control programs.

# CHAPTER I

## INTRODUCTION

### BACKGROUND AND PUBLIC HEALTH SIGNIFICANCE

**Impact of liver cancer.** Worldwide, primary liver cancer (PLC) ranks fifth among cancer incidence, with an estimated 0.5 – 1 million cases diagnosed each year, and third among cancer mortality. It accounts for 5.6% of all human cancers, with 7.5% among men and 3.5% among women. Developing countries generally have a greater share of the burden, however, where it can rank as the leading cause of cancer incidence and mortality among males.<sup>1-5</sup> Hepatocellular carcinoma (HCC) and cholangiocarcinoma are the two major types, accounting for 85% and 10% of all primary liver cancers respectively.<sup>2-4</sup> The following report will focus exclusively on HCC, as it is by far the dominant malignancy and has the greatest relevance to public health.

Cholangiocarcinoma is generally associated with specific occupational exposures and smaller risk groups, whereas the general population is susceptible to HCC, requiring public health programs of a much larger scale.<sup>4</sup>

Approximately 81% of all HCC cases are found in Asia and Africa, with China producing 53% of these cases.<sup>6</sup> In these high-risk regions, rates begin increasing after age 20 and typically peak or stabilize at age 50 and older.<sup>2,4,5</sup> Not only does this lead directly to a drain on health resources, it also contributes to an indirect loss of the

country's productivity. Considering the age group affected and the high rate of mortality, this disease removes people who are the most economically productive and who hold a great deal of social responsibility in terms of caring for children and the elderly. Despite public health's longstanding knowledge of the problem, few strides have been made when compared to other cancers such as breast and prostate cancer. This can be attributed to the complex combination of liver anatomy and the insidious nature of liver cancer's primary risk factors (i.e. hepatitis B virus, hepatitis C virus, aflatoxin exposure, and excessive alcohol intake).<sup>1-5</sup>

**Liver anatomy and function.** The liver, along with its companion biliary tree and gall bladder, is located in the upper-right quadrant of the abdomen (Figure 1.1). Existing at the crossroads between the digestive tract and the other functions of the body, the liver is charged with the enormous task of maintaining metabolic homeostasis. This involves processing normal dietary elements, such as lipids and carbohydrates, as well as detoxifying and excreting waste products and pollutants.<sup>8-9</sup> Consequently, hepatic malfunction can have dramatic repercussions.

The liver holds about one pint (13 percent) of the body's blood supply at any given moment. The liver consists of two main lobes, both of which are made up of thousands of lobules. These lobules are connected to small ducts that connect with larger ducts to ultimately form the hepatic duct. The hepatic duct transports the bile produced by the liver cells to the gallbladder and duodenum (the first part of the small intestine).<sup>8-9</sup> Incoming blood arrives via the portal vein (60%-70% of blood flow) and the hepatic artery (30%-40% of blood flow) through the *porta hepatis*. The common hepatic bile duct exits in this same region. The initial branches of artery, vein, and bile duct lie just

outside the liver, but the remaining branches run roughly in parallel within the liver to form the portal tracts. The terminal vessels of the portal vein and hepatic artery supply blood to the expanse of hepatic parenchyma. Blood is ultimately collected and exits into the inferior vena cava, lying posterior to the liver.<sup>8-9</sup>

The liver performs several vital functions: it processes and stores many of the nutrients absorbed from the intestine, it secretes bile into the intestine to help absorb nutrients, and it also makes some of the clotting factors needed to stop bleeding from a cut or injury. In addition, the liver plays a very important part in removing toxic wastes from the body, of which alcohol and dietary toxins are particularly noteworthy. The enormous functional reserve of the liver often masks the clinical impact of early liver damage.<sup>8-9</sup> With progression of diffuse disease or disruption of bile flow, however, the consequences of liver damage can easily become life-threatening.

The most severe consequence of liver disease is hepatic failure, which will only occur once 80%-90% of hepatic functional capacity is removed.<sup>9</sup> By far the most common route to hepatic failure is the endpoint of chronic hepatitis or alcoholic liver disease ending in cirrhosis. Approximately 85% of patients infected chronically with hepatitis B virus (HBV) will develop cirrhosis, and nearly 95% of patients with chronic hepatitis C virus will develop cirrhosis.<sup>3,10</sup> Cirrhosis can be defined by three main characteristics: parenchymal nodules, disruption of the architecture of the entire organ, and fibrous septa that replace multiple adjacent lobules. In particular, the parenchymal fibrosis is typically diffuse, extending throughout the liver. Once the fibrosis has developed, it is generally irreversible. Although alone cirrhosis is among the leading causes of death in the world, it also serves as an important precursor to HCC.<sup>4</sup>

## HEPATOCELLULAR CARCINOMA: RISK FACTORS

**Hepatitis B Virus.** To date, the dominant risk factor for HCC has been HBV, accounting for nearly 75%-80% of all cases worldwide. There are approximately 350 million hepatitis B carriers worldwide, of which one quarter are expected to develop serious liver disease, including cirrhosis and HCC. In addition, HBV infection directly accounts for nearly 1 million deaths, and indirectly it accounts for 60 million deaths from chronic liver disease annually.<sup>11-13</sup> The problem disproportionately affects developing countries where transmission occurs at an early age, increasing the likelihood of chronic hepatitis and subsequent HCC (Figure 1.2).<sup>14</sup>

Hepatitis B virus (HBV) is a DNA virus of from the Hepadnaviridae family. It is transmitted via blood products and other bodily fluids. It is unique in that all regions of the viral genome encode protein sequences.<sup>11,13-14</sup> The association between HBV and HCC was suggested by the high incidences of HCC in regions where HBV infection is hyper-endemic. The risk of eventually developing HCC is inversely related to the age of acquisition of HBV. Thus, children exposed to HBV have a much greater chance of becoming chronic HBV carriers than do newly infected adults.<sup>14</sup>

In 1994, a working group of the International Agency of Research on Cancer (IARC) officially concluded that HBV is a carcinogen in humans. The incorporation of HBV DNA into tumor DNA has been well documented. Molecular studies have shown that HBV DNA is present in the tumor tissues of HCC patients who are chronic carriers of HBV, and that 10% to 29% of patients with HCC harbor markers of past HBV infection.<sup>15-17</sup> Of particular significance is the protein from the X region (HBX). It is necessary for virus replication and acts as a transcriptional transactivator of the viral



genes and a wide variety of host gene promoters. This protein is thought to play a key role in the causation of HCC.<sup>18-22</sup>

**Hepatitis C Virus.** The hepatitis C virus (HCV) is an enveloped single-stranded RNA virus from the Flaviviridae family. The virus is inherently unstable, leading to multiple types and subtypes, which has seriously limited efforts to develop an effective vaccine.<sup>23-26</sup> Of particular significance is the fact that elevated titers of anti-HCV IgG occurring after an active infection do not confer immunity. Thus, it is characteristic for patients to experience repeated bouts of hepatic damage, resulting from reactivation of preexisting infection or emergence of an endogenous, newly mutated strain. Cirrhosis typically develops within 5-10 years of infection.<sup>26-29</sup>

Hepatitis C virus (HCV) has been suggested as a major cause of HCC in areas where the incidence of HBV infection is declining or already low. The prevalence of anti-HCV antibodies among patients with HCC ranges from around 30% in China and South Africa to 70% to 80% in southern Europe, Egypt, and Japan. Chronic HCV infection coexists with HBV infection or alcohol abuse in 20% of cases.<sup>23-24</sup> In areas where HBV is hyper-endemic, such as Taiwan, the prevalence of anti-HCV antibodies is much higher among hepatitis HBsAg-negative HCC patients than among HBsAg-positive HCC patients. Sheu, et al. found a higher incidence of HCV RNA in serum and liver tissue of HBsAg-negative patients with HCC than in samples from HBsAg-positive patients, suggesting that HCV infection is important in the pathogenesis of HCC in HBsAg-negative patients.<sup>30</sup> Prospective studies have indicated that more adults are long-term carriers of HCV than of HBV.<sup>31-39</sup> In a group of patients with transfusion-associated liver disease, the interval between blood transfusion and diagnosis of anti-HCV

antibody–positive HCC was as long as 29 years.<sup>26</sup> The IARC working group determined that chronic infection with HCV is carcinogenic in humans. It has been hypothesized that HCV is probably the major viral cause of HCC in areas with a low incidence of HBV carriers. Although there is no evidence that the virus itself is oncogenic, it has been suggested that this agent may promote carcinogenesis through the induction of chronic necro-inflammatory hepatic activity and liver cirrhosis.<sup>28, 30-34,37,39</sup> Approximately 90%-95% of patients with HCV-associated HCC also have cirrhosis.<sup>33-34,40</sup>

**Aflatoxin Exposure.** Aflatoxins are mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*. These toxins may contaminate food stored in humid conditions. Animal studies suggest that aflatoxin B<sub>1</sub> (AFB<sub>1</sub>) is a carcinogen.<sup>1-5,8,41-43</sup> Early case-control studies, however, failed to find a significant association between human HCC and aflatoxin exposure. In Mozambique, South Africa, Swaziland, China, and Taiwan, the use of biomarkers has shown a statistically significant correlation between dietary aflatoxin exposure and HCC mortality or morbidity.<sup>43</sup> Areas with high aflatoxin contamination overlap areas with high rates of HBV infection. Some investigators propose that the combination of aflatoxin and HBV infection has a synergistic effect that leads to oncogenesis. In liver cells, aflatoxin is metabolized by the microsomal mixed-function oxidase. Aflatoxin B<sub>1</sub>-2-3-epoxide, a highly reactive metabolite of aflatoxin B<sub>1</sub>, is capable of binding to DNA and RNA and is thought to be the main carcinogenic metabolite of aflatoxin.<sup>8,41</sup> Aflatoxin B<sub>1</sub> is known to induce a mutation at codon 249 of *p53*.<sup>41,43</sup> This mutation could be one of the mechanisms leading to HCC.

**Excessive Alcohol Intake.** Cirrhosis is the most important risk factor for HCC and underlies HCC in more than 80% of the cases. The annual incidence of HCC among patients with cirrhosis ranges from 3% to 5%.<sup>1-5,8-9</sup> Autopsy studies of patients with cirrhosis have shown a prevalence of HCC ranging from 20% to 80%. In Africa and Asia the cause of underlying liver cirrhosis may be related to exposure to HBV or HCV. In developed nations, however, cirrhosis is most commonly linked to alcohol abuse.<sup>1-3,40</sup> Although research providing direct causal inference is not overwhelming, several laboratory and epidemiologic studies conclude that alcohol plays an indirect role in hepatocarcinogenesis.<sup>1-5</sup> The reported risk of developing HCC among individuals with alcoholic cirrhosis ranges from 3% to 15%. The risk may actually increase after cessation of alcohol consumption, but this increase results from an extended lifespan rather than from a direct effect on tumor formation.<sup>5,8</sup> Hepatocellular carcinoma has been observed in 60% to 80% of patients with macronodular cirrhosis and in 3% to 10% of the patients with the micronodular pattern.<sup>4-5</sup>

#### **EGYPT AND LIVER DISEASE**

Egypt holds a unique position in the epidemiology of hepatitis and liver cancer. It has successfully implemented the hepatitis B virus (HBV) vaccine and now boasts 95%-100% coverage.<sup>44-46</sup> Despite this major public health achievement, liver cancer continues to be the second highest cause of cancer incidence and mortality in men. The age standardized rate is approximately 20.6 in males and 5.2 in females. For males, this rate is 7 times the second-highest rate found in the Middle East Cancer Consortium (Israeli Jews) and more than 3 times the rate reported by US SEER.<sup>47</sup> Egypt is also home to the

highest prevalence of hepatitis C virus (HCV) in the world (Figure 1.3), with an overall rate of approximately 22%.<sup>48-60</sup> In populations of blood transfusion recipients over the age of 30, this rate has been reported to be as high as 73%, and in the general population aged 40-60 years this rate can be as high as 55%. At the same time, the rate in children is much lower, ranging between 2-10%. While the rate in children is much lower than that in the older population, it is still considered high by World Health Organization (WHO) standards, where rates greater than about 4% are considered high.<sup>51,53,59</sup> Therefore, as more and more countries adopt the HBV vaccine, HCV could become the dominant force driving liver cancer rates worldwide.<sup>49-51,61</sup> Understanding the HCV epidemic in Egypt will aid in the global effort to fight HCV and liver cancer as well as provide insights into viral etiology and pathogenesis that may help in future situations with newly emerging infectious agents.

Most scholars will agree that the HCV story in Egypt must be divided into two chapters: pre 1980's infection and post 1980's infection. In the population aged older than 20-30 years, the vast majority of infection can be explained by the intravenous treatment for schistosomiasis (PAT) and other iatrogenic exposures such as blood transfusion.<sup>62-69</sup> The tragic irony of the situation in Egypt is that a mass public health campaign designed to dramatically reduce the burden of schistosomiasis led to a new public health crisis: the HCV epidemic. This campaign extended from the 1940's through the 1970's, with probably the peak transmission occurring during the 1960's and 1970's. In 1982, praziquantal, an oral treatment for schistosomiasis, was introduced, and use of PAT rapidly declined. In addition, throughout the 1980's numerous measures were adopted to limit iatrogenic exposures, and they appear largely to have been effective.<sup>63,66</sup>

The question that remains for Egypt, therefore, is what will the shape of the epidemic curve be now that PAT and hospital-based infections no longer contribute to the picture of transmission. Indeed the rates among children are significantly lower, but it nevertheless appears that HCV is capable of sustaining transmission. The problem is that presently we have little explanation for this transmission.

The studies that have tried to examine this tend to divide the study population into groups older than 20 years and younger than 20 years. This has to do with the inherent confounding between age and exposure (namely PAT). Types of exposures examined have included formal medical procedures, informal medical procedures, hookah use, etc. In general, there tends to be limited association between anti-HCV positivity and receiving medical procedures from an informal practitioner, such as circumcision. In all studies reviewed, however, R-squared values were rarely greater than 0.1, which means that 90% of the infections seen in the younger population cannot be effectively explained by the exposures measured.<sup>51,53-59</sup> This is compounded by the fact that the Egyptian population does not appear to have the risk factors seen in other regions of the world with higher HCV prevalence, for example IVDU or HIV/HCV coinfection.

It seems clear, therefore, that researchers need to broaden their scope of potential risk factors for transmission. It has been observed anecdotally that family members in prolonged close contact could have greater risk of infection, but this has yet to be directly studied.<sup>70-73</sup> It is possible that exposure to the virus may require intense close contact and the exposures could be cumulative. To properly study this point, a prospective family cohort study would be desired to follow family members of anti-HCV positive and negative individuals and follow-up for new infections. Suggesting this design would

likely be premature at this point. An appropriate step would be to provide the intermediate link between anecdotal observation and cause-and-effect demonstration.

Another glaring gap in the understanding of the HCV epidemic in Egypt is the complete lack of information regarding incidence rates. Due to the nature of the infection, longitudinal incidence studies would be very difficult to carry out. Initial infection is rarely symptomatic, and chronic carriage is insidious. Nevertheless, it is difficult to predict the burden HCV will have on the community in the future and what priority it should have in health policy if the rates of transmission are unknown. Up to this point, incidence rates have been inferred from prevalence.<sup>48-52,59-60,70</sup> The prevalence among children is much lower than it is among adults who were infected during the PAT campaign, and therefore incidence rates must be lower now. Though it appears clear that transmission rates have declined since the early 1980's, it is still essential to quantitatively characterize the nature of the HCV problem in order to make informed predictions about the future of the epidemic and define appropriate health policy to address the crisis.

Because HCV is such an important predictor of hepatocellular carcinoma (HCC), it frequently receives special attention from the public health community in Egypt. This should not be at the exclusion of all other risk factors, however. There are many recognized risk factors for HCC, including but not limited to HBV, HCV, aflatoxin exposure, alcohol consumption, and tobacco use. In addition, a number of other environmental agents could act as promoters in concert with the above risk factors (pesticides, heavy metals, etc).<sup>74-76</sup> It is necessary to identify the Egypt's HCC profile in terms of risk factor distribution. Identifying clusters of cases associated with certain risk

factors can also help policy makers locate risk groups and design targeted interventions that will improve efficiency while at the same time protecting the largest number of susceptible individuals in the population.

Fortunately, Egypt provides an excellent location for performing studies to understand the epidemiology of liver cancer. It has successfully created a population-based cancer registry that has been functional since 1999. In addition, the Egyptian National Liver Institute is a unit dedicated to understanding liver disease throughout the country. Such a well established infrastructure is a rich resource that should be recognized by the research community. This administrative organization is in addition to the unique exposure status of the population, with the highest levels of HCV in the world, dramatically declining levels of HBV, and large exposures to environmental contaminants, owing to its status as a country in the midst of an industrial transition. Therefore, not only can Egypt serve as host to studies concerning the natural history of HCV, but it should be the focus of subsequent liver cancer studies as well.

Prior cancer studies in Egypt have focused on utilizing qualitative instruments to assess the likelihood that specific exposures led to carcinogenesis.<sup>77-82</sup> They start with liver cancer cases and attempt to document infection with HBV and HCV as well as any exposures to environmental agents. These studies have shown the increasing importance of HCV in liver cancer, estimated to account for 40-50% of liver cancer cases, and the declining influence of HBV and HBV/HCV infection (25% and 15% respectively).<sup>79-80,82</sup> These studies have also documented an overall increase in the prevalence of liver cancer in Egypt, from approximately 4.0% in 1993 to 7.3% in 2003.<sup>79</sup> This increase in liver cancer is generally attributed to HCV. Since chronic HCV does not typically lead to

carcinogenesis for 10-30 years following infection, the rates of liver cancer can be expected to continue increasing until the cohort of PAT-related infected individuals has worked its way through.<sup>49,79</sup> This suggests that the true burden of liver cancer in Egypt has yet to be realized.

### **SUMMARY AND SPECIFIC AIMS**

Though the studies mentioned above have contributed greatly to our understanding of the gravity of the problem in Egypt, they are lacking a more quantitative approach that would provide the data necessary for developing appropriate health policy. Such data would also be useful in making geographic comparisons, either across regions in Egypt and the Arab world or on a global scale. This dissertation offers a series of studies designed to precisely define the nature of hepatitis virus infections and HCC in Egypt, spatially and temporally, as well as their future implications. This approach is necessary to disentangle the complex etiology of liver disease in Egypt and the web of confounders often produced by qualitative assessment of exposures.

In this dissertation, specific aims were to:

- 1) Characterize the descriptive features of HCC cases in the Nile Delta through extensive examination of Egypt's only population-based cancer registry for the period 1999-2003. This study represents the first population-based HCC study in Egypt.
- 2) Determine if heterogeneous spatial patterns in HCC cases exist in the Nile Delta and if these spatial patterns can be linked to those of squamous cell carcinoma of the bladder. This retrospective analysis addresses: 1) the question



of heterogeneity among HCC risk factors; and 2) the relationship between PAT treatment, HCV transmission, and HCC incidence.

3) Determine time trends and other descriptive elements of HBV and HCV infections among the healthy population and HCC cases in Egypt by means of a systematic literature review and meta-analysis for the period 1980-present.

4) Calculate prior HCV incidence in Lower Egypt (Nile Delta) using age-specific sero-prevalence data, and estimate the future health burden due to HCV sequelae using a Markov natural history model.

**Figure 1.1.** Anatomy of the liver.<sup>7</sup>

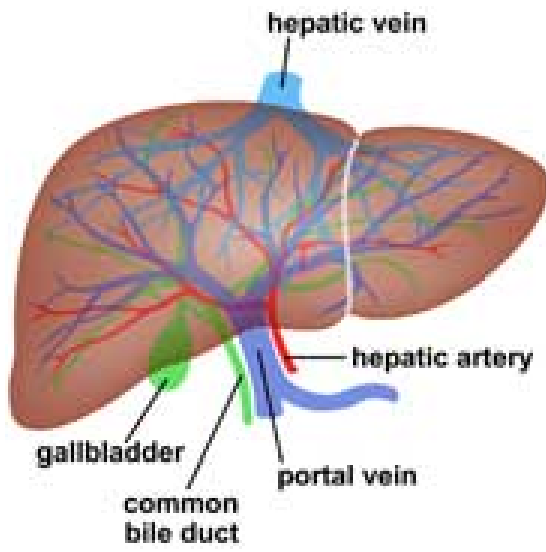
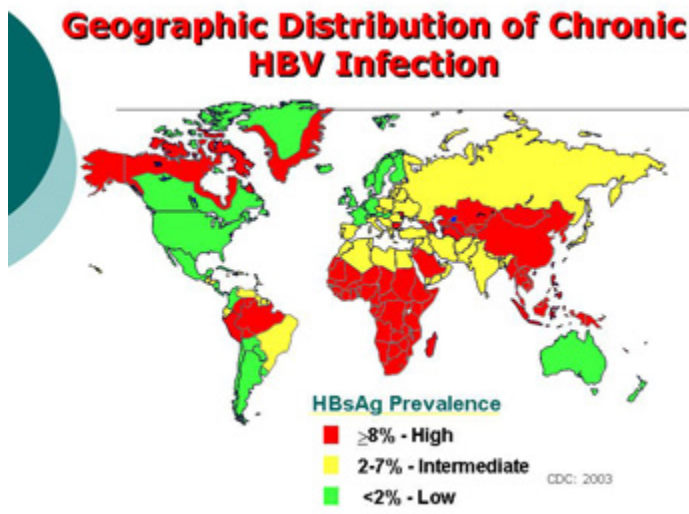
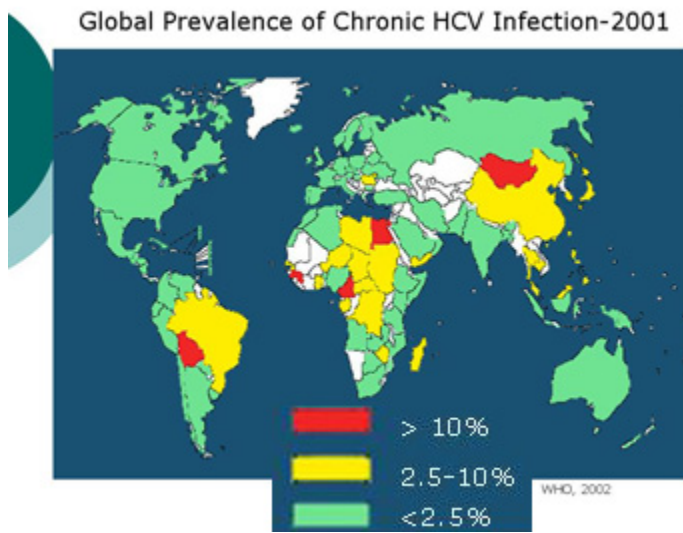


Figure 1.2. Geographic distribution of chronic HBV infection.<sup>6</sup>



**Figure 1.3.** Global Prevalence of Chronic HCV Infection in 2001.<sup>6</sup>



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## CHAPTER II

### PATTERNS OF HEPATOCELLULAR CARCINOMA INCIDENCE IN EGYPT FROM A POPULATION-BASED CANCER REGISTRY

#### INTRODUCTION

Liver cancer rapidly reduces quality of life and typically causes death within six months to one year from diagnosis.<sup>1</sup> Globally, it is the fifth leading cause of cancer and the third leading cause of cancer deaths.<sup>1-2</sup> This cancer varies widely in incidence throughout the world with rising incidence in Egypt. The primary risk factors for hepatocellular carcinoma (HCC) are hepatitis B virus (HBV), hepatitis C virus (HCV), dietary aflatoxin exposure, and chronic alcohol consumption.<sup>1-2</sup>

Prior to the introduction of the HBV vaccine, chronic infection with HBV was generally high, with developing countries sharing the greatest burden.<sup>3</sup> Consequently, HBV was the dominant etiologic factor in the development of HCC. This is largely still true in Egypt, because vaccination programs were not started until the 1980s. More recently, HCV has begun to eclipse HBV in incidence in many countries throughout North America, Europe, and the Middle East.<sup>4-5</sup> Rates of HCV in Egypt are among the highest in the world, with a prevalence rate of up to 20%.<sup>6-7</sup> Although an HBV vaccine program has been successfully implemented, with childhood coverage estimated at 95%-100%, most people born 20 years or more ago in Egypt have not been vaccinated.<sup>8-9</sup>

Hospital-based studies from Egypt have reported an overall increase in the

relative frequency of all liver-related cancers in Egypt (>95% as HCC), from approximately 4.0% in 1993 to 7.3% in 2003.<sup>10</sup> Recent investigations in Egypt have shown the increasing importance of HCV infection in the etiology of liver cancer, estimated to account for 40-50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15% respectively).<sup>10-12</sup>

Few studies in Egypt have fully measured the presence of aflatoxins and their impact on liver disease there. A recent study by Rahman el-Zayadi *et al.* examined 200 HCC cases and 120 healthy controls, and detected aflatoxin B(1) in 17% of the HCC cases compared to 9.4% of the healthy controls (risk ratio = 2).<sup>13</sup> In 2005, Sayed *et al.* performed a cross-sectional analysis on risk factors for liver disease in a rural population south of Cairo to evaluate aflatoxin exposure in serum.<sup>14</sup> They found aflatoxin B(1) to be associated with hepatitis B virus s antigen (HBsAg) seropositive subjects (odds ratio = 6.2) and anti-HCV seropositive subjects (odds ratio = 2.5).<sup>14</sup>

In addition to HBV, HCV and aflatoxins, other risk factors have been linked to HCC, including chronic alcohol consumption and hemochromatosis. Although alcohol plays a significant role in the etiology of HCC in many countries, it was not examined in this study because the prevalence of alcohol consumption in Egypt is extremely low.<sup>15-16</sup> Hemochromatosis also was not investigated in this study, because in Egypt it is extremely rare.<sup>17-18</sup>

With assistance and quality assurance from the United States NCI, Egypt successfully created a population-based cancer registry in Gharbiah province (Figure 2.1) that has been successfully functional since 1999.<sup>19</sup> The first year of the registry data (1999) showed that the age-standardized annual incidence rate of HCC was

~20.6/100,000 in males and ~5.2/100,000 in females. A comparison of data from the Gharbiah Population-based Cancer Registry (GPCR) for the period 1999-2001 with other countries in the Middle East Cancer Consortium showed that the liver cancer incidence rate was 7 times greater than the next-highest country rate, and more than 3 times the rate reported by United States Surveillance Epidemiology and End Results (SEER).<sup>19</sup> Our study was designed to characterize the demographic and geographic patterns of HCC cases in this region to lay the groundwork for future research directed at understanding the complex etiology of this disease. Accordingly, we retrieved and analyzed data of all HCC patients included in the GPCR from January 1, 1999 through December 31, 2003. We analyze the demographic and geographic characteristics of HCC patients and compared patterns with those of HCC cases in the United States SEER registry for this period.

## METHODS

**Characteristics of the Study Region.** Gharbiah Province is located in the middle of the Nile delta, approximately 100 km north of Cairo. The province population (4.2 million people - 5.7% of Egypt) has a density of ~1,752/km<sup>2</sup>, making it the tenth most densely populated province in Egypt (Egypt has a total of 27 provinces). The male:female ratio in the Gharbiah province is 1.02:1, and the age structure resembles that of the rest of the country, with 47% of the population <20 years and 3.6% >65 years.<sup>20</sup> Gharbiah Province is considered an urban-rural Province by the Central Agency for Public Mobilization and Statistics (CAPMAS) with 31% of the population in urban areas and 69% in rural areas.<sup>20</sup> Each of its 8 districts has its own main city. The capital of

Gharbiah Province is the city of Tanta, which serves as the headquarters for the population-based cancer registry. Gharbiah has a total of 316 villages, and is mainly agricultural, but one district (El Mehalla) is a major textile producer in Egypt. Annual incidence rates of HCC were based on population data estimated using linear interpolation between pairs of published government census population information. This preliminary study used an annual linear interpolation from the 1996 census data, and the 2001 and 2005 population projections.<sup>20</sup>

**Gharbiah Population-Based Cancer Registry Data.** The GPCR was established in 1998 as part of the Middle East Cancer Consortium (MECC) Joint Cancer Registration Project, and it is affiliated with the Egyptian Ministry of Health and Population.<sup>21</sup> The registry actively seeks all cases in Gharbiah province, regardless of inpatient/outpatient status or whether patients were seen in public or private hospitals. Information is gathered from medical records of government and private hospitals and clinics, death certificates, and histopathology laboratories and radiology clinics. A team of registrars trained by the International Agency for Research on Cancer (IARC) and Emory University School of Public Health in Atlanta, USA conduct regular visits with each collaborating center to obtain the data and review its quality using IARC's standard review process. Data quality was assessed in 2002 for completeness of coverage and reliability of registration. In addition to computer checks through CanReg software; external auditing was done by Emory University staff in 2002. Coverage was found to exceed 90% of the Gharbiah population.<sup>22</sup>

The registry conducts routine data cleaning by standardizing field entries and removing duplicate entries. Inconsistencies were addressed by reviewing medical

records. We obtained the data of all liver cancer cases diagnosed and included in the registry from January 1, 1999 through December 31, 2003 (n = 1,309). Our case definition was restricted to those with an ICD-10 topology code of 22.0 and a morphology of HCC. Cases were excluded if they did not satisfy both elements of the case definition or if they had missing values and if inconsistencies were not resolved after examining medical records. This process reduced the total number of cases to 1,186 (removing 123 cases). The following variables were obtained for each case: registry patient number, sequence number, age at diagnosis, date of birth, gender, usual residential address, date of diagnosis, basis of diagnosis, primary site code (ICD-O-3), morphology codes, histology type, behavior, grade/differentiation/cell indicator, and summary stage at diagnosis. Additional variables included: family history, marital status, smoking history, religion, occupation, and treatment data. Unfortunately hepatitis data are not currently included in the management protocol for HCC cases at the GPCR. Thus, patient HBV and HCV status were unavailable for this study. Additionally, because this study was retrospective and the cases in our sample were all deceased, there was no way to independently acquire HBV/HCV data.

**Statistical analyses.** The annual and average age- and sex-specific incidence rates for the period 1999-2003 were calculated using the number of HCC cases as the numerator and age-sex-specific population data from Egypt as the denominator. Univariate analyses were used to develop a descriptive profile for cases, using demographic indicators and information regarding the case's geographic location of residence at the time of diagnosis. Interannual variation in number of cases during the five year period in Gharbiah and in individual districts was examined using chi-square



analyses. Rate ratios (RR) were calculated to examine the differences among the eight districts of Gharbiah. We also calculated and compared the incidence rates for HCC among cases residing in urban and rural regions of Gharbiah.

We also compared HCC incidence rates for the years 1999-2003 in Gharbiah to those for 1999-2002 from United States SEER.<sup>23</sup> Age-specific incidence rates for Gharbiah and the United States were adjusted to the World million, and age-adjusted rates of the two regions were compared.<sup>24</sup> Rate ratio (RR) estimates and 95% confidence intervals (CI) were used to estimate age-specific incidence variation between Gharbiah and the United States. All statistical operations were conducted using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC. 2006).

**Ethical Oversight.** Our study protocol was approved by Internal Review Boards at the University of Michigan and the registry ethical committee in Egypt.

## RESULTS

**Incidence and descriptive features of HCC.** The number of HCC cases registered in the GPCR from 1999 through 2003 was fairly stable over the five-year study period, with 200-250 cases per year (Table 1). Cyto-histopathological confirmation of the primary tumor was the basis of diagnosis in 33.5% of cases. Non-microscopic (laboratory marker/radiological) diagnosis was the basis of diagnosis in 43.2% of cases. Approximately 23% of cases were diagnosed by death certificate only. The majority of cases (96.5%) were aged 40 years and older. Of the 1,186 HCC cases, 949 (80%) were males and 237 (20%) were females. Males had consistently higher incidence rates than females for all age groups above 40 years (Table 2).

**Geographic patterns in incidence among districts.** Geographically, 1,062 cases (89.5%) reported being born in Gharbiah, supporting the notion that the population in this region is fairly stable and suggesting that relevant exposures largely occurred in Gharbiah. The eight districts varied considerably in population, representing the following percentages of the total provincial population: Tanta - 23.9%, El Mehalla - 22.6%, Kafr El Zayat - 8.7%, Zefta - 9.7%, El Santa - 9.2%, Samanoud - 7.4%, Kotour - 7.2%, Basyoon - 6.3%.<sup>20</sup> In addition to being the most densely populated districts, Tanta and El Mehalla jointly account for approximately 56.4% of the cases in the registry. The remaining six districts, however, did not all contribute cases in relative proportion to their population densities. Interannual variation in number of cases during the five year period in Gharbiah and in individual districts was examined using chi-square analyses. No significant differences were found except in Zefta ( $P = 0.01$ ; not shown), which had more cases diagnosed in 2000 than in any other year.

A similar proportion of cases came from rural and urban regions of the governorate, accounting for 50.7% and 49.3% respectively. However, mean age-adjusted incidence rates were greater for cases residing in urban (15.8/100,000PY) than in rural (8.6/100,000PY) areas. Urban residents had an incidence rate 1.84 times greater than that among rural residents (95% CI 1.42, 3.13).

The mean age-adjusted incidence rates for HCC in Gharbiah and its eight districts for males, females, and the total population (1999-2003) also varied (Table 3; Figure 2.2). Chi-square analysis of the HCC incidence rates of the 8 districts showed statistically significant variation ( $p < 0.0001$ ), largely the result of variation among males ( $P < 0.0001$ ). Overall, Kotour had the highest incidence rates among males and the total

population (24.1/100,000PY and 12.9/100,000PY respectively), with Samanoud having the lowest rates (10.3/100,000PY and 6.1/100,000PY respectively). Incidence rates among females showed a different distribution, with the highest occurring in Tanta and El Mehalla (5.8/100,000PY) and the lowest in Zefta (1.5/100,000PY). Statistically significant differences were not observed among females, likely a result of small sample size and insufficient power.

The RRs for HCC incidence rates among the eight districts varied considerably (Table 4). Among the largest RRs were those of Kotour compared with Samanoud (2.34 times greater) and Kotour compared with Zefta (1.94 times greater). People in Tanta had 1.94 times the rate of HCC as those in Samanoud. Other significant rate ratios varied between 1.29 and 1.85.

#### **Comparisons between incidence rates in Gharbiah versus the United States.**

Our results showed that the overall age-adjusted HCC incidence rate in Gharbiah (10.6/100,000) is significantly higher than the rate observed in the United States (3.0/100,000), with a rate ratio of 3.53 (95%CI 3.11, 4.67). Incidence rates among males were significantly higher than those among females in both Egypt and the United States. Males in Egypt had incidence rates 4.3 times higher than females (95%CI 3.11, 5.66), and males in the United States had rates 3.3 times higher than females (95%CI 3.16, 4.34). In addition, age-specific incidence rates were significantly higher in Egypt compared to the United States for all age groups older than 40 years (Table 5). Significant differences in rates among the younger age groups were not apparent. These age patterns remained consistent when examining males and females separately.

## DISCUSSION

Analyses from this population-based cancer registry were based on HCC cases that appear to be largely complete and highly representative. Case data from the GPCR were obtained from virtually all sources in the province, and there is no reason why agencies from various sectors would not have participated in the registry. GPCR is financially and scientifically supported by the United States National Cancer Institute, and has quality assurance from SEER and the International Association of Cancer Registries. Standard procedures for training registrars and for data collection, processing, and transmission enhanced the accuracy of data. In addition, the population-based structure improves our ability to draw conclusions about the entire province from the study results. For these reasons, our study is unlike other hospital- or clinic-based reports that have previously appeared for HCC in Egypt, as it includes essentially all cases in the study area. Thus, results represent a high degree of internal and external validity. Furthermore, data on the vast majority of cases included complete demographic and geographic information. It is reassuring that the number of HCC cases reported per year did not vary during the five years of this study. Therefore, the case data that we analyzed should be considered valid for the populations at risk, and the results generalizable at least to the people of Gharbiah Province.

At first glance, the approximately 23% of cases diagnosed using only the death certificate might seem overly high. This percentage, however, is not unusually high for liver cancer.<sup>1</sup> Due to difficulty in diagnosing HCC even in developed countries, many cases are not diagnosed until death.<sup>1,25</sup> This is more a limitation of current technology than specific registry methods. In fact, the overall proportion of cancers diagnosed by

death certificate only in the Gharbiah registry is only 6-7%. It is also unlikely that missed cases would significantly alter our findings. The GPCR has a reported coverage rate of greater than 90% for Gharbiah province.<sup>22</sup> The number of cases could be underestimated due to HCC being misclassified as cirrhosis, but there is no reason to believe that this underestimation is biased in any way. Access to healthcare is similar across the province, and there is nothing to suggest that physicians diagnose differently in any systematic way.

This study presents various findings that are similar to, but also different from, those that have previously addressed HCC in the Middle Eastern region. As has been shown consistently in other studies, male incidence was considerably greater than that for females. Worldwide, estimates show males to be 1.3 to 3.6 times as likely to develop HCC as females.<sup>1</sup> In high-risk countries, sex ratios tend to be higher, and this is demonstrated here by the lower rate ratio seen in the United States versus Egypt. A satisfactory biologic interpretation has yet to be demonstrated. Several hypotheses have been investigated, however, including the interaction of sex hormones with HBV, leading to a different natural history depending on the sex of the individual, or the impact of sex specific exposures.<sup>26-27</sup>

Interestingly, the incidence rate of HCC in Gharbiah was 3.5 times higher than that reported in the United States. Such results were seen in the overall age-adjusted incidence rates, as well as the age-specific rates in age groups older than 40 years, among both males and females. This finding expands upon reports in the United States NCI and MECC publications for a shorter period, 1999-2001.<sup>19</sup> Our similar findings for a longer

period suggest that variation in rates truly reflect different risk factor profiles of these two populations, warranting further prospective studies.

The most notable finding of this study, however, was the statistically significant geographic variation in incidence of HCC among districts within Gharbiah province. Incidence ranged from 12.9/100,000PY (Kotour) to less than half that rate at 6.1/100,000PY (Samanoud). Districts were similar with respect to age distribution and sex-ratios, suggesting that the at-risk populations were fairly homogeneous. Consequently, this observed heterogeneity is likely attributable to variation in local risk factors that future studies may investigate.

Another interesting finding demonstrated HCC incidence among urban individuals was nearly twice that of rural residents. Several studies have postulated that the HCV epidemic in Egypt has disproportionately affected rural populations, which should be reflected in the distribution of HCC cases.<sup>28-29</sup> The HCV epidemic in Egypt is unique. Egypt developed the world's highest rates of HCV infection over a short period of time, largely due to a massive public health campaign. The vast majority of infections among individuals age 30 years and older can be explained by parenteral anti-Schistosomal therapy (PAT) and other iatrogenic exposures.<sup>29-32</sup> The anti-Schistosomiasis campaign extended from the 1950's through the 1980's, with peak transmission probably occurring during the 1960's and 1970's. In 1982, praziquantal, an oral treatment for Schistosomiasis, was introduced, and use of PAT declined. Since Schistosomiasis was a greater problem in rural regions, these populations were more affected by the PAT campaign, and, consequently, HCV transmission.<sup>30,32</sup> With iatrogenic infections nearly eliminated, person-to-person transmission is presently the

dominant route, which should preserve the urban/rural disparity. Higher rates of HCV infection should manifest as higher rates of HCC.

The higher HCC incidence among urban residents could represent better access to medical facilities, resulting in an underestimate of HCC in rural populations. It is also possible that the peak of the cohort with PAT-related HCV transmission has not yet matured, suggesting the incidence of HCC due to HCV is still increasing and competing with HBV-related HCC incidence. This could mean a shift in burden from urban to rural regions is currently underway and will be discernible in the near future. It should also be noted that disease progression from HCV to HCC may take up to 20 years and the incidence of HCC can increase in both urban and rural area in Egypt over the next years. Risk factors of most importance in this region include hepatitis B virus, hepatitis C virus, and dietary aflatoxins. Unfortunately, we were unable to acquire data on the HBV and HCV status of our cases, as testing is not standard protocol for patient intake and management at the GPCR. Due to the retrospective nature of our study, we were unable to independently test cases, as they were all deceased at the time of study initiation. Nevertheless, several studies in Egypt have documented general prevalence of HCV and HBV throughout the country, and we can use these results to help guide our inference.

Several small-scale hospital-based studies have been conducted to examine the etiologic significance of the primary HCC risk factors in Egypt, though it should be noted that they have not assessed geographic variation. These studies have shown the increasing importance of HCV in liver cancer, estimated to account for 40-50% of liver cancer cases, and the declining influence of HBV and HBV/HCV infection, 25% and 15% respectively.<sup>10-12</sup> These studies also have documented an overall increase in the

relative frequency of liver cancer in Egypt, from approximately 4.0% in 1993 to 7.3% in 2003.<sup>10</sup> Such increase in liver cancer is generally attributed to HCV.

Since chronic HCV does not typically lead to carcinogenesis for 10-30 years following infection, the rates of liver cancer can be expected to continue increasing until the cohort of PAT-related infected individuals has worked its way through.<sup>10,29</sup> This suggests that the true burden of liver cancer in Egypt has yet to be realized.

Data on schistosomiasis infection in these cases were unavailable. While the role of hepatic schistosomiasis has long been controversial, the prevailing view today is that it has limited influence in the etiology of HCC in Egypt. Epidemiological studies of HCC clearly identified HBV, HCV or HBV/HCV co-infection as important, but schistosomiasis could not be identified as a statistically significant independent risk factor.<sup>19,33</sup> For these reasons, we do not feel the lack of schistosomiasis infection data influence our conclusions.

As in many developing countries, Egypt is undergoing an epidemiologic transition. With increasing urbanization, smoking rates, environmental exposures, and aging, in addition to the maturing HCV epidemic, it is likely that HCC will continue to rise for the next few decades. Therefore, further studies to assess the magnitude and risk factors of HCC in Egypt and other developing countries seem warranted. Our study produced important preliminary insights that can be used to develop more refined, prospective analyses of HCC risk in Egypt. Ongoing collaborations are building upon these preliminary findings to develop studies that will examine cases from the point of intake and acquire HBV/HCV test results to expand our inference regarding the relative importance of certain risk factors on HCC in Egypt. Such analysis should help define the



complex etiology of HCC, enabling policy makers to create targeted, more efficient prevention programs.

**Table 2.1.** Characteristics of people diagnosed with hepatocellular carcinoma in Gharbiah, Egypt during 1999 through 2003.

Descriptive Feature		Number of Cases	% of Cases
Sex	Male	949	80.0
	Female	237	20.0
Age	0-39	41	3.5
	40-49	178	15.0
	50-59	411	34.7
	60-69	358	30.2
	70-79	180	15.2
	80+	18	1.5
	Religion	Muslim	1162
Christian		24	2.0
Governorate of Birth*	Gharbiah	1062	89.5
	Other	123	10.5
Region of Residence	Tanta	362	30.5
	El Mehalla	307	25.9
	Kafr El Zayat	113	9.5
	Kotour	105	8.9
	El Santa	93	7.8
	Zefta	81	6.8
	Basyoon	75	6.3
	Samanoud	50	4.2
	Urban/Rural Distribution	Urban	585
Rural		601	50.7
Year of Diagnosis	1999	212	17.9
	2000	242	20.4
	2001	236	19.9
	2002	249	21.0
	2003	247	20.8
Basis of Diagnosis	Microscopic	397	33.5
	Non-Microscopic	512	43.2
	Death Certificate Only	277	23.4

\* N = 1,185; missing birth place information for one patient

**Table 2.2** Comparison of mean age-specific incidence rates of hepatocellular carcinoma for males and females in Gharbiah, Egypt during 1999 through 2003.

<b>Age group (years)</b>	<b>Male Cases (#)</b>	<b>Male IR per 100,000PY</b>	<b>Female Cases (#)</b>	<b>Female IR per 100,000PY</b>	<b>RR males vs. females (95% CI)</b>
0-24	0.6	0.4	0.4	0.3	1.42 (0.03, 56.1)
25-29	0.6	0.5	0.2	0.1	3.30 (0.02, 161.0)
30-34	1.0	0.8	1.4	1.1	0.73 (0.1, 13.8)
35-39	2.2	1.8	1.6	1.3	1.42 (0.2, 9.1)
40-44	10.0	9.4	2.2	2.1	4.40 (1.0, 8.7)
45-49	19.4	21.0	4.0	4.8	4.37 (1.5, 7.3)
50-54	30.4	48.1	7.2	11.3	4.24 (1.9, 6.5)
55-59	35.8	73.8	8.8	19.2	3.85 (1.8, 5.9)
60-64	36.0	86.0	6.4	13.9	6.19 (2.7, 8.5)
65-69	23.2	75.6	6.0	19.7	3.84 (1.6, 6.3)
70-74	19.4	101.5	6.8	31.5	3.22 (1.3, 5.6)
75 +	14.8	105.2	2.2	13.6	7.76 (1.9, 11.9)

**Table 2.3.** Mean age-adjusted incidence rates per million for hepatocellular carcinoma in Gharbiah, Egypt and its districts per 100,000 PY (1999 through 2003). Rates standardized to the world million.

Region	Male Incidence Rate	Female Incidence Rate	Total Incidence Rate
Gharbiah (all)	16.7	4.0	10.6
Kotour	24.1	3.3	12.9
Tanta	19.9	5.8	12.3
El Mehalla	18.4	5.8	12.1
Kafr El Zayat	19.0	3.7	11.1
Basyoon	17.9	3.2	10.4
El Santa	15.4	2.6	8.8
Zefta	12.5	1.5	6.9
Samanoud	10.3	2.2	6.1

**Table 2.4.** Mean incidence rate ratios for hepatocellular carcinoma among the districts of Gharbiah per 100,000 PY (1999-2003). Rate Ratio = (Row District / Column District). 95% confidence limits are given in parentheses.

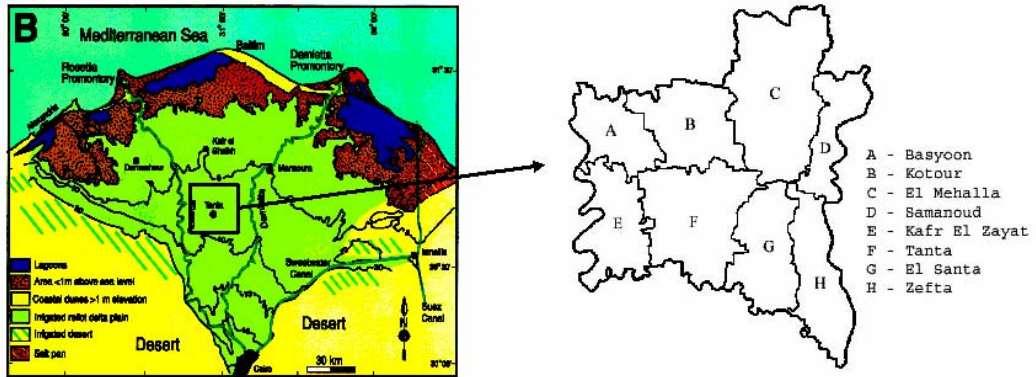
RR=R↓/C→	Kotour	Tanta	El Mehalla	Kafr El Zayat	Basyoon	El Santa	Zefta	Samanoud
Kotour	---	NS	1.31 (1.03, 1.67)	NS	NS	1.57 (1.16, 2.12)	1.94 (1.42, 2.64)	2.34 (1.61, 3.40)
Tanta		---	NS	NS	NS	1.29 (1.01, 1.66)	1.60 (1.23, 2.07)	1.94 (1.39, 2.70)
El Mehalla			---	NS	NS	NS	1.48 (1.13, 1.92)	1.79 (1.28, 2.50)
Kafr El Zayat				---	NS	NS	1.53 (1.12, 2.08)	1.85 (1.28, 2.68)
Basyoon					---	NS	1.43 (1.02, 2.01)	1.74 (1.17, 2.58)
El Santa						---	NS	1.50 (1.02, 2.19)
Zefta							---	NS
Samanoud								---

**Table 2.5.** Age-specific Egyptian HCC incidence rates for 1999 through 2003 compared to SEER data (1999 through 2002) of the United States. Rates standardized to the World Million.

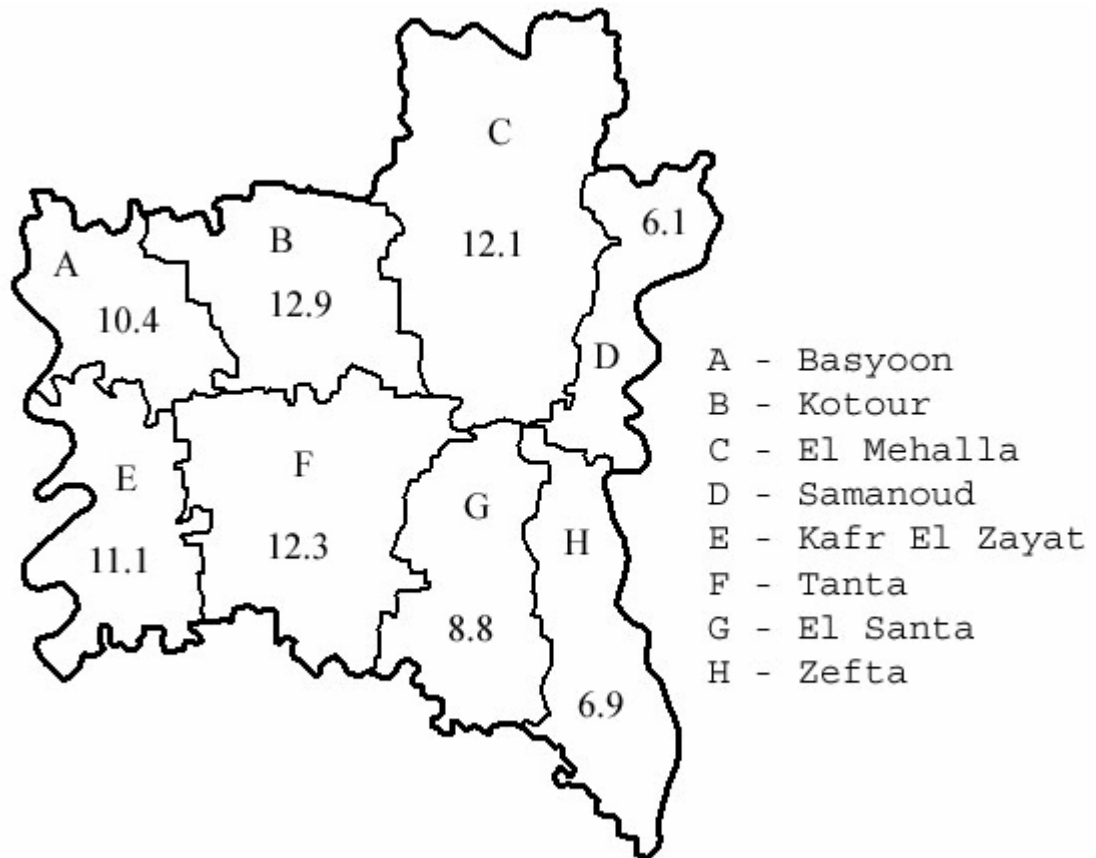
<b>Age Category</b>	<b>Gharbiah IR per 100,000PY</b>	<b>SEER IR per 100,000PY</b>	<b>RR Gharbiah vs. SEER</b>	<b>95% CI</b>
0-24*	---	---	---	---
25-29	0.3	0.3	1.10	0.12, 10.21
30-34	1.0	0.5	2.09	0.57, 7.66
35-39	1.6	0.6	2.47	0.89, 6.87
40-44	6.1	1.8	3.37	1.90, 5.96
45-49	13.9	5.2	2.70	1.79, 4.06
50-54	31.2	7.3	4.28	3.10, 5.93
55-59	49.7	8.9	5.58	4.14, 7.53
60-64	51.1	11.9	4.28	3.10, 5.93
65-69	50.1	16.5	3.05	2.11, 4.39
70-74	67.8	19.6	3.46	2.35, 5.08
75 +	39.1	18.3	2.14	1.19, 3.85
Total	10.6	3.0	3.53	3.11, 4.02

\* Complete SEER data unavailable.

**Figure 2.1.** Northern Egypt (Nile Delta), Gharbiah Province, and its eight districts. (Map: [www.meccegypt.org](http://www.meccegypt.org)).



**Figure 2.2.** Mean age-adjusted incidence rates for hepatocellular carcinoma in the eight districts of Gharbiah (1999-2003), standardized to the World million (Parkin et al, 1997). (Map: [www.meccegypt.org](http://www.meccegypt.org)).





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## **CHAPTER III**

### **SPATIAL ANALYSIS OF HEPATOCELLULAR CARCINOMA IN EGYPT: CLUSTERS LINKED TO SQUAMOUS CELL CARCINOMA OF THE BLADDER**

#### **INTRODUCTION**

Primary liver cancer (PLC) ranks fifth worldwide in incidence of cancers, with an estimated 0.5 to 1 million cases diagnosed each year, and third in cancer mortality.<sup>1-2</sup> In developing countries, however, it often ranks as the leading cause of cancer incidence and mortality among males. Hepatocellular carcinoma (HCC) is by far the dominant PLC malignancy and has the greatest relevance to public health. Reports of rising HCC incidence in many countries throughout the world frequently cite increased burden of hepatitis C virus (HCV) and synergistic interactions among risk factors as contributing causes.<sup>1-2</sup> Risks for HCC are most often associated with hepatitis B virus (HBV), hepatitis C virus (HCV), dietary aflatoxin exposure, and chronic alcohol consumption.<sup>1</sup> Hepatocellular carcinoma in Egypt is currently undergoing a shift in the relative importance of HBV and HCV as primary risk factors. Prior to implementing HBV vaccine in the 1980s, chronic infection with HBV was widespread and considered the dominant etiologic factor in HCC development.<sup>3</sup> Childhood HBV immunization in Egypt today is estimated at 95% to 100%, thus making it likely that HBV-related HCC will steadily decline over the next few decades.<sup>4-5</sup> Despite this major public health

achievement, HCC continues to be the second highest cancer incidence and mortality among Egyptian men. Hospital-based studies from Egypt have even reported an overall increase in the relative frequency of all liver-related cancers in Egypt (>95% as HCC), from approximately 4.0% in 1993 to 7.3% in 2003.<sup>6-8</sup> Recently, the Middle East Cancer Consortium reported that the incidence rate among males was seven times greater than the next highest rate (among Israeli Jews) and more than three times that reported in the United States Surveillance Epidemiology and End Results (SEER) summary.<sup>9</sup>

The future of HCC in Egypt will be a legacy of its unique HCV epidemic. Egypt boasts the highest prevalence of hepatitis C virus (HCV) in the world.<sup>10-11</sup> Egypt developed the world's highest rates of HCV infection over a remarkably short period of time, with a prevalence rate of up to 20%.<sup>10-11</sup> Most infections among individuals age 30 years and older occurred through widespread use of inadequately sterilized needles for administering parenteral anti-schistosomal therapy (PAT), and through other iatrogenic exposures.<sup>12-15</sup> The anti-schistosomiasis campaign took place during the 1950s through the 1980s, with peak HCV transmission probably occurring during the 1960s and 1970s.<sup>13,15</sup> An equal number of people were infected with HBV and HCV during the PAT campaign; however, HBV only caused chronic infections in approximately 5% of infected individuals, whereas chronic HCV infection developed in 70% to 80%.<sup>13,15</sup> This is a function of the natural history of HBV, where the probability of developing chronic infection decreases with age.<sup>2-3</sup> Since most individuals receiving PAT were 10-15 years or older, they were at lower risk for developing persistent HBV infection.<sup>15</sup>

Despite the introduction of an oral treatment in 1982 followed by the elimination of the PAT program, HCV transmission persists. Hepatitis C virus infection is assuming

increasing importance in the etiology of liver cancer in Egypt, and is estimated to account for 40 to 50% of cases, overtaking the influence of HBV and HBV/HCV infection (25% and 15% respectively).<sup>6-8</sup>

Geographic variation in HCC incidence in Egypt has not been studied. Some reports have made simple comparisons between upper and lower Egypt or between urban and rural populations with respect to presumed exposures, but results have been limited.<sup>16-19</sup> More detailed, formal spatial analysis of HCC could be instrumental in identifying areas of high risk and assisting health policy makers to more efficiently allocate resources. Quantifying the current spatial distribution of HCC in Egypt is important to understanding the ongoing transition of primary risk factors. Current distributional data offer a baseline documenting the beginning wave of HCC cases resulting from PAT treatment. Future surveillance can then track spatial trends in HCC incidence, potentially revealing the underlying dynamics of HCV transmission.

Accordingly, we analyzed data collected from 1999 through 2003 by the Gharbiah Population-based Cancer Registry (GPCR) in Gharbiah Province, Egypt in an effort to determine whether spatial variation in HCC incidence exists and whether such variation could be linked to certain environmental factors. We specifically looked at factories and irrigation drainage canals, which could indicate sources of environmental pollutants. We also examined the spatial distribution of squamous cell carcinoma of the bladder in relation to that of HCC.

Despite extensive searches and inquiries, we were unable to identify any sources of PAT distribution data, necessitating the use of a proxy measure. Squamous cell carcinoma of the bladder (SCC-B) is most often attributed to chronic schistosomiasis

infection. In Egypt, chronic schistosomiasis has been estimated to account for more than 95% of all SCC-B cases.<sup>20-21</sup> We hypothesized that spatial variation in both cancers might be similar within this registry cohort, because the cohort represents people heavily burdened by schistosomiasis also previously exposed to the PAT campaign. Areas with higher rates of schistosomiasis may have received more exposures to HCV through PAT treatment, resulting in higher rates of HCC (see Figure 3.1 for a conceptual model). These same high-schistosomiasis areas should also have more SCC-B. An overlap in spatial patterns of the two cancers would suggest pathways involving schistosomiasis, PAT, and HCV for HCC, and schistosomiasis for SCC-B.

## METHODS

**Characteristics of the Study Region.** Gharbiah Province is located in the middle of the Nile delta, approximately 100 km north of Cairo (Figure 3.2).<sup>22</sup> Population density is tenth among the 27 Provinces in Egypt with 4.2 million people (5.7% of Egypt's population). The age and sex structure do not differ significantly from the national average. The male:female ratio is 1.02:1, and 47% of the population is younger than 20 years of age.<sup>23</sup> Gharbiah is considered an "urban-rural" Province by the Central Agency for Public Mobilization and Statistics (CAPMAS), with 31% of the population in urban areas and 69% in rural areas.<sup>23</sup> The Province has 317 villages/cities located within 8 districts, each with a capital city. The capital of Gharbiah Province is the city of Tanta, which serves as the headquarters for the GPCR.

**Gharbiah Population-Based Cancer Registry Data.** The GPCR was established in 1998 as part of the Middle East Cancer Consortium (MECC) Joint Cancer

Registration Project, and it is affiliated with the Egyptian Ministry of Health and Population.<sup>24</sup> The registry uses active case finding, including investigating hospitals in other provinces for cases that reside in Gharbiah. Information is compiled from medical records (government and private hospitals and clinics), death certificates, and histopathology laboratories and radiology clinics. Registrars have been trained by the International Agency for Research on Cancer (IARC) and Emory University School of Public Health in Atlanta, USA, and they conduct regular visits with each collaborating center to obtain case data and review its quality according to IARC's standard review process. The registry conducts routine data cleaning by standardizing field entries and removing duplicate entries. Data quality was assessed in 2002 for completeness of coverage and reliability of registration. In addition to computer checks through CanReg software, external auditing was done by Emory University staff in 2002. Coverage was found to exceed 90% of the Gharbiah population.<sup>25</sup>

We obtained data for all liver cancer cases diagnosed and included in the registry from January 1, 1999 through December 31, 2003 (n = 1,309). We restricted our case definition to those with an ICD-10 topology code of 22.0 and a morphology of HCC. We also obtained data for all bladder cancer cases diagnosed and included in the registry from January 1, 1999 through December 31, 2002 (n = 1,209); review of 2003 data was not final at the time of data collection. For bladder cancer, our case definition was limited to those with an ICD-10 topology code of 67.0 and a morphology of squamous cell carcinoma (n = 256). For both cancers, cases were excluded if they did not satisfy both elements of the case definition or if they had inconsistencies or missing values that



would not be resolved by examining medical records. This selection process resulted in 1,186 (92.4%) HCC cases and 256 (100%) SCC-B cases that were analyzed.

Variables obtained for each case included: registry patient number, age at diagnosis, date of birth, gender, usual residential address, date of diagnosis, basis of diagnosis, primary site code (ICD-O-3), morphology codes, histology type, behavior, grade/differentiation/cell indicator, and summary stage at diagnosis. Additional variables included: family history, marital status, smoking history, religion, occupation, and treatment data. Unfortunately, data on patient HBV and HCV status were unavailable, as these tests are not routinely done. Because of the retrospective nature of this study, and because most of the cases in our sample are deceased, it was not possible to independently acquire HBV/HCV data.

**Population and Geographic Data.** Population abundances were estimated using annual linear interpolation from the 1996 Egypt census and the 2001 population projections, and then the 2001 and 2005 projections. Census data were available at the village level and provided age- and sex-specific population counts.

Geographic data were obtained after digitizing maps from the Egyptian General Survey Authority. Centroid coordinates (latitude, longitude) were acquired for all villages and factories in the Province. In addition, we calculated the shortest distance between each village centroid and its nearest drainage canal. All mapping and visualization was done using ArcGIS (version 9.1, ESRI, Redlands, CA).

**Statistical analyses: Case and Population Description.** We calculated mean annual age-sex-standardized incidence rates for HCC and SCC-B at the Province, district, and village levels. We performed chi-square analyses to test for heterogeneity in

incidences across districts. We also calculated rate ratios among males and females as well as urban and rural cases. To determine comparability of villages for spatial analysis, we examined village population data for sex ratios and age structure.

**Statistical analyses: Environmental Associations.** We calculated the distance from each village to each factory and from each village to its nearest drainage canal. We performed t-tests and chi-square tests to determine if there were any associations among HCC incidence and distance to factory, factory type, and/or drainage canal. For the factory type analysis, each of the 29 factories was placed in one of the following categories: Textile, Agricultural, Industrial, and Water Treatment.

**Statistical analyses: Spatial Analysis.** Because different spatial analytical methods may identify different spatial patterns, we used two independent cluster detection methods to test our hypothesis that spatial clustering of HCC and SCC-B existed in Gharbiah Province. Different methods with consistent results would increase our confidence in the robustness of our findings. Therefore, we applied a spatial autocorrelation method and a spatial scan method and compared the results of local disease cluster patterns, using village centroid as the unit of analysis. We used GeoDa software (version 0.9.5-I, Urbana-Champaign, IL) to generate Anselin's Local Moran (LISA) test statistics for spatial autocorrelation. The Local Moran statistic identifies locations (villages in this case) with disease rates statistically similar to and dissimilar from their neighbors. The null hypothesis is one of no association in HCC incidence rates among neighboring villages. The alternative hypothesis is that neighboring villages have similar rates, i.e. spatial clustering exists.

Because incidence rates for some health events can be unstable, reflecting sizeable variances, we applied Empirical Bayes (EB) smoothing to our raw HCC disease data. This method adjusts village disease rates with respect to the overall mean of the study area. This typically has its greatest impact on those villages with small population sizes. We assigned spatial weights using the *k*-nearest neighbors method, selecting 9 nearest neighbors after comparing weight results for 2 to 13 nearest neighbors. Weights were consistent for 7 to 11 nearest neighbors. We obtained Monte Carlo p-values based on 9,999 conditional randomizations. We compared results for critical p-values ranging between 0.01 and 0.05. We used an alpha level of 0.03 to limit statistical significance due to multiple comparisons.

SaTScan software (version 6, Boston, MA) was employed to calculate scan statistics using the method described by Kulldorff (1997).<sup>26</sup> This method involves moving a circular window around the study region. At each central position of the window, the radius changes between zero and a user-defined upper limit. For each window, the likelihood of finding the observed and expected number of cases inside the circle given the number of observed and expected cases outside of the circle is calculated. The null hypothesis is that the study region has a homogeneous incidence rate. The alternative hypothesis is that at least one window contains an incidence rate significantly different from the mean Province rate.

In this case, raw HCC data were used so as not to bias the likelihood ratio test statistic. We assumed a Poisson probability disease model (case and population data). We examined the number of clusters detected by the method for the range 1 - 10km. At 7km, the number of clusters stabilized and remained the same through 10km.

Consequently, we selected 7km as the parameter value for our scanning window radius. Reported clusters were limited to those representing 20% or less of the population, though the initial test parameter was set at 50% to avoid bias. As with the Moran test, calculations were based on 9,999 Monte Carlo randomizations. Because the test already accounts for multiple testing, we used an alpha level of 0.05.

To determine if the clusters found for HCC were statistically correlated with those found for SCC-B, we used the results from the spatial scan method. We tested the conditional probability that village K is within 7km of a HCC cluster, given that village K is part of a SCC-B cluster. We organized the data by distance to HCC or SCC-B clusters and identified all villages within 7km of a cluster. We then translated the data into binomial distributions: villages in HCC clusters [0 = no; 1 = yes], village within 7km of SCC-B cluster [0 = no; 1 = yes]. This was done for high and low clusters separately. We then used logistic regression to determine if HCC and SCC-B clusters tend to overlap more than we would expect by chance alone.

**Ethical Oversight.** Our study protocol was approved by Institutional Review Board at the University of Michigan and the registry ethical committee in Egypt.

## RESULTS

**Study population.** From 1999 through 2003, there were 1,186 cases of HCC and 256 cases (through 2002) of SCC-B. Table 3.1 presents the number of villages where the age-standardized incidence rates for HCC and bladder SCC-B are greater than zero. Overall, HCC was found in a greater proportion of villages than SCC-B. For both cancers, Tanta was the district where the greatest proportions of villages have at least one

case (HCC = 90.4%; SCC-B = 48.1%), and Zefra was where the fewest proportion of villages had at least one case (HCC = 41.8%; SCC-B = 16.4%).

The 5-year mean annual age-standardized incidence rate for HCC was 8.80 cases per 100,000 individuals, and the 4-year mean annual age-standardized incidence rate for SCC-B was 1.83 cases per 100,000 individuals. District-specific mean annual age-standardized rates are presented in Table 3.2.<sup>27</sup> Chi-square tests found significant variation in incidence across districts for both cancers (p-values < 0.01; not shown).

Overall, HCC and SCC-B were similar with respect to sex and urban/rural rate ratios. Males had 4.13 (95% CI 3.58, 5.29) times the rate of HCC in females and 1.95 (95% CI 1.50, 3.25) times the rate of SCC-B in females. In addition, urban dwellers had 1.83 (95% CI 1.63, 2.95) times the rate of HCC among rural populations and 1.76 (95% CI 1.35, 3.06) times the rate of SCC-B among rural populations.

Examination of the population data revealed no significant differences among villages with respect to sex ratio and age distribution (not shown).

**Incidence rate maps.** Raw and EB smoothed incidence rates for HCC and SCC-B are presented in Figure 3.3. Villages with HCC are distinguished by breakpoints in incidence rates (per 100,000PY) as follows: zero, 0.1-19.9, 20.0-39.9, 40.0-59.9, and  $\geq 60.0$ . Villages with SCC-B are distinguished by breakpoints in incidence rates (per 100,000PY) as follows: zero, 0.1-4.9, 5.0-9.9, and  $\geq 10.0$ . The EB smoothed maps follow the same classification scheme to illuminate the impact of smoothing. The EB smoothed map for HCC appears to show an overall pattern of higher risk in the western side of the Province and lower risk in the east. There do appear to be areas of higher risk in the central northern and southern regions as well as the southwestern corner. Note that the

process of smoothing rates eliminates the possibility of a village having a rate of 0.0 per 100,000PY.

**Environmental Associations.** All tests for associations between HCC incidence and distance to factory, distance to factory type (Textile, Agricultural, Industrial, and Water Treatment), and distance to nearest drainage canal were not significant (not shown).

**Spatial clustering of HCC cases using GeoDa–Anselin’s Local Moran test (LISA).** Formal analysis using the Local Moran test identified significant HCC clustering in the Province ( $p$ -values  $< 0.03$ ). Three clusters of high risk were identified (Table 3.3; Figure 3.4a). As we saw with the EB smoothed map, there were three primary clusters: the central northern region, the central southern region, and the southwestern region. These correspond to the districts of Kotoor, Tanta, and Kafr El Zayat and their EB smoothed rates ranged: 5.2-7.82, 6.24, and 6.40-11.16 cases per 100,000 individuals respectively. (Note that because Anselin’s Local Moran tests each village against each neighbor individually, there are cases when a cluster may only contain one village.)

In addition, three clusters of low risk were also significant ( $p$ -values  $< 0.03$ ; Table 3.3; Figure 3.4b). These were located in the eastern area of the Province as would be expect based on the EB smoothed map. These clusters correspond to the districts of El Mehalla El Kobra, Zefta, and Santa, and their EB smoothed rates ranged from 4.04-4.52, 3.16-4.88, 2.76-5.14 cases per 100,000 individuals respectively.

**Spatial clustering of SCC-B cases using GeoDa–Anselin’s Local Moran test (LISA).** The Local Moran test identified two high risk (Table 3.3; Figure 3.5a) and three

low risk clusters for SCC-B (Table 3.3; Figure 3.5b). The larger high risk region is also in the central northern region of the Province (Kotoor district) with EB smoothed rates ranging from 2.05-14.43 cases per 100,000 individuals. The other high risk location was at a single point in El Mehalla El Kobra with an EB smoothed rate of 3.53. All significant results reported had p-values < 0.03.

The low risk areas also occurred in the eastern side of the Province save one that was located in the southwest corner (p-values < 0.03). As with HCC, two of these clusters were focused in Zefta and Santa with EB smoothed rates ranging from 0.1-0.48 and 0.05-1.20 cases per 100,000 individuals. The other low risk area is centered in Kafr El Zayat, and the rates range from 0.18-0.93 per 100,000 individuals.

#### **Spatial clustering of HCC cases using SaTScan–spatial scan test statistic.**

Table 3.4 and Figures 3.4c and 3.4d show the results from the spatial scan statistic for HCC. This method located 3 high risk regions and 4 low risk areas (p-values < 0.03). These correspond very closely with the clusters identified with the Local Moran method. The EB smoothed rates for the high clusters ranged: 4.84–11.18, 23.98, and 24.38 per 100,000 individuals (1-3 respectively). The EB smoothed rates for the 4 low clusters ranged: 2.04-4.26, 2.20-4.68, 1.22-3.12, and 1.58-6.76 per 100,000 individuals (1 to 4 respectively).

#### **Spatial clustering of SCC-B cases using SaTScan–spatial scan test statistic.**

As with HCC, the spatial scan results for SCC-B corresponded quite well with those from the Local Moran test statistic. Table 3.4 and Figures 3.5c and 3.5d present the results from the spatial scan statistic for SCC-B. This method located 4 high risk regions and 4 low risk areas (p-values < 0.03). The EB smoothed rates for the high clusters ranged:

42.28, 3.86-11.54, 3.08-6.26, and 4.06 per 100,000 individuals (1 to 4 respectively). The EB smoothed rates for the low clusters ranged: 0.04-0.34, 0.02-0.40, 0.06-0.54, and 0.2-0.44 per 100,000 individuals (1 to 4 respectively).

**Spatial clustering of HCC and SCC-B cases.** Figure 3.6 displays the results from both the HCC and SCC-B cluster analyses together as layers, separated by high or low cluster as well as by type of statistical test used. The most striking similarity that was seen among high-risk HCC and SCC-B clusters was the cluster in the central northern region of the Province (Kotoor district). Both the Local Moran and spatial scan methods located clusters in that region, and they clearly overlap. The scan method also located a high-risk cluster for HCC near a cluster for SCC-B in the central southern region of the Province. There are a few other relatively small clusters for each cancer that do not overlap. The dominant high-risk cluster for both cancers occurred in the same location.

Low-risk clusters also showed a great deal of similarity between these two cancers. Both have several clusters in the eastern region of the province. In the southeastern region, corresponding with districts Zefta and Santa, there is significant overlap suggested by both methods of analysis. The scan statistic also suggests some overlap may exist in the northeastern region, which corresponds to the district of El Mehalla El Kobra. Among low risk clusters, only one SCC-B cluster did not overlap well with HCC clusters, in the southwestern region of the Province. This is actually located near a high-risk cluster for HCC.

**Correlation between HCC and SCC-B clusters.** The logistic regression models we used were as follows:



(1) Village in a SCC-B low cluster = Village within 7km of a HCC low cluster;

(2) Village in a SCC-B high cluster = Village within 7km of a HCC high cluster

We found low clusters to be highly correlated with one another ( $P = 0.0010$ ), and high clusters to be marginally correlated with one another ( $P = 0.0692$ ).

## DISCUSSION

**Data Quality.** Our study is unique in comparison to other hospital- and clinic-based reports that have previously been published for HCC in Egypt, as our analyses were based on HCC and squamous cell carcinoma of the bladder cases from a population-based cancer registry and appear to be largely complete and highly representative. GPCR is scientifically and financially supported by the United States National Cancer Institute, and it has quality assurance from US-SEER and the International Association of Cancer Registries. Standard procedures for training registrars and for data collection, processing, and transmission enhanced the accuracy of data. In addition, the population-based structure improves our ability to draw conclusions about the entire province from the study results.

Our results represent a high degree of internal and external validity. Data on the vast majority of cases included complete demographic and geographic information. There was little evidence of interannual variation in case reporting for either cancer for our study period, which was particularly reassuring. In addition, external review of the data collected by the GPCR has found its coverage to exceed 90%, improving our confidence in accurate numerator estimates.<sup>25</sup> Though it is possible the number of cases could be underestimated due to misclassification, there is no reason to believe that this

underestimation is biased in any significant way. Access to healthcare is similar across the province, and there is nothing to suggest that physicians diagnose differently in any systematic way. Therefore, we consider the case data that we analyzed to be valid for the populations at risk, and the results to be generalizable at least to the people of Gharbiah Province.

Denominator estimates were based on census reports from 1996 and published census projections for 2001 and 2005. We believe it was reasonable to calculate population estimates using linear interpolation, as this is a fairly stable population, experiencing the majority of its change through birth and death rates (low population mobility). Data were available at the village level and age- and sex-specific values were provided. We were unable to detect any significant differences between villages and the Province in aggregate with respect to age and sex distributions, suggesting that villages could be compared without serious concern for compromising our conclusions due to these important confounding variables. We are confident that this increases the accuracy of our calculations, since both numerator and denominator data were available at the same high level of resolution.

In addition, we based our spatial data on maps published by the Egyptian General Survey Authority, which followed international guidelines in the creation of their maps. A brief comparison of calculated point coordinates with those obtained from a handheld GPS unit for the same location suggested the maps were internally consistent and geographically accurate.

**Geo-spatial Results.** Significant variation among district-level incidence rates laid the foundation for more formal spatial analyses to test the hypothesis that HCC

spatially clusters in Gharbiah Province. We applied two cluster detection methods that generate test statistics through independent means. Both methods detected significant high- and low-risk clusters that were spatially similar, suggesting our results are indeed robust. When combined with the fact that this population is quite stable and relatively homogeneous in terms of confounders, our findings suggest an underlying clustering of primary HCC risk factors.

Risk factors of most importance in this region include hepatitis B virus, hepatitis C virus, and dietary aflatoxins. Unfortunately, data on these risk factors were unavailable for our cases. Testing for HBV and HCV is not standard protocol for intake at the GPCR, and a review of external medical records for a sample of cases revealed less than 5% to have any test results reported. This is understandable since most infected individuals do not experience symptoms and would have no reason to seek testing. Because our study was retrospective, we were unable to independently acquire risk factor data, as the cases were all deceased at the time of study initiation.

Several studies in Egypt have documented general prevalence of these risk factors throughout the country, and we can use these results to help guide our inference. Reports concerning HBV have suggested a relatively homogeneous geographic distribution of infection, with overall declining rates due to the successful implementation of the HBV vaccine.<sup>4-5</sup> Studies examining HCV have shown higher levels of infection among residents in Lower Egypt versus those in Upper Egypt.<sup>6-7</sup> Higher levels of HCV have also been observed in rural regions as opposed to urban areas.<sup>6-7</sup> Several studies have suggested that HCC is increasingly associated with HCV, and this trend is expected to continue as HBV rates decline.<sup>6-8</sup>

Few studies in Egypt have fully measured the presence of aflatoxins and their impact on liver disease there. Recent studies have documented p53 mutations associated with aflatoxin B(1) among HCC cases, as well as evidence of aflatoxin exposure in the serum of certain rural populations.<sup>16-17</sup>

In addition to HBV, HCV and aflatoxins, other risk factors have been linked to HCC, including chronic alcohol consumption and hemochromatosis. Although alcohol plays a major role in the etiology of HCC in many countries, it was not considered in this study because the prevalence of alcohol consumption in Egypt is extremely low.<sup>18-19</sup> Hemochromatosis also was not considered in this study, because in Egypt it is extremely rare.<sup>28-29</sup>

There is also increasing interest in the role environmental contaminants may play in the development of HCC in Egypt. Studies have shown exposure to pesticides and other chemicals to be associated with HCC in Egypt, often in conjunction with HBV and/or HCV infection.<sup>17-18</sup> It is possible that these exposures may result in an additive interaction and subsequent increase in HCC risk.<sup>18</sup>

To address this concern in an exploratory way, we examined the relationship between village-level HCC incidence and distance to factories and drainage canals. Factories can produce a great deal of contaminants that may be present in the air and water of the surrounding regions. It is also likely that employees of the factories reside in nearby villages. Drainage canals are another potentially point source for environmental contaminants. These waterways receive the vast majority of water-related factory waste as well as any agricultural runoff (pesticides, etc). We were unable to find any association between incidence and distance to possible environmental point sources. This

does not necessarily reject the significance of such factors, but it may suggest that their underlying contribution to HCC burden is obscured by more dominant factors such as HBV and HCV.

The common theme in the discussion of these risk factors is the tendency for them to be found in greater abundance in rural areas, which is contrary to our result of urban cases in Gharbiah having 1.83 times the rate of HCC versus rural cases. The higher HCC incidence among urban residents could represent higher rates of HBV and/or HCV due to iatrogenic transmission during the etiologically relevant time period, which indeed has been documented.<sup>13,15</sup> It is also possible that the peak of the cohort with PAT-related HCV transmission has yet to mature, suggesting the incidence of HCC due to HCV is still increasing. This could mean a shift in burden from urban to rural regions is still ongoing and will be clearer in the near future.

#### **Hepatocellular carcinoma and squamous cell carcinoma of the bladder.**

Schistosomiasis has long been included in the discussion of HCC in Egypt. There is little debate that its treatment was largely responsible for the creation of the HCV epidemic in Egypt, but its relationship with HCC is less clear. Some hypothesize that its chronic presence in the human liver can increase risk for HCC, whether by active promotion or indirectly weakening the liver, reducing its ability to fight HBV and HCV.<sup>12,14</sup> Still others believe schistosomiasis infection itself is not fundamental in liver carcinogenesis, but that the PAT campaign and resulting HCV epidemic is the true culprit.<sup>13</sup>

Due to the limitations of our retrospective data set, we did not attempt to consider the specific mechanisms driving the relationship between schistosomiasis and HCC. Unfortunately, data on schistosomiasis infection as well as receipt of PAT among our

cases were unavailable despite extensive searches and inquiries. Nevertheless, we hypothesized that if such a strong link existed between schistosomiasis and HCC, it should be reflected in the examination of the one type of cancer most associated with schistosomiasis of the urinary bladder: squamous cell carcinoma.

Upon repeating our methods for the cluster analysis of HCC for squamous cell carcinoma, we again found significant high- and low-risk regions with strong agreement across the two methods. In addition we found strong overlap in the primary high-risk cluster for both cancers as well as for all low-risk clusters except one for squamous cell carcinoma. Our test for correlation between spatial clusters of HCC and SCC-B found a strong association among low clusters and a marginal association among high clusters. One possible explanation for why the low-risk clusters were better correlated than high-risk clusters has to do with the interference, though admittedly intended purpose, of PAT. This was a treatment campaign, and it was successful with respect to schistosomiasis. Therefore, the regions with a high burden of schistosomiasis infection that received high frequencies of PAT resulted in a lower risk of chronic schistosomiasis and subsequent SCC-B. Regions of already low risk for schistosomiasis would have received little PAT and, thus, little HCV-related transmission. This scenario would explain the highly significant correlation in low-risk clusters and comparably less significance of high-risk clusters. Data on schistosomiasis infection and PAT distribution for the etiologically relevant time period would be helpful in clarifying this picture, but, unfortunately, these data were not available.

Though we do not suggest that all spatial variation in HCC can be explained by squamous cell carcinoma, the overlap present among the majority of clusters, which was

identified by two separate cluster detection methods, seems hardly a coincidence. Of particular importance is the fact that this cohort of cases represents a population still burdened by schistosomiasis as well as the early wave of individuals affected by the PAT campaign. We believe our findings support the strong connection between schistosomiasis and HCC, but more research is necessary to determine if the association is due to chronic schistosomiasis infection or exposure to PAT treatment.

**Conclusions.** Our study is the first to document spatial clustering of HCC cases in Egypt. Spatial patterns of HCC did not seem to be influenced by environmental point sources of pollutants, but may be linked to the spatial distribution of squamous cell carcinoma of the bladder. Our use of high quality population-based data in addition to rigorous statistical methods suggests our findings are highly robust. Future studies should focus on the dynamics of HCC risk factors in order to better predict regions at greater risk for disease. These initial spatial findings have already stimulated ongoing collaborations to begin developing studies that would include case enrollment and detailed risk factor data collection to improve inference regarding the relative significance of different risk factors in Egypt. We hope these analyses will help untangle the complex etiology of HCC and assist policy makers in generating more efficient prevention and control programs.

**Table 3.1.** Villages in Gharbiah (Total and by District): total number, number with age-standardized incidence rates (ASR) greater than 0.0, and number with ASRs equal to 0.0.

	# Villages	# Villages HCC ASR > 0	% Villages HCC ASR > 0	# Villages SCC-B ASR > 0	% Villages SCC-B ASR > 0
Gharbiah	317	232	73.2	119	37.5
Tanta	52	47	90.4	25	48.1
Mehalla	51	39	76.5	22	43.1
Kafr Zayat	38	29	76.3	15	39.5
Zefta	55	23	41.8	9	16.4
Samanoud	20	18	90.0	8	40.0
Santa	43	27	62.8	16	37.2
Kotoor	30	26	86.7	16	53.3
Basyoon	28	23	82.1	8	28.6



**Table 3.2.** Total age- and sex-adjusted incidence rates (per 100,000PY) for hepatocellular carcinoma (HCC) and squamous cell carcinoma of the bladder (SCC-B) by district. Rates were standardized to the World Million.<sup>25</sup> Chi-square analysis for both HCC and SCC-B showed significant variation across districts ( $P < 0.01$ ).

District	HCC ASR	SCC-B ASR
Gharbiah Province	8.8	1.83
Tanta	11.8	3.20
El Mehalla El Kobra	6.8	2.97
Kafr El-Zayat	9.8	0.65
Zefta	5.9	0.38
Samanoud	6.1	3.13
Santa	8.9	4.07
Kotoor	12.8	10.32
Basyoon	10.4	3.85

**Table 3.3.** HCC and SCC-B clusters identified by the Local Moran’s I statistic. Spatial weights calculated for 9 nearest neighbors. All rates calculated as number of cases per 100,000PY.

Cancer Type	Cluster Risk	No.	Village ID	Raw IR	EB Smoothed IR	Local Moran	<i>p</i> -value
HCC	High Risk	1	151	6.7	6.2	0.2427	0.0300
		2	312	6.7	6.4	0.4029	0.0042
			314	9.2	7.7	0.7665	0.0137
			328	26.5	11.2	2.4658	0.0175
		3	711	9.4	7.8	0.6620	0.0259
			712	5.1	5.2	0.0088	0.0089
			718	7.0	6.3	0.2919	0.0225
			719	8.7	7.1	0.0543	0.0224
			745	10.7	7.6	1.0168	0.0051
		Low Risk	1	244	3.5	4.5	0.1432
	251			3.5	4.0	0.1569	0.0264
	2		238	4.8	4.9	0.0401	0.0119
			246	3.9	4.4	0.1129	0.0250
			265	2.6	3.2	0.2571	0.0258
			272	4.4	4.7	0.0726	0.0140
	3		421	0.0	3.1	0.3673	0.0028
			432	0.0	4.6	0.2039	0.0211
			601	2.7	2.8	0.2764	0.0273
			613	5.0	5.1	0.0053	0.0224
		615	0.0	3.8	0.3157	0.0196	
		617	0.0	3.1	0.3782	0.0277	
		621	0.0	3.4	0.3904	0.0106	

SCC-B	High Risk	1	632	4.2	4.9	0.0557	0.0268		
			701	16.9	14.4	2.3608	0.0292		
			711	2.3	2.1	0.1044	0.0278		
			715	9.3	6.9	0.9179	0.0292		
			722	16.2	8.4	1.3243	0.0296		
			723	9.5	4.8	0.8145	0.0299		
			734	6.1	3.9	0.5981	0.0260		
			2	228	3.8	3.5	0.7005	0.0027	
		Low Risk	1	301	0.5	0.5	0.0920	0.0027	
	310			0.4	0.5	0.0904	0.0104		
	311			0.0	0.3	0.1129	0.0155		
	313			0.5	0.5	0.0907	0.0107		
	319			0.9	0.9	0.0524	0.0244		
	320			0.0	0.2	0.1145	0.0180		
	326			0.0	0.2	0.1076	0.0264		
				2	415	0.0	0.28	0.119092	0.0027
					420	0.0	0.10	0.129472	0.0008
					422	0.0	0.48	0.111987	0.0007
					424	0.0	0.33	0.117180	0.0019
					426	0.0	0.48	0.110472	0.0015
				3	440	0.9	1.0	0.0527	0.0167
					442	1.2	1.2	0.0336	0.0143
					446	0.0	0.4	0.1190	0.0001
					458	0.0	0.1	0.1153	0.0231
					459	1.0	1.0	0.0521	0.0001
				462	0.0	0.1	0.1322	0.0006	
				463	0.0	0.2	0.1112	0.0247	

**Table 3.4.** HCC and SCC-B clusters identified by the scan statistic (Kulldorff method). Scan thresholds: population included is no greater than 20% of the total provincial population, and the circle radius is no greater than 7km. All rates calculated as number of cases per 100,000PY.

Cancer Type	Cluster Type	No.	Reported Cases	Expected Cases	Relative Risk	# Villages	p-value
HCC	High Risk	1	240	119.1	2.27	8	0.0001
		2	25	3.8	6.75	1	0.0001
		3	11	0.8	13.90	1	0.0001
	Low Risk	1	55	109.4	0.48	19	0.0001
		2	0	17.6	N/A	6	0.0001
		3	21	54.4	0.38	5	0.0002
		4	28	71.0	0.38	3	0.0300
SCC-B	High Risk	1	17	0.2	85.69	1	0.0001
		2	22	2.3	10.47	7	0.0001
		3	11	1.5	7.82	2	0.0003
		4	11	2.3	5.03	1	0.0121
	Low Risk	1	0	11.2	0.00	11	0.0009
		2	8	28.6	0.26	8	0.0011
		3	0	8.7	0.00	12	0.0018
		4	4	17.7	0.21	2	0.0266

**Figure 3.1.** Conceptual model showing the pathway by which schistosomiasis may effect hepatitis C virus infection and hepatocellular carcinoma via parenteral antischistosomiasis therapy during the antischistosomiasis campaign in Egypt (1950s – 1980s). The relationship between infectious agents suggests sequelae (cancer) frequency could be measured as indicators of infection burden at the population level. A greater burden of schistosomiasis infection would lead to more frequent exposure to PAT and, thus, a higher probability of HCV transmission.

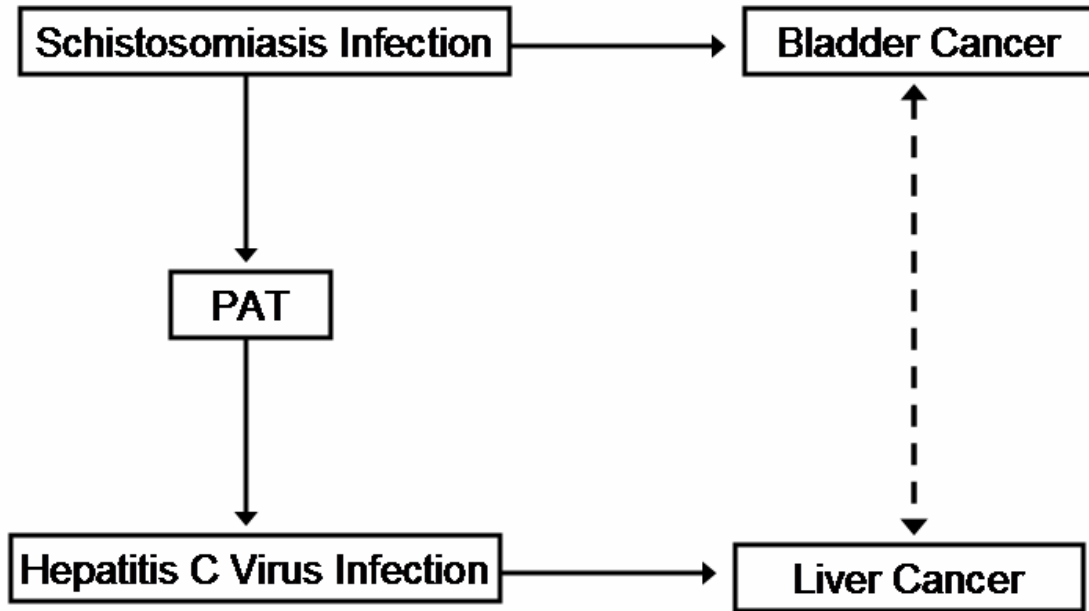
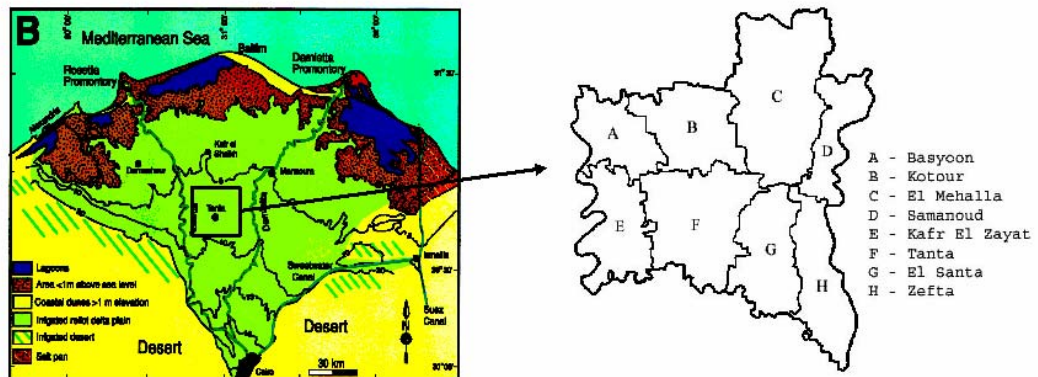


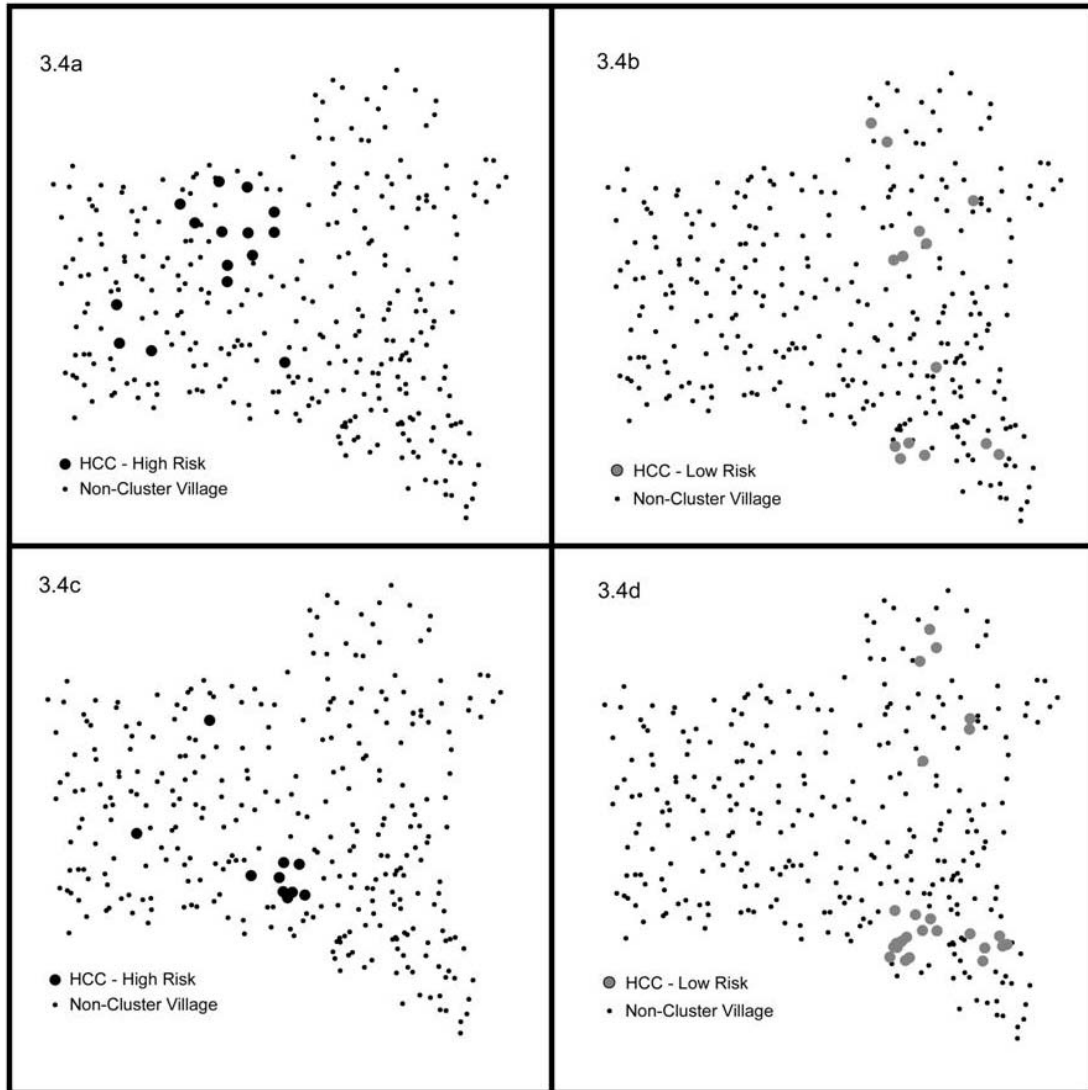
Figure 3.2. Northern Egypt (Nile Delta), Gharbiah Province, and its eight districts.<sup>20</sup>



**Figure 3.3.** Raw and EB smoothed incidence rates (per 100,000PY) for HCC and SCC-B. 3.3a: Raw HCC incidence rates; 3.3b: EB Smoothed HCC incidence rates; 3.3c: Raw SCC-B incidence rates; 3.3d: EB smoothed SCC-B incidence rates.

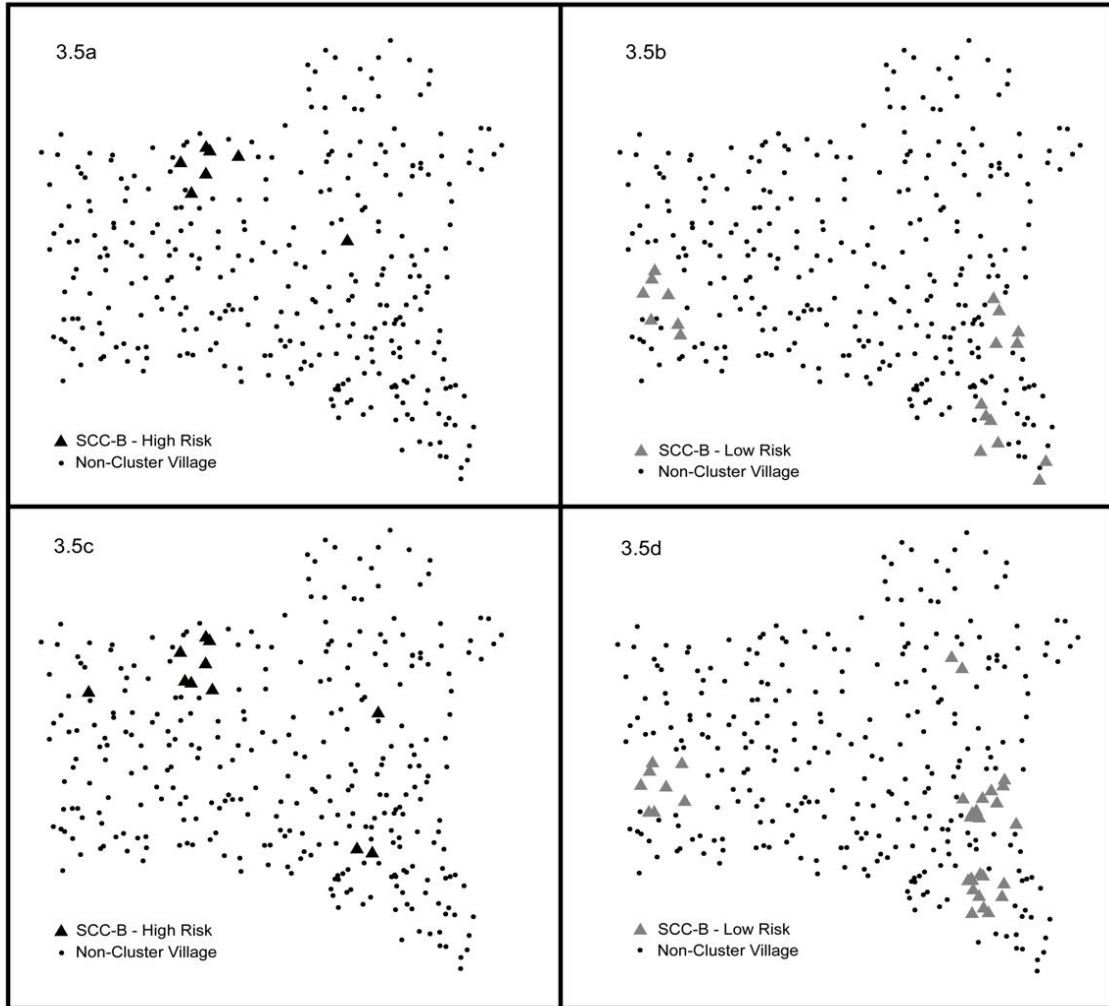


**Figure 3.4.** Clusters of HCC identified by the two methods. 3.4a: High-risk (dark) HCC clusters identified by the Local Moran test statistic; 3.4b: Low-risk (light) HCC clusters identified by the Local Moran test statistic; 3.4c: High-risk (dark) HCC clusters identified by the spatial scan test statistic; 3.4d: Low-risk (light) HCC clusters identified by the spatial scan test statistic.

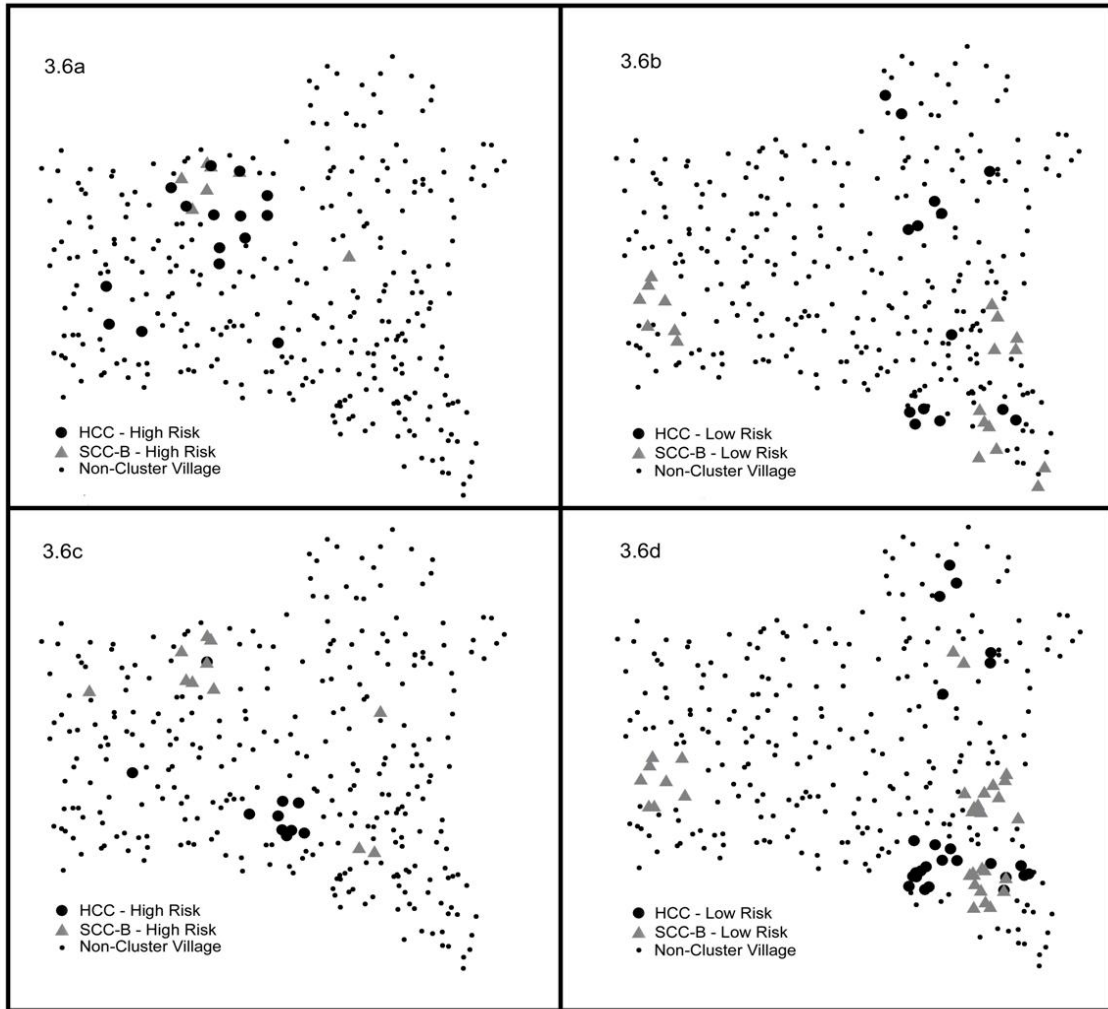




**Figure 3.5.** Clusters of SCC-B identified by the two methods. 3.5a: High-risk (dark) SCC-B clusters identified by the Local Moran test statistic; 3.5b: Low-risk (light) SCC-B clusters identified by the Local Moran test statistic; 3.5c: High-risk (dark) SCC-B clusters identified by the spatial scan test statistic; 3.5d: Low-risk (light) SCC-B clusters identified by the spatial scan test statistic.



**Figure 3.6.** Clusters of HCC (circle) and SCC-B (triangle) identified by the two methods. 3.6a: High-risk (dark) clusters identified by the Local Moran test statistic; 3.6b: Low-risk (light) clusters identified by the Local Moran test statistic; 3.6c: High-risk (dark) clusters identified by the spatial scan test statistic; 3.6d: Low-risk (light) clusters identified by the spatial scan test statistic.



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## **CHAPTER IV**

### **VIRAL HEPATITIS INFECTIONS IN HEPATOCELLULAR CARCINOMA CASES AND THE APPARENTLY HEALTHY POPULATION IN EGYPT: A SYSTEMATIC REVIEW AND META-ANALYSIS**

#### **INTRODUCTION**

Hepatocellular carcinoma (HCC) comprises nearly 6% of all incident cancer cases worldwide, with the overwhelming majority occurring in the developing world.<sup>1</sup> One of the least curable malignancies, HCC is the third most frequent cause of cancer mortality among men worldwide.<sup>1</sup> Chronic infection with hepatitis B virus (HBV) and hepatitis C virus (HCV) have been cited as, by far, the most important etiologic agents.<sup>2</sup> According to the World Health Organisation (WHO), approximately 350 million people are chronically infected with HBV and 170 million are infected with HCV.<sup>3-4</sup> The relative importance of HBV and HCV as causative agents can vary greatly from region to region and over time.<sup>1,5</sup> Selecting appropriate HCC prevention and control methods, therefore, depends on understanding the dynamics of these agents in a specified geographic region. Hepatocellular carcinoma in Egypt is currently undergoing a temporal shift in the relative importance of HBV and HCV as primary risk factors. Hepatocellular carcinoma is the second most frequent cause of cancer incidence and mortality among men in Egypt.<sup>6</sup> Hospital-based studies from Egypt have reported an increase in the relative frequency of all liver-related cancers in Egypt (>95% as HCC), from approximately 4.0% in 1993 to

7.3% in 2003.<sup>7-9</sup> The Middle East Cancer Consortium recently reported that the incidence rate among males was seven times greater than the next highest rate (among Israeli Jews) and more than three times that reported in the United States Surveillance Epidemiology and End Results (SEER) summary.<sup>6</sup> To explain this trend it is necessary to understand the dynamics of HBV and HCV in Egypt.

Prior to implementing the HBV vaccine in the 1980s, chronic infection with HBV was widespread and considered the dominant etiologic factor in HCC development.<sup>10</sup> Childhood HBV immunization in Egypt today is estimated at 95% to 100%, thus making it likely that HBV-related HCC will steadily decline over the next few decades.<sup>11-12</sup> Unfortunately this major public health achievement has been eclipsed by the rise of the HCV epidemic, largely attributed to the mass parenteral anti-schistosomal therapy (PAT) and other iatrogenic exposures.<sup>13-16</sup> Egypt is now plagued by the highest prevalence of HCV in the world, with estimates ranging from 6 to 28% and a reported average of approximately 13.8%.<sup>17-18</sup> Recent investigations in Egypt have also shown the increasing importance of HCV infection in the etiology of HCC, now estimated to account for 40 to 50% of cases.<sup>7-9</sup> Just as many people were infected with HBV as with HCV during the PAT campaign; however, HBV only caused chronic infections in 5% or less of infected individuals, whereas chronic HCV infection developed in 70% to 80%.<sup>14,16</sup> This has to do with the natural history of HBV, where the probability of developing chronic infection decreases with age.<sup>5,10</sup> Since most individuals receiving PAT were older than 10-15 years, they were at less risk for developing persistent HBV infection.<sup>16</sup>

The future of HCC in Egypt and the magnitude of its socio-economic burden will be a legacy of the country's unique HCV epidemic and the shifting dynamics of HBV

and HCV. To date, there has been no systematic review of the literature to synthesize the results of published prevalence studies, including patterns over time. There is, therefore, a need to calculate the fluctuating prevalences of HBV and HCV in Egypt over the past 2 decades among a healthy population-based sample; there is also a need to synthesize data about HCC patients, using appropriate meta-analytic tools. This paper aims to calculate weighted average prevalences of HBV and HCV among two groups in Egypt from 1985 to the present: healthy, population-based samples (non-high risk), and incident HCC cases.

## METHODS

**Literature Search Methods.** MEDLINE , ISI Web of Science, ScienceDirect, and WHO regional indexed databases were used to search for articles published from 1 January 1980 to 31 October 2007, by means of the MeSH terms: ‘hepatocellular carcinoma and Egypt’, ‘hepatitis B virus and Egypt’ and ‘hepatitis C virus or hepacivirus and Egypt’. No language limitation was imposed. For HCC studies, the time and place of subject recruitment were cross-checked to avoid inclusion of the same cases in multiple articles. Articles or reports from non-peer-reviewed sources were not considered for this analysis.

**Data Analyzed.** The key information abstracted from each study included:

- (1) Year(s) conducted (to account for the delay between field work and publication)
- (2) Sample size
- (3) Age range of participants (participants 18 years or older were considered



adults)

(4) Geographic region of the sample population

a. Upper or Lower Egypt

(5) Type of residence

a. Urban or rural

(6) Prevalence of HBsAg

(7) Prevalence of anti-HCV

(8) Generation of HCV serology test used

Unfortunately, we did not examine sex differences in HBV and HCV prevalence, because too few of the studies reported marker prevalence stratified by participant gender.

**Inclusion & Exclusion Criteria.** Healthy population-based studies included the following sample populations: voluntary blood donors (VBD), replacement donors (RBD) (blood donated to replace blood consumed by specific patients, typically from friends or blood relations), healthy antenatal women, and community studies. We excluded studies from the following special groups who were assumed to be at special high risk: patients from sexually-transmitted-disease clinics, thalassemia clinics, hospitalised patients, professional or paid blood donors, sex workers, drug abusers, dialysis patients, and health care workers. Healthy population-based studies with fewer than 100 participants were excluded from this analysis.

HCC studies were limited to those examining incident cases to avoid temporal ambiguity and the possibility that virus infection may have occurred subsequent to the cancer diagnosis. HCC studies with fewer than 25 participants were excluded from this analysis.

Eligible studies had to report prevalence of hepatitis B surface antigen (HBsAg) and/or antibodies against HCV (anti-HCV). Studies were excluded if they failed to indicate the type of test used to assess infection status and if the sensitivity and specificity of the test was unknown. Information regarding HBV infection and immunity status can be obtained from four common seromarkers: hepatitis B surface antigen (HBsAg), hepatitis B surface antibody (anti-HBs), total hepatitis B core antibody (anti-HBc), and IgM antibody to hepatitis B core antigen (IgM anti-HBc). Of these markers, only HBsAg can identify current infection. A positive result indicates either active or chronic infection. Antibody markers cannot distinguish between naturally-acquired immunity and vaccine-related immunity.<sup>19</sup> Since we wanted to measure the prevalence of naturally acquired infection, HBsAg was the seromarker of interest. In addition, these tests have been available for a long time. They are inexpensive, simple to conduct and reliable, allowing them to be fairly standard even in low-resource settings.<sup>19</sup>

HCV infection status can be determined in two primary ways: enzyme immunoassay (EIA) or polymerase chain reaction (PCR). EIA techniques indicate the presence of antibody (anti-HCV) while PCR can serve to confirm infection as well as measure viral load. Both methods are markers of ongoing infection and do not correlate well with resolved infection. As with HBsAg, anti-HCV does not distinguish between acute and chronic infection.<sup>20</sup> While PCR techniques tend to be more accurate, they are far more expensive and, thus, infeasible for low-resource settings or areas with higher prevalence. With a sensitivity greater than 95% and the availability of standardized test kits, the World Health Organisation (WHO) recommends EIA as the primary screening tool, and suggests PCR be used for clinical and case management purposes.<sup>20</sup> For these

reasons, it is rare to find PCR tests reported in the literature from low-resource settings such as Egypt, and we believe the results of EIA are sufficiently consistent and reliable to be used in this analysis. Though some studies did report both anti-HCV and PCR results, we only examined anti-HCV to limit bias due to varying test sensitivity and specificity.

In addition, studies known or likely to have used first-generation ELISA for measuring anti-HCV were not included in the meta-analysis due to known problems of sensitivity and specificity of those assays.<sup>21</sup> To date, there have been three generations of anti-HCV EIAs, with the first developed in 1990. It suffered from poor sensitivity and could not detect antibody prior to 4 to 6 months following initial infection. Second and third generation tests have dramatically improved sensitivity (>95%) and narrowed the window period between infection and anti-HCV detection.<sup>20</sup> Consequently, this paper only considers studies that used second or third generation anti-HCV EIAs. Only 3 studies were excluded based solely on the generation of anti-HCV test used.

We also applied specific definitions when classifying Lower and Upper Egypt as well as urban and rural settings. Figure 4.1 identifies the locations of Upper and Lower Egypt.<sup>22</sup> Generally speaking, Lower Egypt is in the north (designated as “Lower” because it lies downstream on the Nile) and Upper Egypt is in the south. It has been hypothesized that prevalence of HCV infection varies between Lower and Upper Egypt (i.e. north and south).<sup>7-8</sup> To examine this, we focused on studies of these two regions. Though Upper and Lower Egypt do not account for the entire population, they do contain the vast majority. Other areas were *a priori* excluded as the literature is sparse, and we did not want to introduce bias based on differing regional health behaviors, etc. After

completing the literature search, no studies were excluded solely on the basis of governorate.

The Egyptian government classifies governorates as either fully urban or joint urban and rural.<sup>22</sup> The official distinction between urban and rural is reflected in the lower tiers. Fully urban governorates have no districts (*markazes*). The district is specifically defined as a conglomeration of villages around a capital city. In joint urban and rural governorates, therefore, urban locations are comprised of each district's capital city. All other locations in that governorate are considered rural. Some urban governorates consist of just one city which is divided into smaller urban neighborhoods. For example, the governorate of Cairo consists of 23 urban neighborhoods. Since a disparity in HCV prevalence between urban and rural populations has been observed, we wanted to test the hypothesis systematically, using strict and consistent definitions.<sup>13-16,23</sup> We followed this classification scheme in our designation of urban and rural study settings.

**Power Concerns.** We noted difficulties with statistical power could be encountered at two levels: small participant sample size in individual studies as well as small sample size of studies, particularly when stratified by sub-factors of analysis (e.g. geographic region, age group, etc). Pooling 30 studies with sample sizes of 50, for example, may not be as reliable as pooling 5 studies of 300 participants. Nevertheless, it is difficult to compare multiple studies of a particular geographic region to a single study in a different region, regardless of sample size. Since little is available on power calculations for these biomarkers in Egypt, we opted to take a more qualitative approach. Egypt does experience a higher burden of HBV and HCV in its apparently healthy

population as compared to other countries, and it is well known that an overwhelming majority of its HCC cases test positive for HBV and/or HCV. Therefore, we were comfortable setting the lower limit of sample size for healthy population samples at 100 and for HCC cases at 25. This resulted in the exclusion of 2 healthy population studies and 3 HCC studies based solely on the sample size requirement.

**Meta-Analysis.** Data were analyzed according to three different categories: HBV prevalence among healthy population-based samples, HCV prevalence among healthy population-based samples, and HBV and HCV prevalence among incident HCC cases.

Summary prevalence measures were calculated as weighted averages, using a standard method.<sup>25</sup> The individual prevalence rate for each study was multiplied by the corresponding sample size and divided by the total (pooled) sample size of all studies. These summary measures were used to compare differences between sub-groups in aggregate as well as over time. Chi-square tests were performed to determine significant differences in prevalence with respect to age category (child vs. adult), geographic location (Upper vs. Lower Egypt), type of residence (urban vs. rural), and study period (time; treated as a continuous variable), using  $\alpha = 0.05$  as the significance cut-off. We used linear regression models to examine risk factors in combination, with either HBV or HCV prevalence as the dependent variable. The four risk factors mentioned above were all considered for the healthy population studies. Time was the only predictor examined for HBV or HCV among HCC cases. Prevalence was log transformed to obtain a normal distribution, and the models were weighted by study sample size.

All manipulations and analyses were performed using SAS v 9.1.3 (SAS Institute Inc., Cary, NC) and MetaWin v 2.0 (Sinauer Associates, Inc., Sunderland, MA).

## RESULTS

**Literature Search Results.** The initial search generated over 1500 potential articles. After refining the list to relevant articles, we identified a total of 200 unique articles: 107 from MEDLINE, 57 from WHO regional indexed databases, 34 from ISI Web of Science, and 2 from ScienceDirect. A hand search of the reference lists of selected articles identified a few additional relevant studies. Final results from our search yielded a total of 39 unique peer-reviewed studies meeting our inclusion/exclusion criteria (Appendix 4.1). Several studies measured both HBsAg and anti-HCV, and some of them also involved more than one study site, providing prevalence measures for multiple population samples. Of these studies, 25 studied healthy population-based samples, and 14 were specific to HCC cases. The healthy population-based studies included 12 with HBsAg measurements and a total sample size of 7,597, and 24 with anti-HCV measurements and a total sample size of 42,457. The 14 HCC studies with HBsAg and/or anti-HCV information had a total case population of 3,275. Data for HBV among healthy population-based samples covered the time period 1983-2002, HCV studies healthy population-based samples spanned the time period 1991-2004, and HCC studies ranged from 1985-2005. Study details are presented in Tables 4.1-4.2. Chi-square test results for all categories among HBV, HCV and HCC are presented in Table 4.3.

**HBsAg among the healthy population-based samples.** Table 4.4 presents the major findings from examining the prevalence of HBsAg among healthy population-based samples. Overall, the prevalence was 6.7% ( $\pm 1.4\%$ ), with no significant variation

over time ( $P = 0.59$ ). When the studies were separated according to age, we found a similar pattern. Results showed a significant difference between prevalence among adults and children ( $P < 0.0001$ ). Adults averaged a prevalence of 8.0% ( $\pm 1.7\%$ ), with children averaging at 1.6% ( $\pm 0.3\%$ ), likely a function of the introduction of the HBV vaccine (Figure 4.2).

Eleven of the 12 studies provided information regarding individuals from Upper or Lower Egypt. Only one study examined Upper Egypt, while the remaining 11 examined Lower Egypt. A summary measure of the Lower Egypt studies revealed a prevalence of 4.6% ( $\pm 1.3\%$ ), much lower compared to that of the Upper Egypt study (11.7%), which was statistically significant ( $P < 0.0001$ ). Since we only have one study from Upper Egypt, it is difficult to know how representative it is, but it should be mentioned that it was a community-based sample of 1064 individuals.

**Anti-HCV among healthy population-based samples.** Table 4.4 presents the major findings from examining the prevalence of anti-HCV among healthy population-based samples. Overall, from 1990-2004 the prevalence was 13.9% ( $\pm 1.6\%$ ). It was significantly higher among adults ( $15.7 \pm 1.8\%$ ) versus children ( $4.0 \pm 2.1\%$ ;  $P < 0.0001$ ). Unlike our findings with HBsAg, the prevalence of anti-HCV did appear to vary over time, both in summary and by age group (Figure 4.3; Table 4.3). Studies from 1990-1994 showed a higher prevalence of 21.8 ( $\pm 3.3\%$ ) when compared to the time periods 1995-1999 ( $12.5 \pm 3.4\%$ ) and 2000-2004 ( $13.5 \pm 2.0\%$ ), which were not statistically different from one another. This trend continued for adults ( $P < 0.0001$ ), but child studies suggested a continuing decline in prevalence ( $P < 0.0001$ ; Figure 4.3).

Analysis of anti-HCV by geographic region found the reverse trend from what was observed with HBsAg (Figure 4.4). Of the 24 total studies, 19 had specific information on Lower Egypt, and 2 had specific measurements for Upper Egypt. Studies from Lower Egypt showed a higher prevalence ( $15.8 \pm 1.8\%$ ) than those from Upper Egypt ( $6.7 \pm 2.9\%$ ), which was significant ( $P < 0.0001$ ). This pattern remained highly significant after stratifying by age group (Table 4.3).

We were also able to examine urban versus rural populations (Figure 4.5). We found 10 studies examining specifically rural groups and 3 that looked at urban populations. As has been suggested in the literature, we found a significantly higher prevalence of anti-HCV among rural individuals ( $13.4 \pm 2.3\%$ ) compared to urban individuals ( $5.5 \pm 2.0\%$ ;  $P < 0.0001$ ). Again, we found this trend to be highly significant after stratifying by age group (Table 4.3).

**HBsAg and anti-HCV among HCC cases.** Table 4.4 presents our findings from examining the prevalence of HBsAg and anti-HCV among HCC cases. There were 12 studies with HBsAg data covering the time period 1985-2004, and 11 studies with anti-HCV measurements spanning 1991-2004. The overall prevalence of HBsAg was 25.9% ( $\pm 4.7\%$ ), and that of anti-HCV was 78.5% ( $\pm 3.6\%$ ), and this difference was statistically significant ( $P < 0.0001$ ). Both markers showed highly significant changes over time, with HBsAg decreasing (1985-1996:  $32.7 \pm 5.2\%$ ; 1997-2004:  $21.9 \pm 8.9\%$ ;  $P < 0.0001$ ), and anti-HCV increasing (1991-1996:  $68.5 \pm 6.6\%$ ; 1997-2004:  $85.9 \pm 2.6\%$ ;  $P < 0.0001$ ) over time (Figure 4.6a).

Of the HCC studies included, 9 ( $N = 1711$ ) reported both HBsAg and anti-HCV measurements. We repeated the above analysis for just these 9 studies to reduce the



possibility of bias, and we found similar results. The overall prevalence of HBsAg was 24.5% ( $\pm 5.1\%$ ), and that of anti-HCV was 84.1% ( $\pm 2.4\%$ ). The decrease in HBsAg remained significant (1991-1996:  $30.0 \pm 5.3\%$ ; 1997-2004:  $21.9 \pm 8.9\%$ ;  $P = 0.001$ ). A slight increase in anti-HCV prevalence was observed for the revised group of studies, but it was no longer significant (1991-1996:  $80.3 \pm 4.3\%$ ; 1997-2004:  $85.9 \pm 2.6\%$ ;  $P = 0.2015$ ) (Figure 4.6b).

**Linear Regression.** Final models from the regression analysis are presented in Table 4.5. Among the healthy population studies, age was highly significant as a sole predictor, but it lost significance in the presence of study year suggesting that these are not independent predictors for either HBV or HCV prevalence (not shown). Type of residence (urban vs. rural) was not available for the HBV studies, so our final model only examines study year and geographic region (Upper vs. Lower Egypt). Our final model included study year ( $P = 0.03$ ), region ( $P = 0.15$ ), and an interaction term for year and region ( $P = 0.06$ ), suggesting that HBV prevalence has generally decreased over time but has also changed geographically over time. The final model for HCV prevalence included study year ( $P = 0.08$ ), region ( $P = 0.004$ ), residence ( $P = 0.17$ ), and a 3-way interaction term for year, region, and residence ( $P = 0.03$ ), suggesting more complex shifts in prevalence patterns with respect to space (region and residence) and time.

The regression models used to examine HBV and HCV among HCC cases produced results consistent with what we would expect from the chi-square analysis. Study year was significantly associated with HBV, indicating decreasing prevalence over time, but it was not associated with HCV. It is possible that HCV among cancer cases

has increased slightly over time, but we simply do not have the power to detect such a change.

## **DISCUSSION**

This is the first systematic review and meta-analysis of hepatitis B and C virus prevalence in Egypt and should provide some insight for understanding the dynamics of liver disease in Egypt and how best to address the problem. For both viruses among healthy population-based samples, we found adults had a significantly higher prevalence than children, supporting a cohort effect. For HBV, it seems likely that the cohort effect would be related to the introduction of the hepatitis B vaccine in 1992, which was incorporated into the Expanded Programme on Immunisation, and is only given to children, leaving adults at the time of program implementation unvaccinated. The cohort effect seen in HCV is likely related to the early association between the parenteral antischistosomiasis therapy (PAT) campaign and HCV transmission. Oral therapies for schistosomiasis were gradually adopted in the 1980's, dramatically reducing transmission.<sup>14,26</sup> It is still unclear, however, what the rate of HCV transmission is presently, in the absence of the original primary route. Since several of the childhood HCV studies were late enough that none of the children would have been exposed to the PAT campaign, it does appear that HCV has continued to be transmitted. It will be important to understand the emerging routes and rates of transmission to effectively control the burden of liver disease in Egypt.

Our analysis confirmed reports of large scale geographic heterogeneity in HCV prevalence.<sup>7-8</sup> We did find prevalence to be significantly higher in Lower Egypt as

opposed to Upper Egypt, which in turn supports the hypothesis of PAT as the dominant force driving the HCV epidemic. Individuals living in Lower Egypt experienced a greater burden of schistosomiasis, and therefore a greater level exposure to PAT.<sup>7-8</sup> This is in contrast to what we saw with HBV, which appeared to be higher in Upper Egypt versus Lower Egypt. Unfortunately, we did not have access to multiple studies of HBV prevalence in Upper Egypt, so it is best not to draw too many conclusions from that analysis.

We were also able to examine the relationship of HCV with urban and rural populations. We were able to support the hypothesis that HCV prevalence is higher among rural residents than urban residents.<sup>13,23</sup> This is also in line with the PAT hypothesis; that rural residents would have a greater burden of schistosomiasis, and therefore greater exposure to PAT.<sup>13,23</sup> Unfortunately, we were unable to find this type of disaggregated data for HBV for comparison, and since most of the HCC studies were hospital-based, it is difficult to speculate on future effects, though it would be expected that more HCC cases would be found in rural regions. This is contrary to a recent population-based HCC study from Lower Egypt that found cases to be nearly twice as likely to come from urban versus rural areas.<sup>27</sup> Due to the significant lag time between viral infection and development of HCC, it is possible Egypt is witnessing the end of a cohort of higher urban exposure that will soon transition to a population dominated by rural exposure.

Finally, it was illuminating to examine the trends in HBV and HCV prevalence over the past 20 years. As was expected to some degree, HBV prevalence was relatively low overall, but has experienced a slight decrease over the time period included here,

probably due to the implementation of earlier control measures followed by the vaccine. Infection levels in children suggest that this infection may soon be an insignificant element of the liver disease burden in Egypt. Prevalence of HCV, however, showed greater variance over time. Among both children and adults there was a general decline in prevalence over time, with adults stabilizing at just below 15%, and children continuing to drop to nearly 1% by the period 2000-2004. Regression analyses found significant time interactions for both HBV and HCV, suggesting a more complicated shift in prevalence patterns over time that includes geographic changes as well. Detailed risk factor data are needed to further characterize these interactions and distinguish between the effects of population migration, changing risk factor patterns, etc. It would also be valuable to match these prevalence figures with incidence studies to reconstruct the different epidemic curves, shedding light on viral dynamics and guiding predictions about future trends in infection.

We felt it was important to gain a summary understanding of the status of HBV and HCV prevalence in healthy population-based samples, but that this should be complimented by an understanding of these viral dynamics among HCC cases as well. Frequently the burden of HBV and HCV cannot easily be quantified apart from its chronic sequelae. By understanding the degree to which these viruses contribute to HCC, it is possible to see the impact they have on overall population health.

We found the overall prevalence of HBV among HCC cases to be nearly 25%, and the prevalence of HCV infection to be as high as 84%. We also noted some differences within infections over time, though they were not statistically significant, possibly a result of low power. It appeared that HBV infection declined from a

prevalence of 30% between 1991-1996 to 22% between 1997-2004. Conversely, HCV infection among HCC cases may have increased slightly over this same time period from 80 to 86%. This seems fitting when we consider the impact of different cohort effects. Current cancer cases represent individuals being exposed to these viruses 20-30 years prior. These cases may represent the individuals at the end of the pre-HBV vaccine period and the beginning of the PAT campaign. Based on general population estimates, it seems likely that prevalence of HBV will continue to decline and HCV will continue to increase among HCC cases, at least for a while. This has been supported by a projections from a mathematical model designed to predict the burden of HCV in Egypt over the next couple of decades.<sup>23</sup> What will happen after that will depend largely on the new HCV incidence patterns in the absence of the PAT campaign, which remain to be quantified by future research.

In addition to the lack of data regarding the geographic distribution of HBV and HCV prevalence, some limitations of this present review should be considered. It is possible that despite our extensive literature search, the specific search terms used may not have captured all quality papers published in peer-reviewed indexed journals. To counter this problem, we supplemented our electronic search by a hand search of references from selected articles, but there is always the possibility that we missed some data.

We attempted to gain representative samples of the Egyptian healthy population by accepting community-based samples, but we also included some large scale convenience samples such as healthy pregnant women attending clinics and voluntary blood donors. John, et al have questioned the inclusion of voluntary blood donors in

systematic reviews.<sup>28</sup> They refer to a study among voluntary donors at Vellore where the HBV carrier rate was 0.7%, which they suggest is artificially low because voluntary donors are a self-selected group and persons who are found to be positive do not come for repeat blood donation. They suggest using data from replacement donors. Batham, et al. conducted an analysis of different population groups tested in Delhi.<sup>29</sup> On examining the forest plots from these studies, voluntary donors, replacement donors and ante-natal mothers all had prevalences close to the overall mean, suggesting it was appropriate that all these groups be included in systematic reviews.

The extent to which the HCC cases we reported upon are representative is also unclear, since in most cases only hospital-based studies were available. We did focus on cancer studies from the larger, nationally recognized cancer centers, which serve as diagnostic and treatment facilities for the overwhelming majority of cancer cases throughout Egypt.

It is understood that only a large, national epidemiological study can provide a definitive answer regarding the overall prevalence of hepatitis B and C viruses in Egypt. In the absence of such a national sample survey, which would require a large mobilization of resources, a systematic review and meta-analysis of high quality studies previously conducted may provide the best compromise.

**Table 4.1.** Data abstracted from studies examining the general population. Numbers following author name correspond to the full citation in Appendix 4.1. VBD = Voluntary Blood Donors; HPW = Healthy Pregnant Women.

Marker	Author [Citation]	Study Period	Population	Region	Residence	Age	N	Prevalence (%)	
HBsAg	Sherif, et al [1]	1983	Community	Lower	Urban & Rural	18+ yr	1064	11.7	
	Sherif, et al [1]	1983	Community	Lower	Urban & Rural	18+ yr	802	8.0	
	Gumie, et al [2]	1988-1990	VBD	Lower	Urban & Rural	18+ yr	1715	2.5	
	El-Sherbini, et al [3]	1991	School Children	Lower	Urban & Rural	< 18 yr	198	1.5	
	El-Hawey, et al [4]	1991-1992	Community	Lower	Rural	18+ yr	300	15.7	
	Arthur, et al [5]	1993	VBD	Upper & Lower	Urban & Rural	18+ yr	1030	12.7	
	Mabrouk, et al [6]	1993	Army Recruits (Male)	Lower	Urban & Rural	18+ yr	297	1.7	
	Darwish, et al [7]	1993	VBD	Lower	Urban & Rural	18+ yr	163	3.2	
	Darwish, et al [8]	1995	Community	Lower	Urban & Rural	18+ yr	796	8.8	
	El-Sherbini, et al [3]	1995	School Children	Lower	Rural	< 18 yr	300	0.7	
	Reda, et al [9]	1997	Community	Lower	Urban & Rural	< 18 yr	500	2.2	
	El-Sherbini, et al [3]	2002	School Children	Upper	Urban	< 18 yr	470	1.5	
	Anti-HCV	El-Sherbini, et al [10]	1991	School Children	Lower	Rural	< 18 yr	138	15.9
		El-Sherbini, et al [10]	1991	School Children	Lower	Urban	< 18 yr	130	6.2
El-Sherbini, et al [10]		2002	School Children	Lower	Urban	< 18 yr	470	2.1	
Fathalla, et al [11]		1991-1992	VBD	Upper & Lower	Urban & Rural	18+ yr	248	18.2	
Arthur, et al [5]		1993	VBD	Upper & Lower	Urban & Rural	18+ yr	2644	24.8	
Darwish, et al [7]		1993	VBD	Lower	Rural	18+ yr	163	22.0	
El-Sherbini, et al [10]		1994	School Children	Lower	Rural	< 18 yr	294	2.0	
Kumar, et al [12]		1994-1996	HPW	Lower	Urban & Rural	18+ yr	499	15.0	
Darwish, et al [8]		1995	Community	Lower	Urban & Rural	18+ yr	796	40.3	
Kassem, et al [13]		1996	HPW	Lower	Urban & Rural	18+ yr	100	19.0	
Mohamed, et al [14]		1997	Community	Lower	Rural	< 18 yr	1823	8.2	
Mohamed, et al [14]		1997	Community	Upper	Rural	< 18 yr	2808	2.5	
Stoszek, et al [15]		1997-2003	HPW	Lower	Urban & Rural	18+ yr	2587	15.8	
Abdel Aziz, et al [16]		1999	Community	Lower	Rural	18+ yr	3999	24.3	
Nafeh, et al [17]		1999	Community	Upper	Rural	18+ yr	6031	8.7	
Tanaka, et al [18]		1999	VBD	Upper & Lower	Urban & Rural	18+ yr	3608	8.8	
Rizk, et al [19]		2000-2002	HPW	Lower	Urban & Rural	18+ yr	696	15.8	
Bakr, et al [20]		2002	Community	Lower	Rural	18+ yr	4720	19.3	
Mohamed, et al [21]		2002	Community	Lower	Rural	18+ yr	2425	18.5	
Arafa, et al [22]		2002-2003	Community	Lower	Rural	18+ yr	4020	11.8	

Raouf, et al [23]	2002-2003	HPW	Lower	Urban & Rural	18+ yr	1832	10.1
El-Sadawy, et al [24]	2003	Community	Lower	Rural	18+ yr	842	27.4
El-Sadawy, et al [24]	2003	Community	Lower	Urban	18+ yr	580	23.4
El-Raziky, et al [25]	2004	Community	Lower	Urban & Rural	< 18 yr	1042	1.4

VBD = Voluntary Blood Donors; HPW = Healthy Pregnant Women.

Total N for HBsAg studies = 7,597

Total N for anti-HCV studies = 42,457



**Table 4.2.** Data abstracted from studies examining HCC cases. All studies had a mean age between 45–60 yrs.

Author	Study Period	Patient Source	Study N	HBsAg+ (%)	Anti-HCV+ (%)
Ahmed, et al [26]	1985	Tropical Medicine Department – Cairo University	25	28.0	-----
El-Soudani, et al [27]	1989-1992	Al-Azhar University	38	73.7	-----
El-Sherif, et al [28]	1991-1992	Mansoura University	30	30.0	70.0
El-Zayadi, et al [29]	1992-1995	Cairo Liver Center	321	38.6	85.7
Abdel-Wahab, et al [30]	1992-2005	Medical Research Institute – Alexandria University	1012	-----	79.6
Abdel-Wahab, et al [31]	1993	National Cancer Institute – Cairo	60	33.3	-----
Angelico, et al [32]	1993-1995	National Cancer Institute – Cairo	135	15.6	67.4
Attia, et al [33]	1995	Ain Shams University Hospital	429	-----	53.4
Hassan, et al [34]	1995-1996	National Cancer Institute – Cairo	33	15.2	75.8
Mabrouk, et al [35]	1995-1996	Ain Shams University Hospital	34	20.6	94.1
Yates, et al [36]	1997-1998	National Cancer Institute – Cairo	131	61.8	73.3
El-Zayadi, et al [37]	1998-2002	Cairo Liver Center	750	20.5	87.9
El-Kafrawy, et al [38]	1999-2002	Ain Shams University Hospital & NCI – Cairo	41	2.4	87.8
Ezzat, et al [39]	2003-2004	National Cancer Institute – Cairo	236	7.6	86.4

Total N for all studies = 3275.

All studies had a mean age between 45–60 yrs.

**Table 4.3.** Prevalence and chi-square results for all categories of analysis among healthy populations and HCC cases.

Population	Category of Analysis	Comparison Group	Prevalence (%)	N	$\chi^2$ P-value
HBV – Healthy Population	Age	Adult	8.0	6129	< 0.0001
		Child	1.6	1468	
	Time (Total)	1983-1992	6.9	4079	0.5900
		1993-1995	6.5	3518	
	Time (Adult)	1983-1992	7.1	3881	0.0027
		1993-1995	9.3	2248	
	Time (Child)	1991-1993	1.5	198	0.8478
		1994-1997	1.6	800	
		1998-2002	1.5	470	
	Region (Total)	Lower Egypt	4.6	5503	< 0.0001
	Upper Egypt	11.7	1064		
HCV – Healthy Population	Age	Adult	15.7	35752	< 0.0001
		Child	4.0	6705	
	Time (Total)	1990-1994	21.79	3579	< 0.0001
		1995-1999	12.45	19664	
		2000-2004	13.54	16627	
	Time (Adult)	1990-1994	24.7	3017	< 0.0001
		1995-1999	14.8	15033	
		2000-2004	14.8	15115	
	Time (Child)	1990-1994	6.4	562	< 0.0001
		1995-1999	4.8	4631	
2000-2004		1.0	1512		
Region (Total)	Lower Egypt	15.8	27118	< 0.0001	
	Upper Egypt	6.7	8839		

	Region (Adult)	Lower Egypt Upper Egypt	17.6 8.7	23221 6031	< 0.0001
	Region (Child)	Lower Egypt Upper Egypt	5.1 2.5	3897 2808	< 0.0001
	Residence (Total)	Urban Rural	13.4 5.5	27100 1180	< 0.0001
	Residence (Adult)	Urban Rural	15.4 8.1	22037 580	< 0.0001
	Residence (Child)	Urban Rural	4.9 3.0	5063 600	0.0003
HCC Cases	Infection Prevalence (Total)	HBV HCV	19.1 78.5	2846 3152	< 0.0001
	Infection Prevalence (1985-1996)*	HBV HCV	32.7 68.5	676 982	< 0.0001
	Infection Prevalence (1991-1996)**	HBV HCV	30.0 80.3	553 553	< 0.0001
	Infection Prevalence (1997-2004)*	HBV HCV	21.9 85.9	1158 1158	< 0.0001
	Infection Prevalence (1997-2004)**	HBV HCV	21.9 85.9	1158 1158	< 0.0001
	HBV Prevalence (1985-2004)*	1985-1996 1997-2004	32.7 21.9	676 1158	< 0.0001
	HBV Prevalence (1991-2004)**	1991-1996 1997-2004	30.0 21.9	553 1158	0.0010
	HCV Prevalence (1991-2004)*	1991-1996 1997-2004	68.5 85.9	982 1158	< 0.0001

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HCV Prevalence (1991-2004)**	1991-1996	80.3	553	0.2015
	1997-2004	85.9	1158	

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\*Analysis performed on all HCC studies

\*\*Analysis performed only on HCC studies that reported both HBsAg and anti-HCV prevalence

**Table 4.4.** Prevalence and standard error of viral markers among healthy populations and HCC cases.

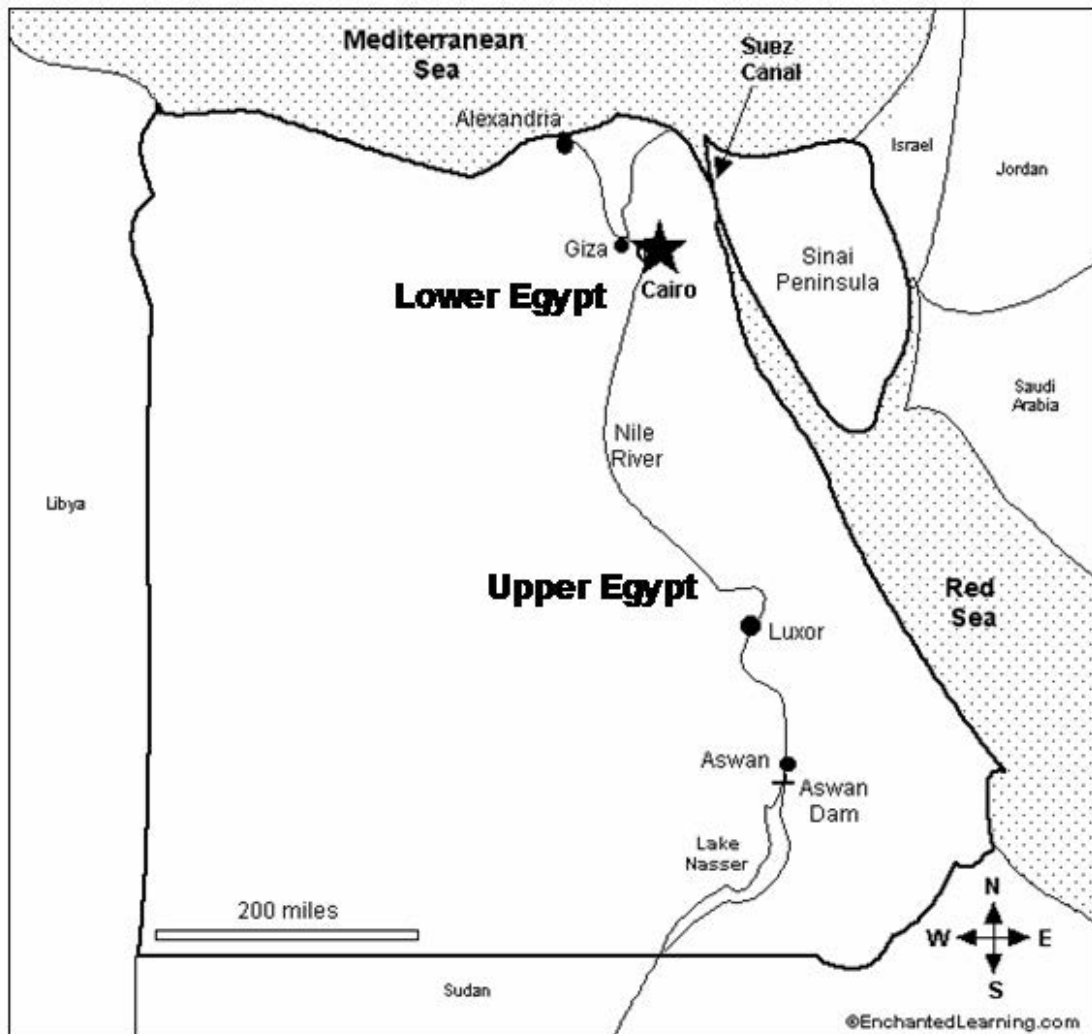
Population	Marker	Category of Analysis	Time Period or Location	HBsAg+ (%)	SE	# Studies	Total N		
Apparently Healthy	HBsAg	Summary	1983-2002	6.7	1.43	12	7597		
			1983-1992	6.9	2.32	5	4079		
			1993-2002	6.5	1.99	7	3518		
		Adult	1983-1995	8.0	1.69	8	6129		
			1983-1992	7.1	2.65	4	3881		
			1993-1995	9.3	2.25	4	2248		
		Child	1991-2002	1.6	0.32	4	1468		
			1991-1993	1.5	-----	1	198		
			1994-1997	1.6	0.74	2	800		
			1998-2002	1.5	-----	1	470		
		Region	Lower Egypt	4.6	1.31	10	5503		
			Upper Egypt	11.7	-----	1	1064		
		Anti-HCV	Anti-HCV	Summary	1990-2004	13.9	1.65	24	42457
					1990-1994	21.8	3.33	6	3579
					1995-1999	12.5	3.45	8	19664
					2000-2004	13.5	2.01	9	16627
				Adult	1990-2004	15.7	1.78	17	35752
					1990-1994	24.7	1.93	3	3017
					1995-1999	14.8	4.07	6	15033
2000-2004	14.8				1.75	7	15115		
Child	1990-2004			4.0	2.13	7	6705		
	1990-1994			6.4	4.02	3	562		
	1995-1999			4.8	2.76	2	4631		
	2000-2004			1.0	0.76	2	1512		

		Region	Lower Egypt	15.8	1.82	19	27118
			Upper Egypt	6.7	2.85	2	8839
		Residence	Rural	13.4	2.31	10	27100
			Urban	5.5	1.99	3	1180
HCC Cases (All studies)	HBsAg	Summary	1985-2004	25.9	4.74	12	1834
			1985-1996	32.7	5.26	8	676
			1997-2004	21.9	8.88	4	1158
	Anti-HCV	Summary	1991-2004	78.5	3.58	10	3140
			1991-1996	68.5	6.64	6	982
			1997-2004	85.9	2.63	4	1158
HCC Cases (Only studies measuring both HBsAg and anti-HCV)	HBsAg	Summary	1991-2004	24.5	5.13	9	1711
			1991-1996	30.0	5.31	5	553
			1997-2004	21.9	8.88	4	1158
	Anti-HCV	Summary	1991-2004	84.1	2.39	9	1711
			1991-1996	80.3	4.36	5	553
			1997-2004	85.9	2.63	4	1158

**Table 4.5.** Results from multivariate linear regression analyses.

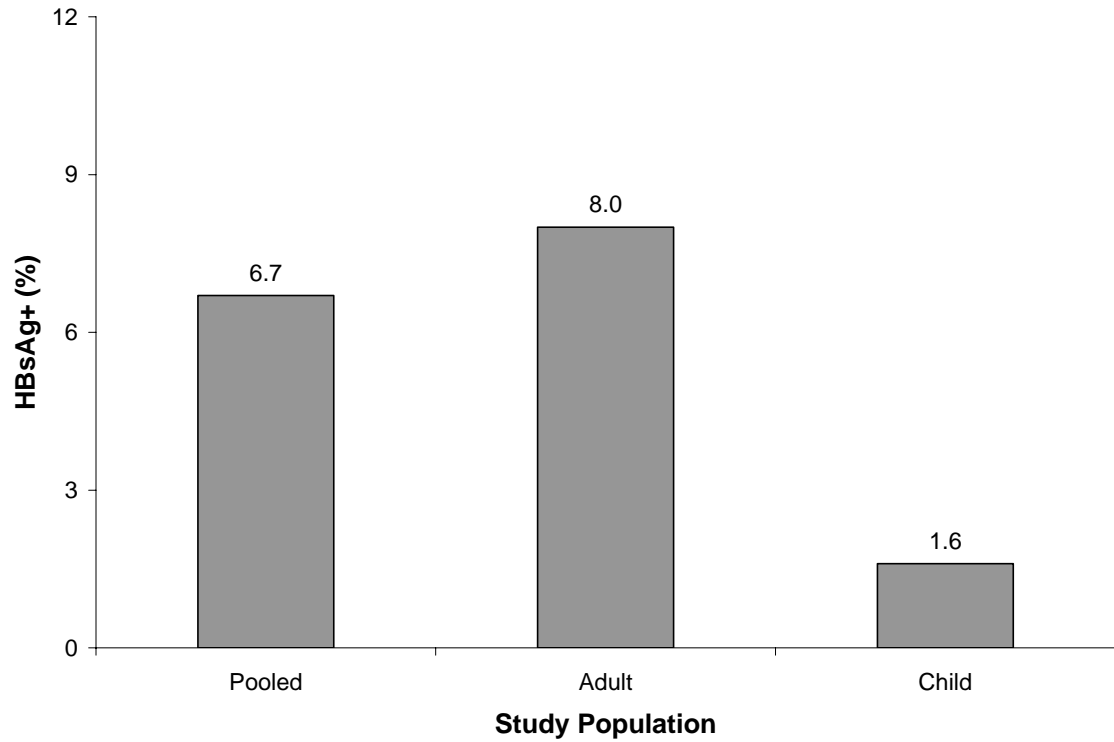
<b>Population</b>	<b>Prevalence (%)</b>	<b>Model Predictors</b>	<b>Parameter (SE)</b>	<b><i>p</i>-Value</b>
Healthy Population	HBV	Year	-0.9049 (0.35)	0.0320
		Region	-1.7035 (1.06)	0.1474
		Year*Region	0.7755 (0.36)	0.0622
	HCV	Year	0.1836 (0.10)	0.0828
		Region	1.3280 (0.40)	0.0039
		Residence	0.8003 (0.56)	0.1678
		Year*Region*Residence	-0.1157 (0.05)	0.0279
HCC Cases	HBV	Year	-0.1675 (0.06)	0.0182
	HCV	Year	0.0340 (0.03)	0.1672

Figure 4.1. Map showing Upper and Lower Egypt regions.<sup>22</sup>

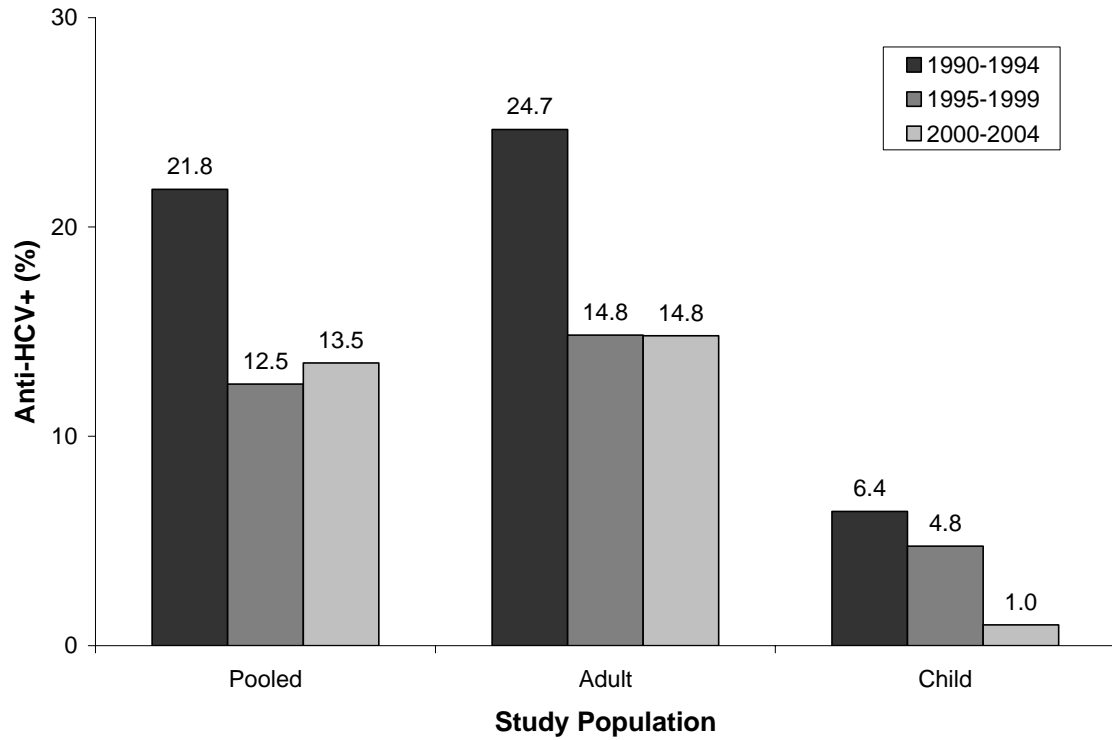




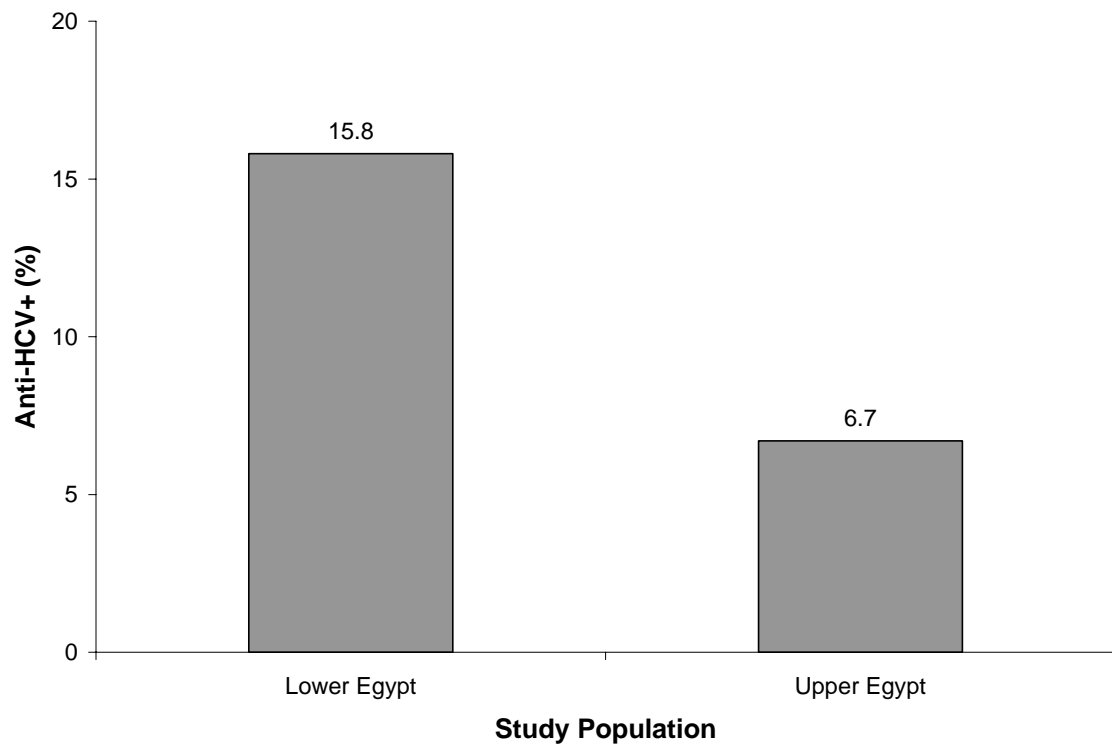
**Figure 4.2.** Prevalence of HBsAg+ individuals. Pooled measurement is based on 12 studies (N = 7,597) and spans 1983-2002. Adult measurement is based on 8 studies (N = 6,129) and spans 1983-1995. Child measurement is based on 4 studies (N = 1,468) and spans 1991-2002. The difference between adult and child populations was significant ( $P < 0.0001$ ).



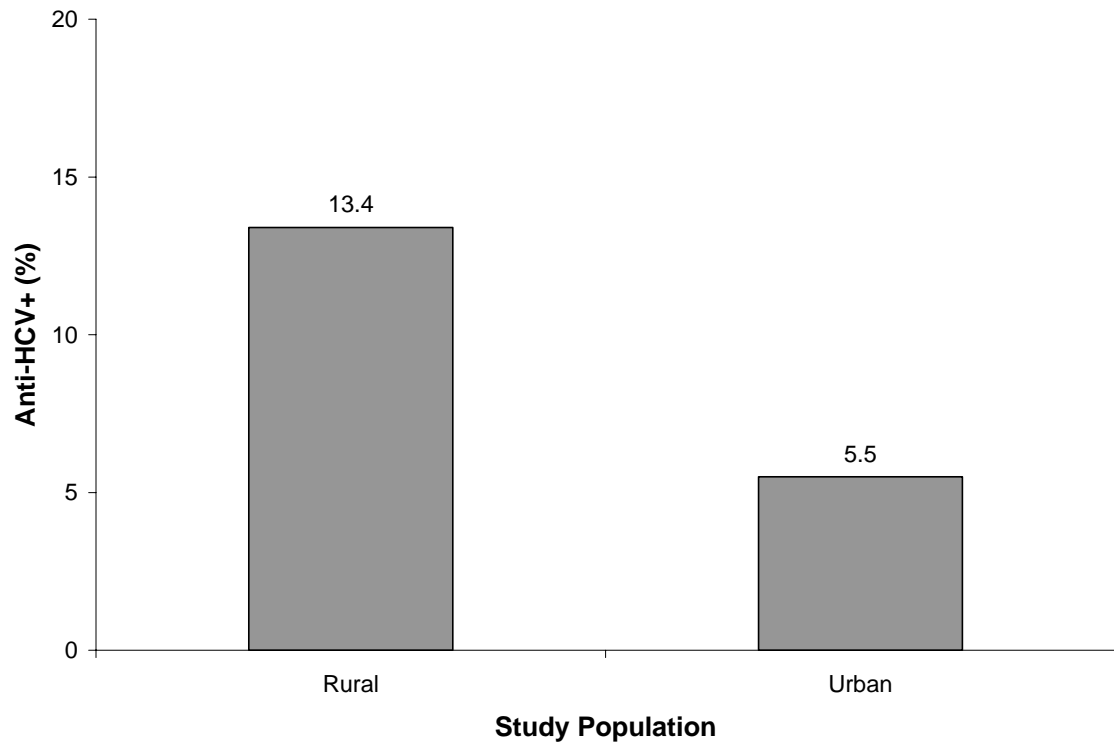
**Figure 4.3.** Prevalence of anti-HCV+ individuals over three time periods: 1990-1994, 1995-1999, and 2000-2004. Pooled measurement is based on 24 studies (N = 42,457). Adult measurement is based on 17 studies (N = 35,752). Child measurement is based on 7 studies (N = 6,705). The difference observed across time period was highly significant for all categories (total, adult, and child;  $P < 0.0001$ ).



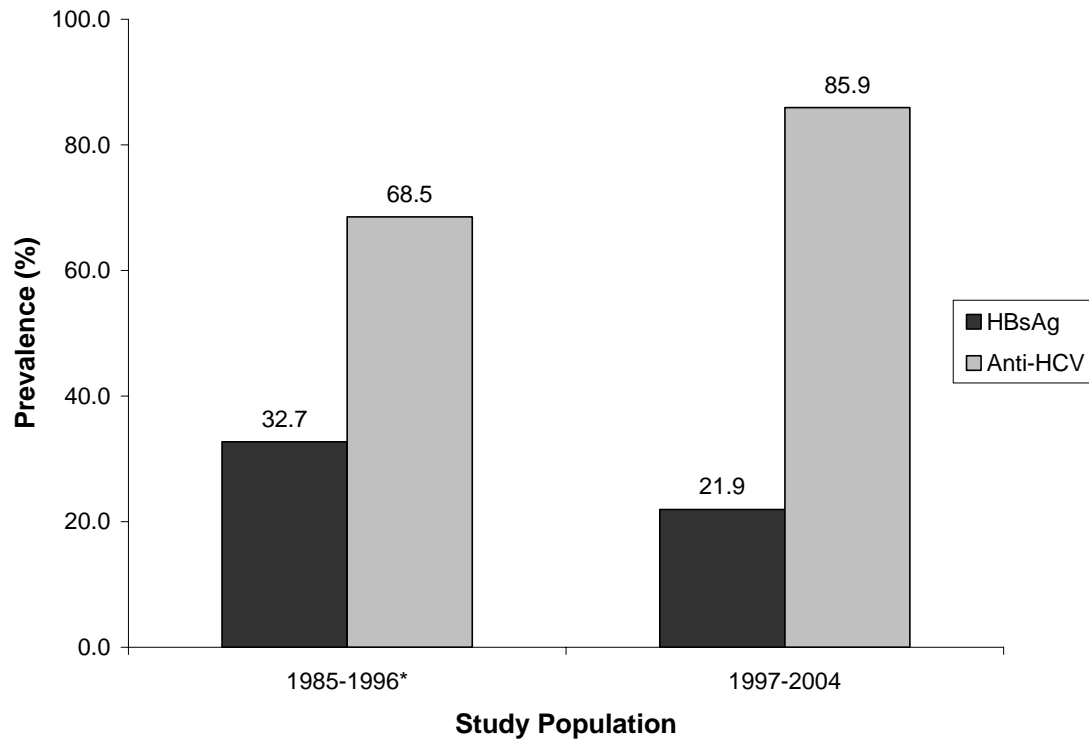
**Figure 4.4.** Prevalence of anti-HCV+ individuals by geographic region: Lower Egypt and Upper Egypt. Lower Egypt measurement is based on 19 studies (N = 27,118). Upper Egypt measurement is based on 2 studies (N = 8,839). Differences between Lower and Upper Egypt were significant ( $P < 0.0001$ ).



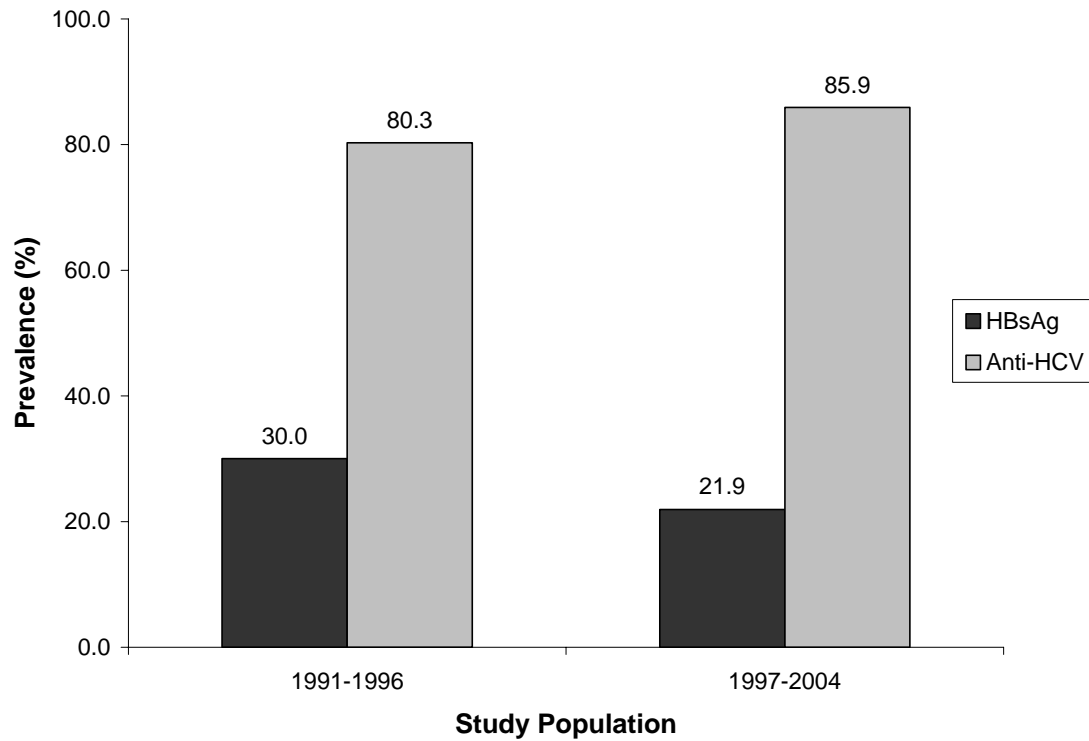
**Figure 4.5.** Prevalence of anti-HCV+ individuals by residence: rural or urban. The rural measurement is based on 10 studies (N = 27,100). The urban measurement is based on 3 studies (N = 1,180). Differences between rural and urban sites were significant (P < 0.0001).



**Figure 4.6a.** Prevalence of HBsAg and anti-HCV+ among HCC cases over two time periods: 1985-1996 and 1997-2004. The HBsAg estimate for 1985-1996 is based on 8 studies (N = 676). The HBsAg estimate for 1997-2004 is based on 4 studies (N = 1,158). The anti-HCV estimate for the first period actually spans 1991-1996 and is based on 6 studies (N = 982). The anti-HCV estimate for 1997-2004 is based on 4 studies (N = 1,158). Differences across biomarkers as well as within biomarkers over time were significant (P < 0.0001)



**Figure 4.6b.** Prevalence of HBsAg and anti-HCV+ among HCC cases over two time periods: 1991-1996 and 1997-2004, using only studies that reported both HBsAg and anti-HCV measurements. The estimates for 1991-1996 are based on 5 studies (N = 553). The estimates for 1997-2004 are based on 4 studies (N = 1,158). Differences between HBsAg and anti-HCV were significant ( $P < 0.0001$ ), as were differences within HBsAg prevalence over time ( $P = 0.001$ ) but time differences within anti-HCV were not significant ( $P = 0.2051$ ).



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## CHAPTER V

### THE HEPATITIS C VIRUS EPIDEMIC IN EGYPT: ESTIMATING PAST INCIDENCE AND PREDICTING FUTURE COMPLICATIONS

#### INTRODUCTION

Hepatitis C virus (HCV) infection is gaining increasing attention as a global health problem, with approximately 3% of the world's population infected.<sup>1-2</sup> These individuals are at greater risk for developing cirrhosis and/or hepatocellular carcinoma (HCC). Egypt reports the highest prevalence of HCV worldwide, ranging from 6 to more than 40% with an average of 13.8%.<sup>3-5</sup> In populations of blood transfusion recipients over the age of 30, this rate has been reported to be as high as 73%, and in the general population aged 40-60 years this rate can be as high as 55%. At the same time, the rate in children is much lower, ranging between 2-10%.<sup>3-5</sup> While the rate in children is much lower than that in the older population, it is still considered high by World Health Organization (WHO) standards, where rates greater than about 4% are considered high.<sup>2</sup>

In Egypt, the major route of exposure to HCV appears to be the mass parenteral antischistosomal treatment (PAT), with more than 35 million injections given over a 20-year period (1960–1980).<sup>3</sup> Despite termination of this program and the implementation of measures designed to reduce hospital-related infection, HCV transmission continues.

Because the prevalence of HCV is so much higher than that of HBV infection, the attributable fraction of HCV (60%-70%) and the anti-HCV rate (75%-90%) has become

the leading risk factor for HCC in Egypt.<sup>6</sup> Research has indicated an increase in the relative frequency of all liver-related cancers in Egypt (>95% as HCC), from approximately 4.0% in 1993 to 7.3% in 2003.<sup>7</sup> Now the second highest cause of cancer incidence and mortality among men, HCC in Egypt occurs at rates significantly greater than those seen in surrounding Middle Eastern countries as well as the United States.<sup>8</sup>

Incidence data are limited, however, because researchers have been plagued by logistical and methodological difficulties. Initial infection is rarely symptomatic, and chronic carriage is insidious. Nevertheless, it is essential to characterize the HCV situation in Egypt in order to predict the future health burden and guide appropriate health policy to confront the issue.

Mathematical techniques provide a practical approach for making reasonable estimates about the history of the HCV epidemic and future predictions of HCV-related morbidity and mortality. Methods for indirectly calculating age-specific incidence using sero-prevalence data were developed throughout the 1980's.<sup>9-11</sup> Saidel et al (1996) successfully validated one of these methods for HIV by comparing its predictions with observations from an incidence study.<sup>12</sup> Such a method is advantageous, as it uses data from a single cross-sectional survey to estimate incidence, and is particularly appropriate for developing countries where incidence data are frequently unavailable or unreliable.

The purpose of this study was to indirectly estimate the past incidence of HCV infections in Egypt using sero-prevalence data and estimate future HCV-related morbidity and mortality using a Markov natural history model.

## **METHODS**

**Prevalence and population data.** Age-sex-specific population and mortality data for five-year age groups were obtained from Egypt's Central Agency for Public Mobilization and Statistics (CAPMAS) and the World Health Organization (WHO).<sup>13-14</sup>

Prior systematic review of the literature identified high quality HCV prevalence studies for the period 1992-2005. A detailed description of these studies and their methods are presented in Chapter 4. We selected five studies from the period 2001-2002, which we considered to have the greatest amount of detail and the highest methodological quality (Table 5.2).<sup>4,15-18</sup> All studies were conducted on apparently healthy community-based populations. Third generation ELISA was used in all cases to test for HCV antibody. These studies covered ages 5-60+ years as well as geographic regions of interest (Upper and Lower Egypt; see Chapter 4 for detailed discussion). Since it is known that prevalence varies significantly between children and adults as well as between Upper and Lower Egypt, this sample of studies is highly representative of Egypt when considered in aggregate.

Age-specific prevalences across studies were compared using the chi-square test to determine if pooling results would be appropriate. No significant differences were found among adults from Lower Egypt, children from Lower Egypt, adults from Upper Egypt, or children from Upper Egypt. Summary age-specific prevalence measures as well as male:female ratios from these studies were calculated as weighted averages, using a standard method.<sup>19</sup> The individual prevalence rate for each study was multiplied by the corresponding sample size and divided by the total (pooled) sample size of all studies. To populate the initial values of the HCV Infection health state in the Markov model, these



summary age-specific prevalence proportions (adjusted for sex) were multiplied by their respective census population values.

**Incidence estimates.** The method for estimating age-specific incidence from prevalence data was originally described by Leske (1981), Podgor (1983), and Podgor and Leske (1986).<sup>9-11</sup> Though initially developed for studying glaucoma, Laidel (1996) tested model predictions against observed incidence for HIV/AIDS and determined the model can provide crude estimates of one-year age-specific incidence rates that are similar to those observed in longitudinal studies of HIV/AIDS.<sup>12</sup> For these calculations, we chose to select only one of the studies mentioned above, so as not to introduce bias. Since we are inferring incidence measures, it is best not to pool studies where HCV seroprevalence was measured by different researchers on different populations. We chose to focus our investigation of incidence on the data from Arafa, et al (2005), which presents age-specific prevalence from an area in Lower Egypt, the region known to have higher levels of HCV in Egypt.<sup>6,17</sup>

Incidence is derived by using estimated prevalence of disease at the beginning and end of an age interval and the known age-specific mortality rates during the interval. The model is based on the functional relation between a risk (CI) and a rate (ID) that:

$$CI = 1 - e^{-(ID \times \Delta t)}$$

Where ID is incidence density, CI is cumulative incidence, and  $\Delta t$  is the elapsed time (which could be defined by age differences), as long as the assumption of constant incidence within a time interval is not violated.<sup>12</sup> After accounting for age-specific mortality within age intervals, Leske (1981) proposes the following:<sup>9</sup>

$$I_x = \frac{\ln(1-\Pi_x)}{\ln(1-q_x) + \ln(1 - \Pi_x)} \times \frac{(1 - (1 - \Pi_x)(1 - q_x))}{(1 - \frac{1}{2}q_x)}$$

Where

$I_x$  = Incidence rate for the age interval

$P_x$  = Prevalence proportion at the beginning of the interval

$q_x$  = Probability of dying during the age interval

$x+1$  = Age interval immediately following  $x$

$$\Pi_x = \frac{P_{x+1} - P_x}{1 - P_x}$$

After solving for  $I_x$ , it is divided by the number of years in the age interval to estimate the mean annual rate for time interval  $x$ . In 1983, Podgor validated this model against other, more established methods.<sup>10</sup> For more details on the derivation and validation of this method, see Leske (1981), Podgor (1983), Podgor and Leske (1986), and Saidel (1996).<sup>9-12</sup> Variance and standard error for model estimates were calculated using the “delta method” described by Armitage (1971) and Leske (1981).<sup>9,20</sup>

$$\text{Var}(I_x) = [\partial I_x / \partial \Pi_x]^2 \text{Var}(\Pi_x)$$

In order to use this model, it is necessary to address its assumptions.<sup>9-10,12</sup> First, we assume that infection is lifelong and irreversible. We limit our analysis to chronically infected individuals, as the rate of remission is quite low. In addition, treatment for HCV in Egypt is limited and rarely successful in treating infection. The second assumption is that mortality rates are constant throughout the interval of analysis. By limiting age intervals to 5- or 10-year periods, it is reasonable to assume little fluctuation in mean mortality rates. In sensitivity analyses, we found the estimates to be fairly stable in the presence of varying mortality rates. The final assumption states that disease incidence and population composition with respect to risk factors for disease are constant during the

interval of analysis. Since the prevalence of HCV is high, it is possible that the composition of risk groups may change over time. Nevertheless, by restricting our age intervals to 5- or 10-year periods, we are limiting the degree to which we violate the constancy assumption. Unfortunately, the model estimates are not robust when one age interval is followed by an interval with lower prevalence (results in a negative incidence). For the purpose of this analysis, we excluded estimates under those conditions.

**Future HCV-related morbidity/mortality.** We used a Markov simulation model to predict HCV-related morbidity and mortality among a cohort representative of the HCV-infected population in Egypt (Figure 5.1). Cohort members move among health states over 1-year time steps for 20 years. By tracking the proportion of the cohort developing sequelae, we are able to estimate the future health burden. The likelihood of health state transitions was based on probabilities derived from a review of the literature as well as publications from the WHO Hepatitis C Working Group (Table 5.1) [Appendix 5.1]. Based on error estimates reported in the literature and cited by the WHO Hepatitis C Working Group, we performed sensitivity analysis on four of the transition parameters. The upper and lower bounds used in that analysis are presented in Table 5.1.

The model is set up to include ages 0 to 80 (time step = 1 year). Anyone alive at age 80 years dies by age 81. Since life expectancy in Egypt is 69 for males and 73 for females, and the population over 80 years accounts for only 0.3% of the total population, we found this age structure to be reasonable.<sup>14</sup> In order to incorporate aging into the model, we added the probability of remaining in a health state from one time step to the next, which is simply  $[1 - \text{sum}(\text{transition probabilities to different health states})]$ . This exists for all health states except the original HCV Infection. No one remains in the HCV

infection health state after the first time step, when people are either moved to chronic infection, or removed from the system in the resolution health state.

Age-sex-specific background mortality rates were obtained from the WHO and are applied to the following health states: chronic HCV infection, compensated cirrhosis, and decompensated cirrhosis.<sup>14</sup> All deaths from hepatocellular carcinoma are considered to be attributed to it. No deaths occur in the first time step. Typically, hepatitis C infection resolves or becomes chronic within the first year of infection.<sup>1-2</sup> Since our prevalence estimates are population-based, the first time step is designed to appropriately calculate the number of chronically infected individuals.

Sex is incorporated only insofar as generating the initial population structure and applying age-sex-specific background mortality. Though we hypothesize that transition probabilities may indeed be different by sex, they have yet to be clearly defined in the literature and from prospective cohort studies. Since previous research has indicated a greater proportion of males are infected in Egypt versus females (Chapter 4), sex is included to reflect these different initial values. For the Markov model alone, it provides little beyond the initial values and the ability to project sex-specific health outcomes.

We estimated the burden related to disability or mortality from hepatitis C. We used the computer simulation to determine the years of life spent by the cohort with decompensated cirrhosis or hepatocellular carcinoma. To estimate the years of life lost, we also compared life expectancy estimates for the chronic hepatitis C population with those for an age- and sex-matched general population. These estimates calculated premature mortality and assumed permanent disability once patients developed decompensated cirrhosis or hepatocellular carcinoma but did not consider quality-of-life

deficits, as no such studies have been reported from Egypt that would provide appropriate quality of life information for HCV infection.

We chose not to extend the model beyond 20 years for two reasons: 1) the natural history beyond 20 years is less certain weakening our confidence in parameter estimates; and 2) estimates of disease and death are less meaningful as they would not include incident cases who became infected during the period of analysis (due to the unavailability of incidence data).

## RESULTS

**Age-specific prevalence.** All studies used in calculating summary measures had sample sizes greater than 1400, reducing the probability of bias in prevalence estimates due to low power (Table 5.2). Chi-square tests revealed no significant differences between studies measuring redundant populations (i.e. adults from Lower Egypt), so we proceeded with calculating summary measures for the natural history model (not shown).

Summary prevalence estimates were calculated for the following age groups: 5-9, 10-14, 15-19, 20-29, 30-39, 40-49, and 50+. As expected, prevalence consistently increases with age except for a decrease between the age groups 40-49 and 50+. The decrease in the final age group could be related to lower exposure to the anti-schistosomiasis therapy so tightly linked to HCV infection. Pooled estimates also revealed a male:female ratio of 1.2 for ages 0-19 years and 1.6 for individuals 20 years or older.

**Incidence estimates.** Raw prevalence measures used in the calculation of incidence (from Arafa et al, 2005) were smoothed by fitting them to a logistic curve ( $1 /$

$e^{(-a-bx)}$ ), where  $a = -4.172$  and  $b = -0.076$  (Figure 5.2). Table 5.3 presents the results from the age-specific incidence calculations. Mean annual incidence rates were obtained by dividing the model estimate by the number of years included in the age interval (5 years). Model estimates ranged from 2.01 to 25.47 per 1,000 person-years. The highest incidence rates were calculated for those 35 years and older. Such high incidence for those 35 years and older is consistent with the age cohort effect seen with respect to the PAT campaign. We were unable to estimate the incidence for those older than 50 years, because the prevalence significantly decreases beginning with the 55-59 year age group. This particular method for estimating incidence loses accuracy under these conditions and frequently results in the calculation of a negative incidence rate.

**Future HCV-related morbidity and mortality.** Table 5.4 presents the major predictions from the Markov model estimating future HCV-related morbidity and mortality in Egypt. Upper and lower bound estimates from the sensitivity analysis are also included. Based on review of the prevalence studies, approximately 8.84 million individuals (4.98 million males and 3.86 million females) are estimated to be infected with HCV in Egypt, resulting in 6.62 million with chronic infection. Our model predicted this cohort would yield a total of 127,821 deaths due to decompensated cirrhosis and 117,556 deaths due to hepatocellular carcinoma over the next 20 years. The highest proportion of deaths related to HCV will occur 10-20 years from now. Deaths due to HCC appear to peak within 15-16 years, whereas deaths due to decompensated cirrhosis only begin to flatten out in the final two years of analysis (Figure 5.3). Deaths from HCC are greater than deaths from decompensated cirrhosis earlier in the model, due to the fact that individuals diagnosed with HCC in Egypt typically do not live past one or

two years, whereas an individual with decompensated cirrhosis can live several years before succumbing to the condition.

In addition to the 244,377 HCV-related deaths predicted by the model, when compared with an age-matched general population over the same time period, the HCV-infected cohort would have accumulated 3,862,643 years of compensated cirrhosis, 750,210 years of decompensated cirrhosis, 132,894 years of hepatocellular carcinoma, and the loss of 32.86 million years of life.

## **DISCUSSION**

The objective of this study was to estimate past incidence of HCV using an indirect method and make projections about the future health burden using a natural history model. Despite Egypt's status as the country with the highest levels of hepatitis C virus in the world, few studies have been done to characterize the dynamics of transmission, how they may have changed over time, and what this means for the future of health in Egypt. There is little debate within the scientific community that the HCV epidemic is largely the result of using unsterilized needles during the PAT campaign between the 1960's and the 1980's. Numerous cross-sectional studies have found age to be a significant confounder between HCV and history of medical procedures such as receiving injections and blood transfusions.<sup>3,6,21-22</sup> Prior to the introduction of oral schistosomiasis treatments, formal medical procedures explained an overwhelming majority of HCV infections.

Unfortunately little has been done to characterize HCV dynamics now that sterilization and blood screening programs have nearly eliminated that mode of

transmission. Prevalence studies show a significantly lower level of HCV infection among younger individuals, with rural rates near 12% and urban rates at a far lower 2-3%.<sup>3-4,15-18</sup> This still exceeds the WHO's definition of high endemicity (4%), leaving Egypt's position as a leading country in the burden of HCV unchanged. Recent cross-sectional studies have found little evidence that formal medical procedures still contribute to transmission. In addition, informal medical procedures, such as circumcision performed by a barber, show significant associations but can only explain between 10-20% of the infections.<sup>3-4,6</sup> Some have suggested that intrafamilial transmission may be crucial in transmission, but the hypothesis has yet to be specifically tested.<sup>23-25</sup> Mohamed et al. (2005) found a significant association between incident cases and individuals with HCV positive family members, but they did not investigate specific routes for intrafamilial transmission. Though illuminating, such reports bring us no closer to inference regarding the future of HCV, and consequently the future of liver disease, in Egypt.

**Incidence estimates.** To address the knowledge gap noted above, we calculated past age-specific HCV incidence rates using a method that analyzes HCV prevalence data from a one-time cross-sectional survey. Though originally developed for studying chronic diseases like glaucoma, Saidel et al demonstrated the validity of this method when compared with observational data from a HIV/AIDS study.<sup>12</sup> As was expected, we identified very high incidence rates among the population older than 30 years. In the population younger than 30 years, which would be largely comprised of individuals unaffected by the PAT campaign, we noted a continuous decline in incidence rates. Despite lower incidence rates, they are still between 2 and 3 per 1,000PY among 5-14



year olds. This suggests that transmission is indeed still occurring among the younger population despite the elimination of PAT and intense measures to reduce other iatrogenic exposures. It is imperative that we conduct longitudinal studies to gather observational data both to validate these findings as well as to test hypotheses regarding the current dominating modes of transmission.

The authors are aware that the incidence estimates are only as valid as the model assumptions are robust. The model is subject to some limitations, the most important being the assumption of constant incidence rates.<sup>9-12</sup> The degree to which violation of the constancy assumption may have affected model estimates is unpredictable and difficult to assess. We were unable to acquire prevalence data for age intervals less than 5 years. It is possible that the constancy assumption was violated in some of these age groups, but based on our knowledge of the natural history of HCV and the strong influence of widespread PAT and iatrogenic exposures as the driving force of infection among the older age groups, we feel it is not unreasonable to have 5-year age intervals for this population. Since the primary modes of transmission among younger individuals have yet to be clearly identified, the probability of violating the constant incidence assumption is likely greater, but we believe we have reasonable resolution in examining incidence among the younger populations as well.

In addition, because HCV is an infectious disease with a high prevalence in Egypt, it is possible that the composition of risk groups will change as the population at risk becomes saturated with infection. Because the steady-state condition assumed by the model is only approximately met, the relation between prevalence, incidence, and duration that drives the model will only produce rough estimates.<sup>12</sup>

The Podgor and Leske-based method can result in negative estimates of the incidence rates if prevalence at an older age is sharply less than at an earlier age.<sup>9,12</sup> Such an estimate is obviously invalid, and indicates either of two things: 1) a lack of power and/or too much variability between the age groups in the data used; or 2) the model assumptions do not hold in the age range being examined. For this reason we were unable to get incidence estimates for age groups over 50 years when age-specific prevalence begins to decline. This decline among older populations supports the hypothesis that the population aged 30-50 years was the most affected by the PAT campaign. It would be valuable to gain prevalence data of the older population in smaller age group intervals and in different geographic locations to examine HCV incidence and PAT trends more closely.

Lastly, although the prevalence of HCV infection in Egypt is relatively high, the incidence of new infections needed to maintain prevalence is still low enough that it is difficult to obtain precise incidence estimates. Therefore, the model should not be used to measure changes in incidence rates over time or differences in incidence rates between risk groups. Again, this was largely the reason we chose to estimate incidence from only one study. While it was attractive to consider calculating estimates from multiple studies, it would be difficult to tease apart possible violations of assumptions when attempting to compare results or draw broader conclusions. True longitudinal incidence studies are still the best way to identify nuances in incidence trends across age, time, and space.

**Future HCV-related morbidity/mortality in Egypt.** Our natural history model predicted 127,821 deaths from decompensated cirrhosis and 117,556 deaths from hepatocellular carcinoma due to the HCV epidemic over the next 10 to 20 years. We also

calculated that the infected cohort would spend 750,210 years in the health state of decompensated cirrhosis, 132,894 years in the health state of hepatocellular carcinoma, and lose 32.86 million years of life in comparison to an age-sex-matched non-infected cohort.

Our projections may have underestimated total HCV-related morbidity and mortality for a few reasons. We decided to exclude all cases of acute hepatitis. Egypt does not have highly accurate data regarding acute infection and its natural history. For this reason, we chose to limit our natural history model and projections to those with chronic HCV infection.

In addition, we did not consider the possibility of accelerated progression among older cases or those co-infected with HBV or HIV, all of whom may be more likely to develop hepatic complications.<sup>26</sup> To date there has only been one other model constructed to look at HCV specifically in Egypt, and it fitted a model to historic data.<sup>27</sup> Though there was evidence for age and sex influencing parameter estimates, confirmatory observational data are presently not available for comparison. Consequently, we elected to work with a more conservative model, assuring a baseline, or underestimated, projection of future morbidity and mortality. The same number of people were infected with HBV and HCV during the PAT campaign; however, HBV only caused chronic infections in approximately 5% of infected individuals, whereas chronic HCV infection developed in 70% to 80%.<sup>3,6</sup> This can be explained by the natural history of HBV, where the probability of developing chronic infection decreases with age.<sup>28</sup> Since most individuals receiving PAT were 10-15 years or older, they were at less risk for developing persistent HBV infection.<sup>6</sup>

Our analysis suggested the health burden related to premature mortality or disability from decompensated cirrhosis or hepatocellular carcinoma may be very high. Despite the remarkable decline in hepatitis C since the end of the PAT campaign, mortality related to existing cases will likely continue to increase over the next 10 to 20 years, similar to the results of Deuffic-Burban et al (2006).

There is an urgent need to address this problem as HCV infection is frequently asymptomatic until cirrhosis develops, when treatment is less effective. More research regarding cost-effectiveness of screening for HCV infection and indications for treatment is needed to help guide public health policy in this area. Continued research on the natural history of hepatitis C and the development of new treatments should remain top priorities not only for Egypt, but for global health as well.

**Conclusion.** The value of these estimates is that they inform policy makers about the rates at which age-specific groups are becoming infected with HCV and what these estimates will mean in terms of future morbidity and mortality. This information is crucial, because it can lead to conclusions about the level and direction of the HCV epidemic in Egypt that might be less readily apparent from the prevalence data alone.

**Table 5.1** Health state transitions and transition probability estimates used in the Markov simulation. Upper and lower bound estimates for sensitivity analysis were determined based on error associated with parameter estimates found in the literature.

Health State (i)	Health State (i+1)	Annual Probability	Lower Bound	Upper Bound
HCV Infection	Chronic Infection	0.75	0.50	0.85
	Infection Resolution	$1 - P(\text{Chronic})$		
Chronic Infection	Compensated Cirrhosis*	0.05/20 (<40 years)		
	Background Mortality**	0.20/20 (40+ years)		
Compensated Cirrhosis	Decompensated Cirrhosis	0.065	0.04	0.092
	Hepatocellular Carcinoma	0.035	0.024	0.046
Decompensated Cirrhosis	Background Mortality**			
	Death from D Cirrhosis	0.186	0.137	0.300
Hepatocellular Carcinoma	Background Mortality**			
	Death from HCC***	0.95		

\* The compensated cirrhosis transition probability is 0.05 over 20 years if the individual was initially infected with HCV prior to 40 years of age. The transition probability is 0.2 over 20 years if the individual was aged 40 years or older at the time of initial HCV infection [Appendix 5.1].

\*\* Background mortality is defined by age-sex-specific rates obtained from WHO [14].

\*\*\* All death from hepatocellular carcinoma is assumed to be attributed to it.

**Table 5.2.** Data sources for calculating summary age-specific prevalence of HCV infection used in both the incidence estimates and Markov models.

<b>Author</b>	<b>Year</b>	<b>Region of Egypt</b>	<b>N</b>
Mohamed, et al <sup>15</sup>	2001	Upper	2808
Mohamed, et al <sup>16</sup>	2001	Lower	1823
Arafa, et al <sup>17</sup>	2002	Upper	2261
Medhat, et al <sup>18</sup>	2002	Lower	6033
El-Sadawy, et al <sup>4</sup>	2002	Lower	1422

**Table 5.3.** Indirectly estimated age-specific incidence rates for HCV in Lower Egypt using smoothed age-specific prevalence measures. Data were fit to a logistic curve:  $1 / e^{(-a-bx)}$  where  $a = -4.172$ ;  $b = -0.076$ .

Age Group (Yrs)	Prevalence (%) [Raw]	Prevalence (%) [Smooth]	Incidence (SE)	Mean Annual Incidence per 1,000PY
5-9	1.0	2.2	0.010 (0.01)	2.01
10-14	3.0	3.2	0.014 (0.01)	2.89
15-19	5.0	4.6	0.021 (0.01)	4.13
20-24	7.5	6.5	0.029 (0.01)	5.85
25-29	7.5	9.3	0.041 (0.03)	8.19
30-34	12.5	13.0	0.056 (0.03)	11.28
35-39	22.0	17.9	0.076 (0.04)	15.21
40-44	33.0	24.1	0.10 (0.05)	19.99
45-49	35.5	31.7	0.127 (0.06)	25.47
50-54	40.5	40.4	-----	-----
55-59*	32.0	49.8	-----	-----
60+*	22.5	59.1	-----	-----

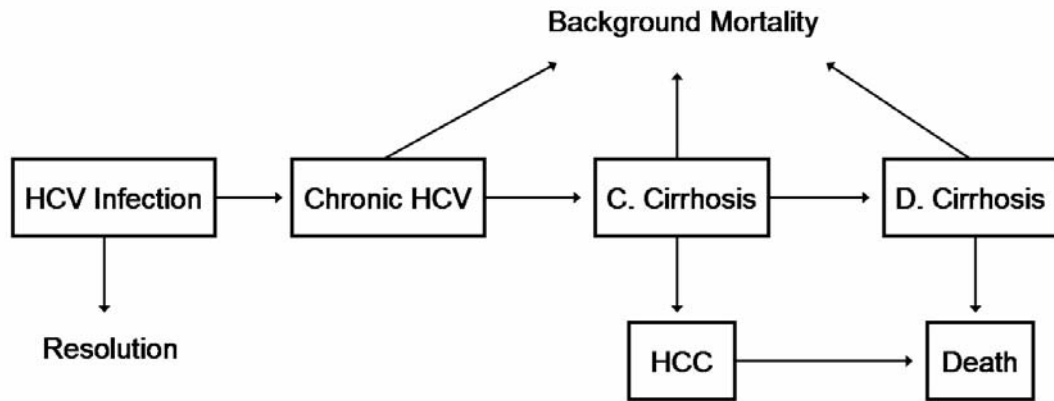
\*Not included in incidence calculations to preserve integrity of the smoothed prevalence estimates

**Table 5.4.** Predicted HCV-related morbidity and mortality, measured as life years or number of deaths. Upper and Lower bound estimates were obtained by varying parameter estimates in accordance with their margin of error as indicated by the literature.

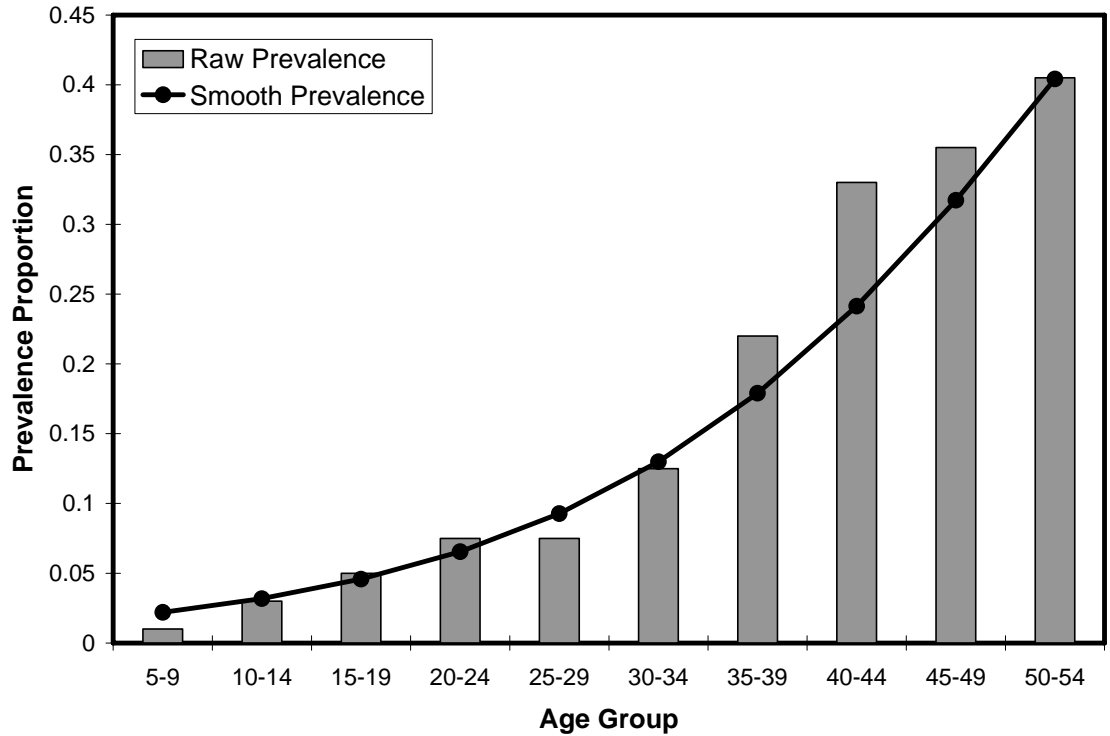
<b>Health State</b>	<b>Estimate</b>	<b>Lower Bound</b>	<b>Upper Bound</b>
Compensated Cirrhosis (Life Years)	3,862,643	2,902,917	3,881,476
Decompensated Cirrhosis (Life Years)	750,210	392,042	818,895
Hepatocellular Carcinoma (Life Years)	132,894	68,132	176,333
Decompensated Cirrhosis (Mortality)	127,821	48,728	227,701
Hepatocellular Carcinoma (Mortality)	117,556	59,960	156,713



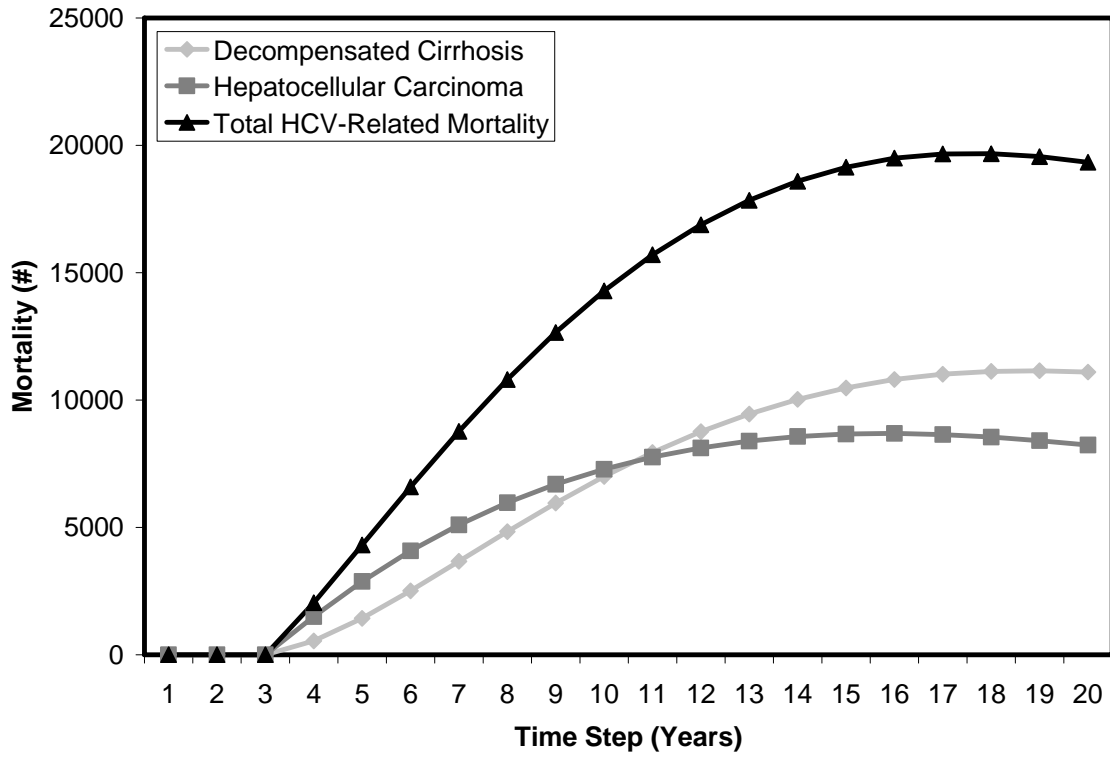
**Figure 5.1.** Natural history model for hepatitis C virus infection and its sequelae.



**Figure 5.2.** Raw age-specific HCV prevalence proportion in Lower Egypt, 2002 (Data from Arafa et al, 2005) plotted with smoothed age-specific prevalence estimates.<sup>17</sup> Prevalence data were fit to a logistic curve:  $1 / e^{(-a-bx)}$  where  $a = -4.172$ ;  $b = -0.076$ .



**Figure 5.3.** Predicted mortality due to HCV-related decompensated cirrhosis and hepatocellular carcinoma over a 20 year period.



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## APPENDIX 5.1: LITERATURE USED IN CREATING MARKOV MODEL PARAMETERS

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## **CHAPTER VI**

### **CONCLUSIONS**

#### **SUMMARY OF MAJOR FINDINGS AND RESEARCH IMPLICATIONS**

This study presents various findings that are similar to, but also different from, those that have previously addressed HCC in the Middle Eastern region. As has been shown consistently in other studies, male incidence was considerably greater than that for females. Additionally, the incidence rate of HCC in Gharbiah was 3.5 times higher than that reported in the United States. Such results were seen in the overall age-adjusted incidence rates, as well as the age-specific rates in age groups older than 40 years, among both males and females. This finding expands upon reports in the United States NCI and MECC publications for a shorter period, 1999-2001.<sup>1</sup> Our similar findings for a longer period suggest that variation in rates truly reflect different risk factor profiles of these two populations, warranting further prospective studies.

The most notable finding of this study, however, was the statistically significant geographic variation in incidence of HCC among districts within Gharbiah province. Incidence ranged from 12.9/100,000PY (Kotour) to less than half that rate at 6.1/100,000PY (Samanoud). Districts were similar with respect to age distribution and sex-ratios, suggesting that the at-risk populations were fairly homogeneous. Consequently, this observed heterogeneity is likely attributable to variation in local risk

factors that future studies may investigate. One feature that was consistent across districts was that HCC incidence among urban individuals was nearly twice that of rural residents, in contrast with reports that HCV prevalence is higher among rural residents.

Significant variation in incidence at the district-level provided the motivation for more formal spatial analyses to test the hypothesis that HCC spatially clusters in Gharbiah Province. We applied two cluster detection methods that generate test statistics through independent means. Both methods detected significant high- and low-risk clusters that were spatially similar, suggesting our results are robust. When combined with the fact that this population is quite stable and relatively homogeneous in terms of confounders, our findings suggest an underlying clustering of primary HCC risk factors.

In the absence of detailed natural history or incidence studies, we used seroprevalence and cross-sectional data to help guide our inference. Reports concerning HBV have suggested a relatively homogeneous geographic distribution of infection, with overall declining rates due to the successful implementation of the HBV vaccine.<sup>2-3</sup> Studies examining HCV have shown higher levels of infection among residents in Lower Egypt versus those in Upper Egypt.<sup>1,4</sup> Higher levels of HCV have also been observed in rural regions as opposed to urban areas.<sup>1,4</sup> Several studies have suggested that HCC is increasingly associated with HCV, and this trend is expected to continue as HBV rates decline.<sup>1, 4-5</sup>

Aside from understanding viral dynamics, there is also increasing interest in the role environmental contaminants may play in the development of HCC in Egypt. To address this concern in an exploratory way, we examined the relationship between village-level HCC incidence and distance to factories and drainage canals. Factories can

produce a great deal of contaminants that may be present in the air and water of the surrounding regions. It is also likely that employees of the factories reside in nearby villages. Drainage canals are another potentially point source for environmental contaminants. These waterways receive the vast majority of water-related factory waste as well as any agricultural runoff (pesticides, etc). We were unable to find any association between incidence and distance to possible environmental point sources. This does not necessarily reject the significance of such factors, but it may suggest that their underlying contribution to HCC burden is obscured by more dominant factors, i.e. HBV and HCV.

Since information regarding schistosomiasis and/or HCV infection were unavailable for the HCC cases included in this study, we opted to examine the PAT hypothesis from a new angle. We hypothesized that if such a strong link existed between schistosomiasis and HCC (via PAT), it should be reflected in the examination of the one type of cancer most associated with schistosomiasis of the urinary bladder: squamous cell carcinoma.

Upon repeating our methods for the cluster analysis of HCC for squamous cell carcinoma, we again found significant high- and low-risk regions with strong agreement across the two methods. In addition we found strong overlap in the primary high-risk cluster for both cancers as well as for all low-risk clusters except one for squamous cell carcinoma. Our test for correlation between spatial clusters of HCC and SCC-B found a strong association among low clusters and a marginal association among high clusters. Though we do not suggest that all spatial variation in HCC can be explained by squamous cell carcinoma, the overlap present among the majority of clusters, which was

identified by two separate cluster detection methods, seems hardly a coincidence. Of particular importance is the fact that this cohort of cases represents a population still burdened by schistosomiasis as well as the early wave of individuals affected by the PAT campaign. We believe our findings support the strong connection between schistosomiasis and HCC, but more research is necessary to determine if the association is due to chronic schistosomiasis infection or exposure to PAT treatment.

Motivated by the absence of viral data for the HCC cases as well as the general lack of natural history or incidence studies characterizing the dynamics of HBV and HCV in Egypt, we conducted a systematic review and meta-analysis of HBV and HCV seroprevalence. Our analysis confirmed reports of geographic heterogeneity in HCV prevalence among the apparently healthy population.<sup>1,4</sup> We found prevalence to be significantly higher in Lower Egypt as opposed to Upper Egypt, which in turn supports the hypothesis of PAT as the dominant force driving the HCV epidemic. Individuals living in Lower Egypt experienced a greater burden of schistosomiasis, and therefore a greater level exposure to PAT.<sup>1,4</sup> This is in contrast to what we saw with HBV, which appeared to be higher in Upper Egypt versus Lower Egypt.

We were also able to examine the relationship of HCV with urban and rural populations, something we had been unable to probe in our earlier GPCR studies. Findings were consistent with the hypothesis that HCV prevalence is higher among rural residents than urban residents.<sup>6-7</sup> This is also in line with the PAT hypothesis; that rural residents would have a greater burden of schistosomiasis, and therefore greater exposure to PAT.<sup>6-7</sup> It is in contrast, however, with our earlier results showing greater risk for HCC among urban residents. Due to the significant lag time between viral infection and

development of HCC, it is possible Egypt is witnessing the end of a cohort of higher urban exposure that will soon transition to a population dominated by rural exposure. Unfortunately, we were unable to find this type of disaggregated data for HBV for comparison.

For both viruses among healthy population-based samples, we found adults had a significantly higher prevalence than children, supporting a cohort effect. For HBV, it seems likely that the cohort effect would be related to the introduction of the hepatitis B vaccine in 1992, which was incorporated into the Expanded Programme on Immunisation, and is only given to children, leaving adults at the time of program implementation unvaccinated. The cohort effect seen in HCV is likely related to the early association between the parenteral antischistosomiasis therapy (PAT) campaign and HCV transmission. Since several of the childhood HCV studies were late enough that none of the children would have been exposed to the PAT campaign, it does appear that HCV has continued to be transmitted.

In addition to age differences, we examined time (year) trends in HBV and HCV prevalence. As was expected to some degree, HBV prevalence was relatively low overall, but has experienced a slight decrease over the time period included here, probably due to the implementation of earlier control measures followed by the vaccine. Infection levels in children suggest that this infection may soon be an insignificant element of liver disease in Egypt. Prevalence of HCV, however, showed greater variance over time. Among both children and adults there was a general decline in prevalence over time, with adults stabilizing at just below 15%, and children continuing to drop to nearly 1% by the period 2000-2004. Regression analyses found significant time

interactions for both HBV and HCV, suggesting a more complicated shift in prevalence patterns over time that includes geographic changes as well.

Among HCC cases, we found the overall prevalence of HBV among HCC cases to be nearly 25%, and the prevalence of HCV infection to be as high as 84%. We also noted some differences within infections over time, though they were not statistically significant, possibly a result of low power. It appeared that HBV infection declined from a prevalence of 30% between 1991-1996 to 22% between 1997-2004. Conversely, HCV infection among HCC cases may have increased slightly over this same time period from 80 to 86%. This seems fitting when we consider the impact of different cohort effects. Current cancer cases represent individuals being exposed to these viruses 20-30 years prior. These cases may represent the individuals at the end of the pre-HBV vaccine period and the beginning of the PAT campaign. Based on general population estimates, it seems likely that prevalence of HBV will continue to decline and HCV will continue to increase among HCC cases, at least for a while. This has been supported by a projections from a mathematical model designed to predict the burden of HCV in Egypt over the next couple of decades.<sup>7</sup> What will happen after that will depend largely on the new HCV incidence patterns in the absence of the PAT campaign, which remain to be quantified by future research.

Based on the results of our meta-analysis, we pursued mathematical techniques to indirectly calculate past HCV incidence to further probe the epidemic and PAT cohort hypothesis. As was expected, we identified very high incidence rates among the population older than 30 years. In the population younger than 30 years, which would be largely comprised of individuals unaffected by the PAT campaign, we noted a continuous

decline in incidence rates. Despite lower incidence rates, they are still between 2 and 3 per 1,000PY among 5-14 year olds. This suggests that transmission is indeed still occurring among the younger population despite the elimination of PAT and intense measures to reduce other iatrogenic exposures.

In addition to reconstructing previous incidence rates and identifying a cohort of exceptionally high sero-prevalence, we constructed a Markov model to predict the burden of HCV-related liver disease in Egypt over the next 10 to 20 years, which would largely quantify the outcome of this special cohort. Our natural history model predicted 127,821 deaths from decompensated cirrhosis and 117,556 deaths from hepatocellular carcinoma due to the HCV epidemic over the next 10 to 20 years. We also calculated that the infected cohort would spend 750,210 years in the health state of decompensated cirrhosis, 132,894 years in the health state of hepatocellular carcinoma, and lose 32.86 million years of life in comparison to an age-sex-matched non-infected cohort.

**Data considerations.** Our HCC studies from Gharbiah are unique in comparison to other hospital- and clinic-based reports that have previously been published for HCC in Egypt, as our analyses were based on HCC and squamous cell carcinoma of the bladder cases from a population-based cancer registry and appear to be largely complete and highly representative. The GPCR is scientifically and financially supported by the United States National Cancer Institute, and it has quality assurance from US-SEER and the International Association of Cancer Registries. Standard procedures for training registrars and for data collection, processing, and transmission enhanced the accuracy of data. In addition, the population-based structure improves our ability to draw conclusions about the entire province from the study results.

Our results represent a high degree of internal and external validity. Data on the vast majority of cases included complete demographic and geographic information. There was little evidence of interannual variation in case reporting for either cancer for our study period, which was particularly reassuring. In addition, external review of the data collected by the GPCR has found its coverage to exceed 90%, improving our confidence in accurate numerator estimates.<sup>8</sup> Though it is possible the number of cases could be underestimated due to misclassification, there is no reason to believe that this underestimation is biased in any significant way. Access to healthcare is similar across the province, and there is nothing to suggest that physicians diagnose differently in any systematic way. Therefore, we consider the case data that we analyzed to be valid for the populations at risk, and the results to be generalizable at least to the people of Gharbiah Province.

Denominator estimates were based on census reports from 1996 and published census projections for 2001 and 2005. We believe it was reasonable to calculate population estimates using linear interpolation, as this is a fairly stable population, experiencing the majority of its change through birth and death rates (low population mobility). Data were available at the village level and age- and sex-specific values were provided. We were unable to detect any significant differences between villages and the Province in aggregate with respect to age and sex distributions, suggesting that villages could be compared without serious concern for compromising our conclusions due to these important confounding variables. We are confident that this increases the accuracy of our calculations, since both numerator and denominator data were available at the same high level of resolution.



Spatial data were based on maps published by the Egyptian General Survey Authority, which followed international guidelines in the creation of their maps. A brief comparison of calculated point coordinates with those obtained from a handheld GPS unit for the same location suggested the maps were internally consistent and geographically accurate.

Much of the work for Chapters IV and V was based on a systematic review of the literature. It is possible that despite our extensive literature search, the specific search terms used may not have captured all quality papers published in peer-reviewed indexed journals. To counter this problem, we supplemented our electronic search by a hand search of references from selected articles, but there is always the possibility that we missed some data.

We attempted to gain representative samples of the Egyptian healthy population by accepting community-based samples, but we also included some large scale convenience samples such as healthy pregnant women attending clinics and voluntary blood donors. John, et al have questioned the inclusion of voluntary blood donors in systematic reviews, suggesting data from replacement donors would be less biased.<sup>9</sup> Batham, et al. conducted an analysis of HBV studies in Delhi that used voluntary donors, replacement donors and ante-natal mothers.<sup>10</sup> Analyses revealed that all populations had prevalences close to the overall mean, suggesting it was appropriate that all these groups be included in systematic reviews.

The extent to which the HCC cases we reported upon are representative is also unclear, since in most cases only hospital-based studies were available. We did focus on cancer studies from the larger, nationally recognized cancer centers, which serve as

diagnostic and treatment facilities for the overwhelming majority of cancer cases throughout Egypt.

In addition to the data sources used in the systematic review and calculations, it is important to consider the restrictions on inference associated with the modeling methods we selected. The incidence method used in Chapter V is subject to some limitations, the most important being the assumption of constant incidence rates.<sup>11-14</sup> It is possible that the constancy assumption was violated in some of these age groups, but based on our knowledge of the natural history of HCV and the strong influence of widespread PAT and iatrogenic exposures as the driving force of infection among the older age groups, we feel it is not unreasonable to have 5-year age intervals for this population. Since the primary modes of transmission among younger individuals have yet to be clearly identified, the probability of violating the constant incidence assumption is likely greater, but we believe we have reasonable resolution in examining incidence among the younger populations as well.

In addition, because HCV is an infectious disease with a high prevalence in Egypt, it is possible that the composition of risk groups will change as the population at risk becomes saturated with infection. Because the steady-state condition assumed by the model is only approximately met, the relation between prevalence, incidence, and duration that drives the model will only produce rough estimates.<sup>14</sup>

The Podgor and Leske-based method can result in negative estimates of the incidence rates if prevalence at an older age is sharply less than at an earlier age.<sup>11,14</sup> Such an estimate is obviously invalid, and indicates either of two things: 1) a lack of power and/or too much variability between the age groups in the data used; or 2) the model

assumptions do not hold in the age range being examined. For this reason we were unable to get incidence estimates for age groups over 50 years when age-specific prevalence begins to decline.

Lastly, although the prevalence of HCV infection in Egypt is relatively high, the incidence of new infections needed to maintain prevalence is still low enough that it is difficult to obtain precise incidence estimates. Therefore, the model should not be used to measure changes in incidence rates over time or differences in incidence rates between risk groups. Again, this was largely the reason we chose to estimate incidence from only one study. While it was attractive to consider calculating estimates from multiple studies, it would be difficult to tease apart possible violations of assumptions when attempting to compare results or draw broader conclusions. True longitudinal incidence studies are still the best way to identify nuances in incidence trends across age, time, and space.

Our Markov model projections of future burdens may have underestimated total HCV-related morbidity and mortality. We excluded all cases of acute hepatitis. Egypt does not have highly accurate data regarding acute infection and its natural history. For this reason, we chose to limit our natural history model and projections to those with chronic HCV infection.

In addition, we did not consider the possibility of accelerated progression among older cases or those co-infected with HBV or HIV, all of whom may be more likely to develop hepatic complications.<sup>15</sup> To date there has only been one other model constructed to look at HCV specifically in Egypt, which fitted parameters to historic data.<sup>7</sup> Though there was evidence for age and sex influencing parameter estimates, confirmatory observational data are presently not available for comparison.

Consequently, we elected to work with a more conservative model, assuring a baseline, or underestimated, projection of future morbidity and mortality.

### **SUGGESTIONS FOR FUTURE RESEARCH**

**Cancer Studies.** As in many developing countries, Egypt is undergoing an epidemiologic transition. With increasing urbanization, smoking rates, environmental exposures, and aging, in addition to the maturing HCV epidemic, it is likely that HCC will continue to rise for the next few decades. Therefore, further studies to assess the magnitude and risk factors of HCC in Egypt and other developing countries seem warranted. Our research produced important preliminary insights that can be used to develop more refined, prospective analyses of HCC risk in Egypt. Since chronic HCV does not typically lead to carcinogenesis for 10-30 years following infection, the rates of liver cancer can be expected to continue increasing until the cohort of PAT-related infected individuals has worked its way through.<sup>1,6</sup>

Our research suggested that spatial patterns of HCC did not seem to be influenced by environmental point sources of pollutants, but may be linked to the spatial distribution of squamous cell carcinoma of the bladder. Our use of high quality population-based data in addition to rigorous statistical methods suggests our findings are highly robust. Future studies should focus on the dynamics of HCC risk factors in order to better predict regions at greater risk for disease. These initial spatial findings have already stimulated ongoing collaborations to begin developing studies that would include case enrollment and detailed risk factor data collection to improve inference regarding the relative significance of different risk factors in Egypt. We hope these analyses will help untangle

the complex etiology of HCC and assist policy makers in generating more efficient prevention and control programs.

**Viral Dynamics.** It is understood that only a large, national epidemiological study can provide a definitive answer regarding the overall situation of hepatitis B and C viruses in Egypt. Incidence data are essential to clarify the future of the HCV epidemic in Egypt. The PAT crisis has ended; no new infections are being generated through that route. It is imperative that we determine present modes and rates of transmission to refine prevention efforts. Detailed risk factor data are needed to further characterize the shapes of the HBV and HCV infection curves and distinguish between the effects of population migration, changing risk factor patterns, etc. It would also be valuable to match prevalence figures with incidence studies to reconstruct the different epidemic curves, shedding light on viral dynamics and guiding predictions about future trends in infection.

There is an urgent need to address this problem as HCV infection is frequently asymptomatic until cirrhosis develops, when treatment is less effective. More research regarding cost-effectiveness of screening for HCV infection and indications for treatment is needed to help guide public health policy in this area. Continued research on the natural history of hepatitis C and the development of new treatments should remain top priorities not only for Egypt, but for global health as well.

## CONCLUSIONS

Our findings highlight the significance of continuing prevention of HBV through vaccination campaigns as well as the development of an integrated strategy for the

prevention of HCV infection that should include screening of blood donations, safe injection practices, and avoidance of unnecessary injections. The value of results from mathematical techniques such as those presented here is that they begin to inform policy makers about the rates at which age-specific groups are becoming infected with HCV and what these estimates will mean in terms of future morbidity and mortality until the observational data are available to further refine estimates and predictions.

Ongoing collaborations are building upon these preliminary findings to develop studies that will examine HCC cases from the point of intake and acquire HBV/HCV test results to expand our inference regarding the relative importance of certain risk factors on HCC in Egypt. We have also embarked upon discussions of incidence and natural history studies focused on HCV. This information is crucial, because it can provide clues about the level and direction of the HCV epidemic in Egypt that might be less readily apparent from the prevalence data alone. Such analyses should help define the complex etiology of liver disease in Egypt, enabling policy makers to create targeted, more efficient prevention and control programs.

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