Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: Possibilities for prevention

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Review

Changing pattern of hepatocellular carcinoma (HCC) and its risk factors in Egypt: Possibilities for prevention

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Abstract

The burden of hepatocellular carcinoma (HCC) has been increasing in Egypt with a doubling in the incidence rate in the past 10 years. This has been attributed to several biological (e.g. hepatitis B and C virus infection) and environmental factors (e.g. aflatoxin, AF). Other factors such as cigarette smoking, occupational exposure to chemicals such as pesticides, and endemic infections in the community, such as schistosomiasis, may have additional roles in the etiology or progression of the disease. Estimates of the burden of cancer caused by these factors provide an opportunity for prevention. Previously, there was strong evidence that hepatitis B virus (HBV) was the major cause of HCC in Egypt, but more recently HCV has become the predominant factor associated with the more recent epidemic of HCC. It has been well documented that Egypt has one of the highest prevalence rates of HCV infection in the world. The natural history of HCV infection and disease progression, however, are influenced by additional factors such as duration of infection, age at infection, sex, co-infection with HBV, the level of HCV viraemia and its genotype. The role of exposure to aflatoxins and development of HCC in Egypt was historically less clear. Nevertheless, recent food sampling surveys and population-based studies indicated that exposure to aflatoxins in Egypt may have been underestimated in the past. Recent results indicated that both local and imported samples were positive for aflatoxin B1 (AFB1, 17.5% and 20%, respectively), with concentrations ranging from 3 to 25 μg/kg. The level of AFB1 was dependent on the area of collection as well as the season of the year. In a population-based study, the level and frequency of aflatoxin M1 (AFM1, a major metabolite of aflatoxin B1 excreted in breast milk) was assessed as a biomarker of maternal exposure. The samples were collected from a selected group of 388 Egyptian lactating mothers during May–September 2003. Non-working status, obesity, high corn oil consumption, and the number of offspring contributed to the variability in occurrence of AFM1 in breast milk. Prevention and intervention approaches directed to risk factors of HCC can play a critical role in its prevention. In the case of HCV infection a prevention programme can be achieved by changing personal behaviors and/or cultural habits which are risk factors for HCV transmission, such as injection with contaminated syringes, blood transfusion, surgical operations, venous catheterization, use of common syringes, dental treatment and circumcision at home. Prevention of exposure to aflatoxins can be achieved either at community (via good agriculture practices) or individual levels (treatment or dietary interventions). In conclusion, due to the alarming increase in the incidence of HCC in Egypt, there is a need to further investigate the contribution of these emerging risk factors to the development of HCC in Egypt. This may enable us to determine the susceptibility to HCC among high-risk groups and to provide these individuals with effective measures for early prevention or intervention.

Keywords: Hepatocellular carcinoma; Prevalence; Risk factors; HCV; HBV; Aflatoxins; Prevention

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Contents

1. Introduction

Hepatocellular carcinoma (HCC) is a major cause of cancer death worldwide, accounting for over half a million deaths per year [1,2]. The geographic pattern of HCC incidence is parallel to exposure to viral etiologic factors. Its incidence is increasing, ranging between 3% and 9% annually depending on the geographical location [3], and variability in the incidence rates correspond closely to the prevalence and pattern of the primary etiologic factors. According to recent reports, the incidence of HCC has increased sharply in the last 5–10 years [3–5], with an especially high incidence in Egypt [6].

Chronic infections with HBV or HCV have both been recognized as human liver carcinogens with a combined attributable fraction of at least 75% of all HCC cases [1]. HBV is considered as a major risk factor for the progression to liver cirrhosis and HCC [7]. The relative risk of developing HCC for HBV carriers may be 100–200-fold higher than that for non-carriers [8]. HCV is also an important etiologic factor for HCC, with an estimated attributable fraction of 23% [9]. Yates et al. [10] reported that 67% of HCC patients were HCV seropositive compared to 30% of the controls. Several lines of evidence indicate a strong causal association between HCV and HCC, as shown by the raised prevalence of anti-HCV [3,11] and/or HCV-RNA [12] in patients with HCC.

The role of HBV infection in pathogenesis of HCC differs from that of HCV infection; HBV-DNA genome integrates in hepatocyte chromosomes [13]. The fundamental mechanism by which HCV is related to HCC is not definitely known. Most researchers believe that HCV plays an indirect role in hepatocarcinogenesis through production of cirrhosis with chronic inflammation leading to severe liver damage [14,15]. More recently, however, some reports suggest a direct role of HCV in hepatocarcinogenesis, due to the possible carcinogenic action of the HCV core protein in inducing liver cell proliferation [13,16]. However, it seems that cirrhosis is the common pathway by which several risk factors exert their carcinogenic effect [17].

Significant variations occur in the risk for HCC and in the pathological and natural history of the disease. The pathways by which HCC develop are influenced by a variety of environmental and host factors such as the age or gender of the infected person and the genetic characteristics of the virus in cases of HBV or HCV infections. The role of other carcinogens, such as aflatoxin exposure, as additional risk factors for the development of HCC remain to be fully explored in Egypt, despite evidence elsewhere that such exposures may damage the DNA in liver cells and lead to mutations in the p53 tumor suppressor gene [16]. Critical evaluation of molecular markers of viral (HBV, HCV), environmental (aflatoxin) and genetic factors in well characterized HCC cases and appropriate control groups may provide an improved mechanistic understanding of hepatocarcinogenesis.

In this paper, we summarize several research studies on the relationship between HCC, viral hepatitis and aflatoxins which were carried out in Egypt. Our goal is to summarize the emerging pattern and distribution of exposures to etiologic factors for the development of preventive interventions.

2. Epidemiology of liver cancer in Egypt

In Egypt, several attempts were made to establish cancer registries. Among these attempts in 1998, the Egyptian Ministry of Health and Population in collaboration with several partners established a population-based Cancer Registry (NCR) in Gharbiah Governorate, in addition to a multi-institutional cancer statistics collected in collaboration with the National Cancer Institute of Cairo University [6]. This was intended to estimate the size of the problem nationally. The NCR data confirmed the high incidence of HCC in Egypt and the change in the trends during the last decade (Figs. 1 and 2). The National

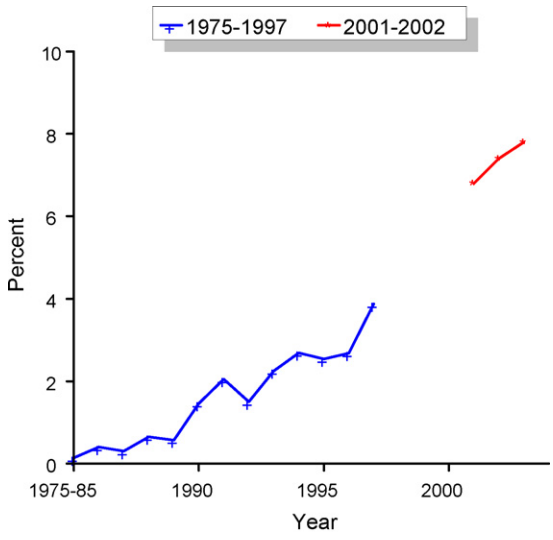

Fig. 1. Trends in frequency of liver cancer, in Egypt according to the National Cancer Institutes records, NCI 1975–2003 [6].

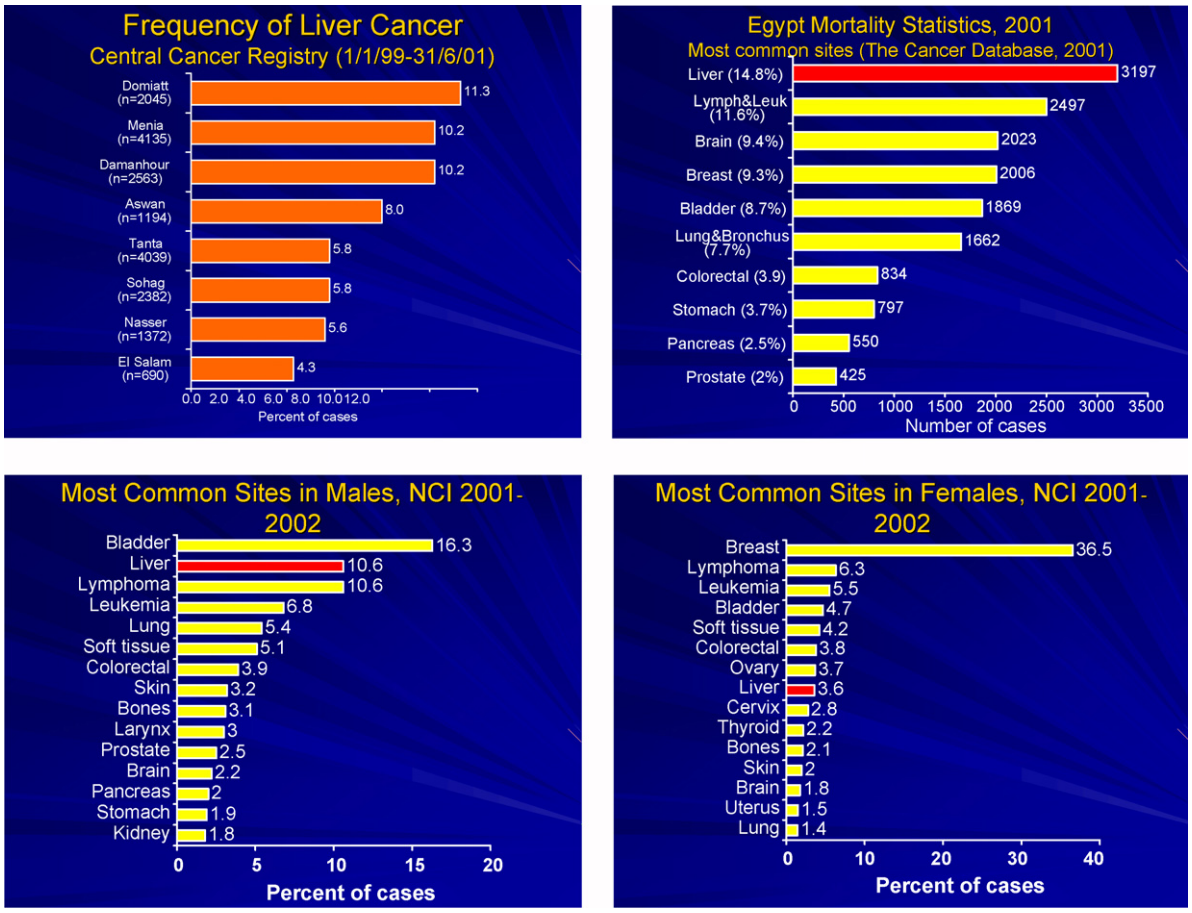

Fig. 2. Frequency of liver cancer, in Egypt according to the National Cancer Institutes records, NCI 1975–2003 [6].

Cancer Institute Pathology Registry indicated that liver cancer formed 11.75% of the malignancies of all digestive organs and 1.68% of total malignancies. Liver tumors, as seen in Fig. 3, were mostly HCC (70.48%), while hepatoblastoma constituted 10.24%, non-Hodgkin's lymphoma 4.21% of hepatic malignancies and adenocarcinoma unspecified 9.03% [18].

In El Zayadi's study in 2005 [19], the trends and pattern changes of HCC in Egypt over the past decade, as well as the possible risk factors were studied. The duration of the study was divided into two periods of 5 years each: period I (1993–1997) and period II (1998–2002). Over this decade, 1328 HCC patients (5.9%) out of 22,450 chronic liver disease (CLD) patients were diagnosed. The annual proportion of HCC showed a significant rising trend from 4.0% in 1993 to 7.2% in 2002. In the statistical analysis, HCC was significantly more prevalent in rural residents, and patients with a history of schistosomiasis and/or blood transfusion. There was a significant decline of HBsAg from 38.6% to 20.5%, and a slight increase of HCV-Ab from 85.6% to 87.9% in periods I and II, respectively. HBV conferred a higher risk to develop HCC more than HCV in period and period II, but the relative contribution of HBV for development of HCC declined in period II compared to period I.

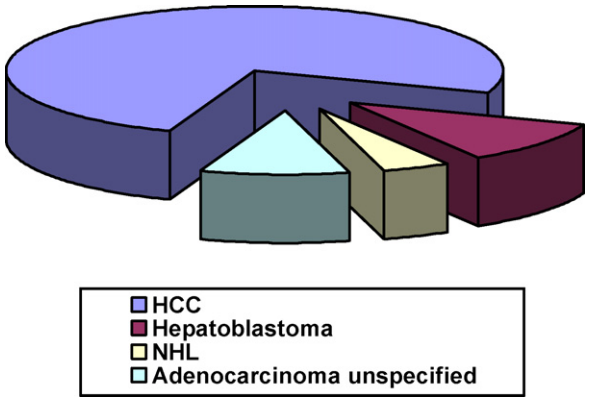

Fig. 3. Proportion of types of malignant liver tumors in Egypt [18].

This rising incidence of HCC in Egypt may be explained by the increasing prevalence of risk factors such as the emergence of hepatitis C virus (HCV) over the same period of time [20], the contribution of HBV infection, and improvements in screening programmes and diagnostic tools [21], as well as the increased survival rate among patients with cirrhosis allowing time for some of them to develop HCC. In part to assess the relative contributions of all these risk factors, Hassan and colleagues [22] studied the attributable risk of HCV in Egyptian patients with HCC. Thirty-three patients with HCC and 35 healthy controls who had a similar socioeconomic status were prospectively enrolled at the University of Cairo National Cancer Institute. Anti-HCV antibodies were present in 75.8% of the patients and in 42.9% of the controls; HBsAg was present in

15.2% of the patients and in 2.9% of the controls. They estimated the attributable fraction of HCC to HCV to be 64% in this study population and 48% in the general Egyptian population. The study concluded that both HCV and HBV infection increase the risk of HCC in Egyptian patients, whereas isolated schistosomal infection does not. A much larger case–control study with over 200 patients in each group recently reported similar findings [23] but with an even higher estimate of the attributable fraction (around 90%) of current HCC due to chronic HCV infection. This likely represents the current status of HCV as the predominant and increasing cause underlying the epidemic of HCC in Egypt.

Earlier research efforts to discover the causes of HCC in Egypt focused on a variety of tropical diseases, including schistosomiasis and its relation to HCV infection. The changes in prevalence of HCV is very well correlated in time and geographical regions to the mass treatment injection campaigns for Schistosomiasis during the years 1950–1980 [20,24,25], Schistosomiasis also induces immune suppression, which could result in increased persistence viraemia, following acute infection of either hepatitis B or C [23]. The combination of Schistosomiasis and HCV infection was found to have more severe effects on liver pathology and progression into worsening complications than HCV infection alone. Fortunately, recent epidemiologic surveys have reported a declining prevalence of Schistosoma infection [26].

3. Viral hepatitis and HCC

Extensive collaborative research was carried out during the last decade to explore the independent and combined effects of HBV and HCV and other factors in the etiology of HCC. Although HBV is considered worldwide as a major risk factor for liver cirrhosis and HCC, the prevalence of HBV infection in Egypt has been declining over the last two decades [19], while HCV has increased [4]. Egypt has possibly the highest HCV prevalence worldwide [27], estimated among the general population to be around 14% [28]. Yet, much of the HCV prevalence data are limited by variability in and selectivity of the populations studied, inconsistent HCV testing methods, and a lack of data regarding mode of transmission.

In 1997, the Egyptian Ministry of Health and Population initiated the National Hepatitis C Research Laboratory at the National Tropical Diseases Institute, the first in Egypt designed to assess the prevalence of HCV infection and evaluate intervention programmes, with the main goal of preventing chronic liver disease and HCC. According to a report from this programme in 2004 [20], high HCV rates were observed among rural residents, reaching up to 20%. Estimates of national prevalence of HCV and HBV infections were derived from two large community-based surveys in 1996 for estimation of viral hepatitis B and C prevalence in Egypt. In the first study, serum HCV antibody was analyzed for 5000 individuals in a survey of Egyptian workers tested in pre-employment health examination. In the second national survey, serum HCV antibody and HBsAg were analyzed for 7357 individuals in a cluster household study design of representative Egyptian communities in 10 selected governorates representing the four major regional areas of Egypt: metropolitan areas, Lower Egypt, Upper Egypt, and seashore and border areas. The overall anti-HCV prevalence was 18%. The overall adjusted prevalence of anti-HCV and HBsAg was 13.5% and 4.5%, respectively. Rural areas had higher age and gender adjusted prevalence (17.2% and 5.5%) than urban areas (7.8% and 3.8%) of these markers respectively. In the same study [20], individuals living in rural areas had significantly more anti-HCV seropostivity than those in urban areas (36.1% and 24.7%, respectively). Also individuals living in Cairo and seashore governorates had lower HCV seropostivity (14.7% and 12.7%, respectively) than those living in governorates of Upper Egypt and Lower Egypt (29.3% and 36.3%, respectively) (Table 1).

At the same time that the above prevalence surveys were carried out, studies of the HCV genome confirmed a uniquely high proportion of genotype 4 (over 90%) in Egypt [29,30], and have traced back the genomic mutation for HCV RNA in different samples in Egypt analyzed by both restriction fragments length polymorphism (RFLP) and phylogenic tree construction. To further explore the genetic diversity of HCV in Egypt, sera from 131 Egyptians (56 from community studies, 37 chronic hepatitis patients, 28 HCC patients and 10 patients with non-Hodgkin's lymphoma) were examined [31]. The majority of the viruses (83 of 131; 63%) were of subtype 4a, but five other subtypes within genotype 4 were also observed, as

Table 1
HCV seropostivity, gender and age groups (national workers surveys, 1996) [20]

Age (year) group	Total no.	HCV+ve (%)	Total males no.	HCV+ve (%)	Total females no.	HCV+ve (%)
<5 years	908	12.8	684	14.3	224	8.0
>5 years	4105	35.7	3738	37.6	367	16.3
Residence						
Rural	2987	36.1	2776	37.8	211	13.7
Urban	1978	24.7	1606	27.5	372	12.6
Region						
Cairo	456	14.7	310	18.4	146	6.8
Upper Egypt	1308	29.3	1219	31.0	89	6.7
Lower Egypt	2998	36.3	2687	38.4	311	18.0
Seashores	220	12.7	183	13.1	37	10.8

well as three genotype 1b, five genotype 1g and one-genotype 3a samples. Interestingly, subtype 4o, which was easily identifiable in all three genomic regions, showed an association with HCC, which merits further investigation.

Other surveys conducted during this same time period were designed to assess the modes of HCV transmission in Egypt. The major identified risk factors for acquisition of HCV in Egypt include intravenous injection, body piercing and invasive health care practice (surgeries catheterization dental practice, etc.) and occupational exposure [32]. For many individual cases, however, there is no identifiable risk factor, highlighting the critical need for further research on this issue. Due to the large reservoir in this population, HCV is likely to remain prevalent in Egypt for several decades [33].

4. Aflatoxins and HCC

While HBV and HCV may account for the majority of HCC in Egypt, there is suggestive evidence for an additional etiologic role of aflatoxin in hepatocarcinogenesis. Aflatoxins are toxic and carcinogenic metabolites of moulds, mainly *Aspergillus flavus* and *parasiticum* that contaminate a variety of agricultural commodities, particularly peanuts, maize and cottonseed, in countries with hot and humid climates. Aflatoxin B1 (AFB1) is the major metabolite produced by these moulds. Aflatoxins are classified as human carcinogens based on sufficient evidence of carcinogenicity (IARC, 1987) [34].

Mohamed et al. [35] detected a significant higher percent of aflatoxins in the serum of Egyptian patients with HCC compared to their controls; with a twofold increased risk. Aflatoxins may cause mutations in the tumor suppressor gene p53 that act as initiating agents leading to liver cell hyperplasia and HCC. In support of this hypothesis, Kafrawy et al. [36] documented the presence of p53 codon 249 mutations associated with aflatoxin exposure in a sample of HCC tumor tissues analyzed by gene chip analysis in Egypt.

Hifnawy et al. [37] suggested that the progressive nature of HCV-related liver diseases was influenced by aflatoxin exposure. The quantitative identification of the possible aflatoxins contamination in six urban and 11 rural areas using high performance liquid chromatography technique, revealed that the prevalence of AFB1 contamination in corn, wheat, peanut, lupine, white rice, cowpea, fava bean and brown rice was 64.7%, 53%, 53%, 47%, 47%, 41%, 29.4% and 29.4%, respectively. A positive correlation was found between aflatoxin and positive HCV-PCR together with liver disease progression to stage G3S3, that was indicative of HCC.

Several studies were carried out to evaluate the level of aflatoxins in food products in different governorates in Egypt [38]. From Qaluobia and Kafr El-Sheikh Governorates, 100 samples of imported and local wheat grains were collected (*Triticum sativum*) and examined for the natural occurrence of aflatoxins during 2000–2001. Results indicated that both local and imported samples were positive for aflatoxin B1 (17.5% and 20% respectively), and the concentration ranged from 3 to 25 μg/kg. The level of aflatoxins was dependant on the area of collection as well as the season of the year.

During 2004, samples were collected from Menofeya Governorate (four different districts: Mnuf, Quisna, Shibin El-Kom and Tala) to survey the natural occurrence of aflatoxins in local and imported corn grains and some corn products. The results showed that the local and imported corn grains were naturally infected with fungi belonging to genera of *Aspergillus*. The concentrations of aflatoxin B1 (AFB1) ranged between 5.8 and 7.5 μg/kg corn grains, while the concentration of aflatoxin B2 (AFB2) varied from 2.7 to 4.1 μg/kg corn grains. On the other hand, the concentrations of aflatoxins G1 and G2 were 8.1 and 3.2 μg/kg corn grains, respectively. On the other hand, out of 15 samples of corn products (snacks, cornflakes and popcorn) collected from local markets, only one sample of cornflakes contained AFB1 and AFB2 at concentrations of 3.4 and 2.7 μg/kg cornflakes, respectively.

Data in Table 2 indicated that, out of 110,150,120 and 100 *Aspergillus* spp. isolates which could be isolated from local and imported corn grain samples from Mnuf, Quisna, Shibin El-Kom, and Tala towns, the investigators found 35%, 40%, 45 and 40% of them were *A. flavus*, respectively.

Ten isolated *A. flavus* were randomly tested for producing aflatoxins. The results indicated that 60%, 50%, 25% and 45% of the isolates *A. flavus* were producing aflatoxins from local and imported corn grains samples.

In a study to screen for biomarkers of aflatoxin exposure in Egypt, Polychronaki et al. [39] assessed the level and frequency of breast milk AFM1 as a biomarker of maternal exposure. Breast milk samples were collected from a selected group of 388 Egyptian lactating mothers of children attending the New El-Qalyub Hospital, Qalyubiyah governorate, Egypt, during May–September 2003. Approximately 36% of mothers tested positive for AFM1. Non-working status, obesity, high corn oil consumption, number of children, and early lactation

Table 2
Natural occurrence of *A. flavus* producing aflatoxins in local and imported corn grains

Sources of corn grains	No. of tested *Aspergillus* spp. isolate	Frequency of *A. flavus* isolate (%)	Frequency of *A. flavus* isolate producing aflatoxins (%)[a]	Aflatoxins concentration (mg/l)	
				Range	Mean
1. Mnuf	110	35	25	2.7–0.5	4.8
2. Quisna	150	40	60	4.8–15.2	9.4
3. Shibin El-Kom	120	45	50	3.9–10.4	7.9
4. Tala	100	40	45	5.3–8.7	6.2

[a] These percentages were counted for *A. flavus* isolates producing aflatoxins from 10 isolates of *A. flavus*.

stage (<1 month), contributed to the occurrence of AF in breast milk.

The same research group continued their study [40], following up with 50 of those women who were AFM1 positive at baseline; they were revisited monthly for 12 months to assess the temporal variation in breast milk AFM1. In a multilevel regression model of the data there was a highly significant ($p < 0.001$) effect of month of sampling on the frequency of AFM1 detection with summer months having the highest frequency (>80%) and winter months the lowest frequency (<20%) of detection. The duration of lactation and peanut consumption also contributed to the model. The identification and understanding of factors determining the presence of toxicants in human milk is important and may provide a knowledge driven basis for controlling the transfer of chemicals to infants.

Hassan et al. [41] assessed the presence of aflatoxin (AFM1) in both mothers' milk and the infants' sera. Fifty healthy breast lactating mothers and their infants who were exclusively breast fed for at least 4 months were included. Twenty-four mothers (48%) and their infants had detectable levels of aflatoxin with the following mean contamination levels (ng/ml); mothers' serum of 8.9 ± 4.2, mothers' milk of 1.9 ± 0.6 and infants' serum of 1.8 ± 0.9.

5. Role of pesticides in the etiology of HCC

Occupational exposure pesticides may have a contributory role in the etiology or progression of HCC. According to Ezzat et al. [23] a major segment of the Egyptian population is employed in agriculture and uses pesticides routinely to control insects, weeds, rodents, and fungal infections of crops and livestock. A case–control study was carried out to investigate pesticides as environmental risk factors for HCC while taking into account viral risk factors. 236 subjects with confirmed HCC were recruited from the National Cancer Institute, Cairo University, Egypt, and 236 controls matched on sex, age group and urban–rural status recruited from the Orthopaedic Department, Cairo University Hospital, Egypt. Among rural males, the adjusted odds ratio (OR) for organophosphorus compounds was 2.7 and for carbamates it was 2.9. No statistically significant associations between HCC and household application of pesticides were observed for urban males or for females. As expected, the strongest risk factors for HCC in this study were HCV RNA and current HBV infection. This study therefore suggested that exposures to organophophorus and carbamate pesticides are additive risk factors to current HCV and HBV infection among rural males. Future investigation should address the possible hepatocarcinogenicity of pesticides using biomarkers of exposure and other techniques to better estimate dose–response relationships.

6. Approaches to HCC prevention

With recognized etiologic factors and improved mechanistic understanding, rational interventions to reduce the incidence and morbidity associated with HCC can be designed and implemented. Vaccination against HBV in infancy is the most effective approach to prevent HBV-related HCC, particularly in developing countries [42]. HB vaccine has now been given to millions of persons worldwide and is one of the most safe and efficacious vaccines in use [43]. However, vaccines for prevention of HCV are not available.

Antiviral treatment against HBV or HCV may interrupt or delay progression to HCC. The lengthy asymptomatic period prior to HCC diagnosis at advanced stages provides opportunities for earlier HCC detection. Key to the success of these secondary preventive interventions will be identifying the target groups for which antivirals or screenings are appropriate. The quantitative plasma 249ser p53 mutation is a prototypical example of a marker of both exposure and HCC risk. Screening and prevention strategies could be tailored to individuals based on 249ser p53 levels. Similarly, recent data on the predictiveness of HBV DNA levels for HCC suggest that appropriate management should be tailored to the specific levels and persistence of HBV viral load. Analysis of multiple markers can result in a 'molecular profile' of each stage of progressive liver disease from chronic viral infection to significant fibrosis/cirrhosis to HCC. These 'molecular profiles' may further delineate individuals for whom aggressive monitoring may be the most beneficial. Unfortunately, once advanced HCC is diagnosed, limited options exist for limiting complications or reducing mortality from HCC [2].

Prevention programmes can also be implemented against HCV infection by changing personal behavioral patterns and/or cultural habits which are considered as risk factors for HCV such as injection with contaminated syringes, blood transfusion, surgical operations, venous catheterization, use of common syringes, dental treatment and circumcision at home.

In parallel, reduction of exposure to AFB1, although a difficult task, may also prove an effective primary prevention measure. This can be achieved either at community (via good agriculture practices) or individual levels (treatment or dietary interventions). Easier methods for field detection and quantification of aflatoxin contamination of foodstuffs could aid epidemiologic studies of exposure and behavioral intervention trials aimed at reducing contamination [44]. Chemoprevention trials have also provided evidence that agents which modulate the effective level of aflatoxin exposure by increasing metabolic detoxification (as with oltipraz) or by reducing the bioavailability (as with chlorophyllin) may reduce levels of aflatoxin exposure [45].

Amra et al. [38] studied the effect of processing steps of corn products on the destruction of aflatoxins for popcorn and porridge. The results of the trial (Table 3) indicated that the process of popcorn and porridge preparation had a significant effect on aflatoxins' destruction. The temperature of preparing and treatment with 5% salt (sodium chloride) yielded the highest destruction rates (ranging from 90% to 97% depending on the specific compound measured). Such effects of processing of corn grains on the fate of residual aflatoxins should stimulate more trials aiming at prevention of aflatoxins health hazards.

Several probiotic bacteria are able to bind AFB1 in vitro, including *Lactobacillus rhamnosus* LC-705 and *Propionibacterium freudenreichii* subsp. *shermanii* JS [46]. A mixture of

Table 3
Effects of popcorn preparation on aflatoxins B1, B2, G1 and G2 destruction

Treatments (preparing stages)	Aflatoxin B_1		Aflatoxin B_2		Aflatoxin G1		Aflatoxin G2	
	Conc. (μg/kg + S.D.)	Destruction (%)	Conc. (μg/kg + S.D.)	Destruction (%)	Conc. (μg/kg + S.D.)	Destruction (%)	Conc. (μg/kg + S.D.)	Destruction (%)
Without salt added								
Corn after aflatoxins addition	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
After mixing with corn grain	80.5 + 0.41[a]	19.5	78.0 + 0.82	22.0	79.0 + 0.89[a]	21.0	75.4 + 0.90	24.6
After popcorn preparation	6.2 + 0.16	92.3	5.3 + 0.2 × 4	93.2	7.5 + 0.35	90.5	5.5 + 0.30	92.7
With 1% sodium chloride								
Corn after aflatoxins addition	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
After mixing with corn grain	79.4 + 0.73	20.6	77.7 + 0.57	22.3	75.9 + 0.74	24.1	70.9 + 0.85	29.1
After popcorn preparation	5.4 + 0.33	93.2	3.9 + 0.33	94.9	4.3 + 0.24	94.3	2.5 + 0.18	96.5
With 5% sodium chloride								
Corn after aflatoxins addition	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
After mixing with corn grain	78.3 + 0.82	21.7	75.2 + 0.39	24.8	74.2 + 0.61	25.8	69.7 + 1.1	30.3
After popcorn preparation	4.4 + 0.24	94.3	2.4 + 0.16	96.8	2.9 + 0.32	96.1	1.9 + 0.29	97.3

[a] These values are means of triplicate + S.D.

these two probiotics is used by the food and feed industry as biopreservative (Bioprofit), making it a promising candidate for future applications. Consequently, ongoing studies in Egypt and elsewhere are investigating the in vitro and ex vivo ability of this probiotic mixture to bind AFB1. In vitro experiments, 57–66% of AFB1 was removed from the solution by the probiotic mixture, but only 38–47% could be extracted from the bacterial surface. In ex vivo experiments, only up to 25% of AFB1 was bound by bacteria, and tissue uptake of AFB1 was significantly reduced when probiotic bacteria were present in the duodenal loop. Furthermore, the effect of intestinal mucus on the bacterial binding ability was investigated in vitro and was found to significantly reduce AFB1 binding by the probiotic mixture. Further work needs to assess the detoxifying potential of probiotics in different experimental setups, and in different dietary interventions. For example, a recent study [47] was carried out among 90 healthy young males from Guangzhou, People's Republic of China, to determine whether administration of probiotic bacteria could block the intestinal absorption of aflatoxin B1 and thereby lead to reduced urinary excretion of aflatoxin B1-N7-guanine (AFB-N7-guanine), a marker for a biologically effective dose of aflatoxin exposure. Probiotic consumption led to a statistically significant decrease in the urinary excretion of AFB-N7-guanine (36% reduction at week 3 and 55% at week 5). Additionally, the likelihood for negative AFB-N7-guanine at weeks 3–5 was 2.89 times bigger in the probiotic arm compared with placebo.

Such probiotic supplementation reduces the biologically effective dose of aflatoxin exposure, and may thereby offer an effective dietary approach to prevent the development of liver cancer. To assess this possibility, an intervention study is ongoing now in Egypt in a group of human subjects proven to be exposed to aflatoxins at baseline.

7. Conclusions and public health implications

In Egypt, extensive research over the past decade has documented high and increasing HCC incidence resulting from chronic HBV and HCV infections, and possibly augmented by agricultural pesticide exposures and dietary aflatoxin exposure. It is clearly shown that HB vaccination can be implemented in the national immunization programmes and that immunization is highly effective in the preventing chronic HBV infection. In the absence of vaccination for HCV, further investigation of the risk factors and modes of transmission of HCV is required to reduce infection rates and to prevent future cases of HCC. Using population-based sampling of markers of HBV, HCV and AFB1 exposure could provide useful data in formulating reasoned, cost-effective and country-specific intervention strategies to reduce HCC. Antiviral therapies hold some promise for interrupting or slowing progression to HCC. Further, the development of early detection markers is a vital missing component in strategies to reduce HCC mortality. With the growth in discovery driven technologies, including genomics and proteomics, systematic evaluation of biomarkers representing the complex process of hepatocarcinogenesis appear promising. As in many human cancers, molecular epidemiology studies may provide a better understanding of the temporal occurrence of genetic and epigenetic alterations in the natural history of liver carcinogenesis [48–50].

References

[1] D.M. Parkin, F.I. Bray, S.S. Devesa, Cancer burden in the year 2000: the global picture, Eur. J. Cancer 37 (Suppl. 8) (2001) S4–S66.
[2] G.D. Kirk, E. Bah, R. Montesano, Molecular epidemiology of human liver cancer: insights into etiology, pathogenesis and prevention from The Gambia, West Africa, Carcinogenesis 27 (10) (2006) 2070–2082.
[3] R.E. Velazquez, M. Rodriguez, C.A. Navascues, A. Linares, R. Perez, N.G. Sotorrios, I. Martinez, L. Rodrigo, Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis, Hepatology 37 (2003) 520–527.
[4] H.B. El-Serag, Hepatocellular carcinoma: an epidemiologic view, J. Clin. Gastroenterol. 35 (Suppl. 2) (2002) S72–S78.
[5] J. Bruix, M. Sherman, J.M. Llovet, M. Beaugrand, R. Lencioni, A.K. Burroughs, E. Christensen, L. Pagliaro, M. Colombo, J. Rodes, EASL panel of experts on HCC. Clinical management of hepatocellular carci-

noma. Conclusions of the Barcelona 2000 EASL conference, J. Hepatol. 35 (2001) 421–430.
[6] Inas Elattar, Website of the National Cancer Institute, Egypt: http://www.nci.edu.eg (2003).
[7] K. Ohata, K. Hamasaki, K. Toriyama, H. Ishikawa, K. Nakao, K. Eguchi, High viral load is a risk factor for hepatocellular carcinoma in patients with chronic hepatitis B virus infection, J. Gastroenterol. Hepatol. 19 (2004) 670–675.
[8] J. Xiong, Y.C. Yao, X.Y. Zi, J.X. Li, X.M. Wang, X.T. Ye, S.M. Zhao, Y.B. Yan, H.Y. Yu, Y.P. Hu, Expression of hepatitis B virus X protein in transgenic mice, World J. Gastroenterol. 9 (2003) 112–116.
[9] P. Pisani, D.M. Parkin, N. Munoz, J. Ferlay, Cancer and infection: estimates of the attributable fraction in, Cancer Epidemiol. Biomarkers Prev. 6 (1997) 387–400 (1990).
[10] S.C. Yates, M. Hafez, M. Beld, V.V. Lukashov, Z. Hassan, G. Carboni, H. Khaled, M. McMorrow, M. Attia, J. Goudsmit, Hepatocellular carcinoma in Egyptians with and without a history of hepatitis B virus infection: association with hepatitis C virus (HCV) infection but not with (HCV) RNA level, Am. J. Trop. Med. Hyg. 60 (1999) 714–720.
[11] A. El-Zayadi, Rahman, H. Abaza, S. Shawky, M.K. Mohamed, O.E. Selim, H.M. Badran, Prevalence and epidemiological features of hepatocellular carcinoma in Egypt—a single center experience, Hepatol. Res. 19 (2001) 170–179.
[12] T.J. Liang, V. Rustgi, E. Galun, H.E. Blum, Viral pathogenesis of hepatocellular carcinoma in the United States, Hepatology 18 (1993) 1326–1333.
[13] G.M. El-Nady, R. Ling, T.J. Harrison, Gene expression in HCV-associated hepatocellular carcinoma-upregulation of a gene encoding a protein related to the ubiquitin-conjugating enzyme, Liver Int. 23 (2003) 329–337.
[14] F.X. Bosch, J. Ribes, J. Borras, Epidemiology of primary liver cancer, Semin. Liver Dis. 19 (1999) 271–285.
[15] M. Colombo, Hepatitis C virus and hepatocellular carcinoma, Semin. Liver Dis. 19 (1999) 263–269.
[16] E. Szab, C. Paska, P. Kaposi Novak, Z. Schaff, A. Kiss, Similarities and differences in hepatitis B and C virus induced hepatocarcinogenesis, Pathol. Oncol. Res. 1 (2004) 5–11.
[17] G. Fattovich, Progression of hepatitis B and C to hepatocellular carcinoma in Western countries, Hepatogastroenterology 45 (Suppl. 3) (1998) 1206–1213.
[18] M. Nadia, G. Iman, A. Iman, Cancer Pathology Registry: 2003–2004, and Time Trend Analysis, National Cancer Institute, Cairo University, 2007.
[19] A.R. El-Zayadi, H.M. Badran, E.M.F. Barakat, M.E.D. Attia, S. Shawky, M.K. Mohamed, O. Selim, A. Saeid, Hepatocellular carcinoma in Egypt: a single center study over a decade, World J. Gastroenterol. 11 (33) (2005) 5193–5198.
[20] K. Mostafa, Epidemiology of HCV in Egypt, Afro-Arab Liver J. 3 (2) (2004) 41–52.
[21] H.B. El-Serag, Epidemiology of hepatocellular carcinoma, Clin. Liver Dis. 5 (2001) 87–107.
[22] M.M. Hassan, A.S. Zaghloul, H.B. El-Serag, O. Soliman, Y.Z. Patt, C.L. Chappell, R.P. Beasley, L.Y. Hwang, The role of hepatitis C in hepatocellular carcinoma: a case control study among Egyptian patients, J. Clin. Gastroenterol. 33 (2) (2001) 123–126.
[23] S. Ezzat, M. Abdel-Hamid, S.A. Eissa, N. Mokhtar, N.A. Labib, L. El-Ghorory, N.N. Mikhail, A. Abdel-Hamid, T. Hifnawy, G.T. Strickland, C.A. Loffredo, Associations of pesticides, HCV, HBV, and hepatocellular carcinoma in Egypt, Int. J. Hyg. Environ. Health 208 (5) (2005) 329–339.
[24] C. Frank, M.K. Mohamed, G.T. Strickland, D. Lavanchy, R. Arthur, L.S. Magder, T. El Khoby, Y. Abdel-Wahab, E.A. Ohn, W. Anwar, I. Sallam, The role of parenteral antischistosomal therapy in the spread of hepatitis C in Egypt, Lancet 355 (2000) 887–891.
[25] Y.A. Ghaffar, S.A. Fattah, M. Kamel, R.M. Badr, F.F. Mahomed, G.T. Strickland, The impact of endemic schistosomiasis on acute viral hepatitis, Am. J. Trop. Med. Hyg. 45 (1991) 743–750.
[26] Taha El Khoby, Schistosomiasis in Egypt, Am. J. Trop. Med. Hyg. 62 (2000) 88–99.
[27] WHO Global surveillance, control of hepatitis C. Report of a WHO Consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J. Viral. Hepat. 6, 35–47 (1999).
[28] T. Heintges, J.R. Wands, Hepatitis C virus: epidemiology and transmission, Hepatology 26 (1997) 521–526.
[29] S.C. Ray, R.R. Arther, A. Carella, J. Bukh, D.L. Thomas, Genetic epidemiology of hepatitis C virus throughout Egypt, J. Infect. Dis. 182 (3) (2000) 698.
[30] Y. Tanaka, S. Agha, N. Saudy, F. Kubanov, E. Orito, T. Kato, M. Abo-Zeid, M. Khalaf, Y. Miyakawa, M. Mizokami, Exponential spread of hepatitis C virus genotype 4a in Egypt, J. Mol. Evol. 58 (2) (2004) 191.
[31] M. Abdel-Hamid, M. El-Daly, V. Molnegren, S. El-Kafrawy, S. Abdel-Latif, G. Esmat, G.T. Strickland, C. Loffredo, J. Albert, A. Widell, Genetic diversity in hepatitis C virus in Egypt and possible association with hepatocellular carcinoma. J. Gen. Virol. 88, 1526–1531 (2007).
[32] M.M. El Gaafary, C. Rekacewicz, A.G. Abdel-Rahman, M.F. Allam, M. El Hosseiny, M. Abdel Hamid, F. Colombani, Y. Sultan, S. EI-Aidy, A. Fontanet, M.I. Mohamed, Surveillance of acute hepatitis C in Cairo, Egypt. J. Med. Virol. 76 (2005) 520–525.
[33] O.G. Pybus, A.J. Drummond, T. Nakano, B.H. Robertson, A. Rambaut, The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: a Bayesian coalescent approach, Mol. Biol. Evol. 20 (3) (2003) 381–387.
[34] International Agency for Research on Cancer. IARC' S7. Monographs on the Evaluation of Carcinogenic Risks to Humans. Overall Evaluations of Carcinogenicity. 440 pp. Lyon, France (1987).
[35] M.K. Mohamed, A.R. El Zayadi, S.A. Anoun, H. Abaza, O.E. Selim, H.M. Badran, G.T. Strickland, Trend of hepatocellular carcinoma and associated risk factor in Egypt in the Pan Arab Association of Gastroentrology 12–16 September Egypt (Abstract) (1999).
[36] S.A. El-Kafrawy, M. Abdel-Hamid, M. El-Daly, O. Nada, A. Ismail, S. Ezzat, S. Abdel-Latif, A. Abdel-Hamid, P.G. Shields, C. Loffredo, P53 mutations in hepatocellular carcinoma patients in Egypt, Int. J. Hyg. Environ. Health 208 (4) (2005) 263–270.
[37] M.S. Hifnawy, A.M. Mangoud, M.H. Eissa, E. Nor Edin, Y. Mostafa, Y. Abouel-Magd, E.I. Sabee, I. Amin, A. Ismail, T.A. Morsy, S. Mahrous, A.F. Afefy, E. el-Shorbagy, M. el-Sadawy, H. Ragab, M.I. Hassan, G. el-Hady, M. Saber, The role of aflatoxin-contaminated food materials and HCV in developing hepatocellular carcinoma in Al-Sharkia Governorate, Egypt. J. Egypt Soc. Parasitol. 34 (Suppl. 1) (2004) 479–488.
[38] H.A. Amra, S.F. Masheal, M.M. Ebba, A.A. Bakr, Q.A. Nogaim, W.A. Anwar, Aflatoxins level in some Egyptian crops collected from different governorates: possibilities for detection and prevention (Poster No. 26), in: 5th International Conference on Environmental Mutagens in Human Populations: "Identification of Environmental Hazards and Promotion of Health", Antalya, Turkey, 20–24, May, 2007.
[39] N. Polychronaki, C.P. Turner, H. Mykkanen, Y. Gong, H. Amra, M. Abdel-Wahhab, H. El-Nezami, Determinants of aflatoxin M1 in breast milk in a selected group of Egyptian mothers, Food Addit. Contam. 23 (7) (2006) 700–708.
[40] N. Polychronaki, R.M. West, P.C. Turner, H. Amra, M. Abdel-Wahhab, H. Mykkanen, H. El-Nezami, A longitudinal assessment of aflatoxin M(1) excretion in breast milk of selected Egyptian mothers, Food Chem. Toxicol. 45 (7) (2007) 1210–1215.
[41] A.M. Hassan, H.A. Sheashaa, M.F. Abdel Fatah, A.Z. Ibrahim, O.A. Gaber, Does aflatoxin as an environmental mycotoxin adversely affect the renal and hepatic functions of Egyptian lactating mothers and their infants? A preliminary report, Int. Urol. Nephrol. 38 (2) (2006) 339–342.
[42] J.H. Kao, D.S. Chen, Global control of hepatitis B virus infection, Lancet Infect. Dis. 2 (2002) 395–403.
[43] G.D. Kirk, Hepatitis B vaccination and liver cancer, in: B.W. Stewart, P. Kleihues (Eds.), World Cancer Report, IARC Press, Lyon, 2003, pp. 144–147.
[44] M.A. Carlson, C.B. Bargeron, R.C. Benson, A.B. Fraser, T.E. Phillips, J.T. Velky, J.D. Groopman, P.T. Strickland, H.W. Ko, An automated, handheld biosensor for aflatoxin, Biosens. Bioelectron. 14 (2000) 841–848.
[45] T.W. Kensler, P.A. Egner, J.B. Wang, et al., Chemoprevention of hepatocellular carcinoma in aflatoxin endemic areas, Gastroenterology 127 (2004) S310–S318.

[46] S. Gratz, H. Mykkanen, H. El-Nezami, Aflatoxin B1 binding by a mixture of Lactobacillus and Propionibacterium: in vitro versus ex vivo, J. Food Protect. 68 (11) (2005) 2470–2474.
[47] H.S. El-Nezami, N.N. Polychronaki, J. Ma, H. Zhu, W. Ling, E.K. Salminen, R.O. Juvonen, S.J. Salminen, T. Poussa, H.M. Mykkänen, Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from Southern China, Am. J. Clin. Nutr. 83 (5) (2006) 1199–1203.
[48] R. Montesano, P. Hainaut, C.P. Wild, Hepatocellular carcinoma: from gene to public health, J. Natl Cancer Inst. 89 (1997) 1844–1851.
[49] C.P. Wild, G.R. Law, E. Roman, Molecular epidemiology and cancer: promising areas for future research in the post-genomic era, Mutat. Res. 499 (2002) 3–12.
[50] S.P. Hussain, C.C. Harris, Molecular epidemiology of human cancer, Toxicol. Lett. 102/103 (1998) 219–225.